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Bio Activity of Sesbania Grandiflora against Hepatic Damage in Albino Rats

Article by K. Padmalochana and M.S. Dhana Rajan

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Abstract

A phytotherapeutic approach to modern drug development can provide many invaluable drugs from traditional medicinal plants. Medicinal plants have been considered as important therapeutic aid for alleviating ailment of humankind. Numerous plants and polyherbal formulations are used for the treatment of liver diseases. This present investigation was aimed to assessing the hepatoprotective activity of aqueous, ethanol and acetone extract of Sesbania grandiflora leaves against carbon tetra chloride (CCl₄) induced liver damage in albino rats. Silymarin as standard drug for comparing the activity. The activity was assessed by comparing the biochemical parameters in serum levels such as serum glutamate pyruvate transaminase, serum glutamate oxalate transaminase, total bilirubin, alkaline phosphatase of plant extracts treated group with carbon tetrachloride treated animals. Results showed, ethanolic extract treated group showed highly significant activity (p<0.001), whereas aqueous extract treated group has shown the significant (p<0.01) action but less compared with ethanolic extract, acetone treated group showed moderate action. Plant extracts restores biochemical enzymes and brings down to normal as compared to standard drug silymarin. This results shows and confirms the significant protective activity against CCl₄ induced hepatotoxicity.

Keywords: Phytotherapeutic, Sesbania Grandiflora, Antioxidant, Carbon Tetra Chloride, Hepatotoxicity.

Introduction

Liver is very important organ in the human body. It regulates metabolic functions such as detoxification and play vital role in bio-chemical conversion. During the process of elimination there is chance of accumulation different kinds of toxic materials inside the hepatocytes and there is chance of liver infection, and hepatic disorders such as hepatitis ¹. Liver diseases caused by various toxic chemicals, chemotherapeutic agents, excessive consumption of alcohol and microorganisms. Hepatotoxicity is an acute adverse effect in liver in caused by over dosages of drugs, toxic chemicals, viruses, bacteria and parasites ².

Hepatotoxicity is a slight changes in hepatic structure and function which may result hypertension, ascites, jaundice, increased bleeding and cause multiple metabolic changes affecting other organs ³, ⁴. The magnitude of derangement of liver by disease or hepatotoxin is generally measured by the level of glutamate pyruvate transaminase (ALT), glutamate oxaloacetate transaminase (AST), alkaline phosphatase (ALP), bilirubin, albumin, and whole liver homogenate ⁴, ⁵.

CCl₄ is a widely used industrial chemical and a potent hepatotoxin. It induces hepatotoxicity by producing free radical, putting oxidative stress hence causing lipid peroxidation in liver tissues, consequently necrotic liver damage ⁶, ⁷. Liver diseases such as hepatitis, cirrhosis and fatty liver are worldwide. Various commercial synthetic drugs are used to treat liver disorders also cause side effect to the liver. Hence, Herbal drugs have become increasingly popular and their use is widespread. Herbal medicines have been used in the treatment of liver diseases for a long time. In India numerous medicinal plants are used for treatment of liver disorders ⁸. Hepatoprotective effect of some plants like Spirulina maxima ⁹, Eclipta alba ¹⁰, Boehmerianivea ¹¹, Cichorium intybus ¹², and Picrorhiza kurroa ¹³, Boswellia Serrata ⁴, Psidium quajava ¹⁴, Coccinia indica ¹⁵ has been well documented.
**Sesbania grandiflora** Figure. 1 fast growing tree belongs to the family, Fabaceae, is commonly known as agathi in regional language Tamil. The leaves, used as greens for cattle and poultry, have got anthelmintic property against selected helminthes.16, 17. The bark, leaves, flowers and roots are also used medically herbs distributed in the tropical regions of the globe 18. Juice of leaves and flowers is popular remedy for nasal catarrh and headache when it is sniffed up the nostrils. Juice of the flowers is squeezed into the eyes to relieve the dimness of vision.

Juice of flower is ideal as expectorant 19. The leaves of the plant have been reported to have anxiolytic and anticonvulsant effect while the flowers have been reported to have anti-microbial activity 20. It shows hypolipemic, anti-ulcer and anti-inflammatory properties as well. Therefore, to justify the traditional claims, we have assessed the hepatoprotective effect of *Sesbania grandiflora* leaves extract in albino rats using biochemical enzyme based analysis.

![Sesbania grandiflora leaves](image)

**Figure 1.** Sesbania grandiflora leaves

**Materials and methods**

**Chemicals:** Analytical grade carbon tetra chloride, Silymarin and other chemicals were purchased from Himedia laboratories private limited, Mumbai. *Sesbania grandiflora* plant was collected from, Tiruvannamalai, South India.

**Preparation of plant extracts**

*Sesbania grandiflora* leaves were collected and shade dried at room temperature. The shade dried leaves were powdered and extracted by using aqueous, ethanol and acetone. Aqueous extracts was prepared by subjecting a 100 g of dried powdered leaves in to 100 ml of distilled water and incubated in water bath shaker for 12 h at 40°C. Ethanol and acetone extract prepared by the coarsely powdered leaves was extracted using soxhlet and extracted with 80 % ethanol and 70 % acetone for 24 h at 60 °C and55°C, respectively. The extracted were collected and concentrated by drying under vacumm and semisolid suspensions were obtained. These suspensions were used to assess hepatoprotective activity.

**Experimental design for hepatoprotective activity of sesbania grandiflora**

Adult male Wister albino rats maintained at the college weighing between 150g-170g were used for the hepatoprotective studies. Animals were divided into six groups in six rats each:

- **Group I (Normal):** Orally received distilled water for 7 days.
- **Group II (Induced):** Orally received carbon tetra chloride (2g/kg body weight) only for 7 days.
**Group III (Standard):** Orally received Silymarin (20 mg/kg body weight) along with CCl4 (2g/kg body weight) for 7 days.

**Group IV (Treatment):** Orally received Aqueous leaf extracts (300mg/kg body weight) along with CCl4 (2g/kg body weight) for 7 days.

**Group V (Treatment):** Orally received ethanol leaf extracts (300mg/kg body weight) along with CCl4 (2g/kg body weight) for 7 days.

**Group VI (Treatment):** Orally received acetone leaf extracts (300mg/kg body weight) along with CCl4 (2g/kg body weight) for 7 days.

Silymarin was used as positive control for comparing hepatoprotective potential of different leaves extract of *Sesbania grandiflora*.

**Hepatoprotective activity of S. grandiflora**

**Collection of blood and biochemical analysis**

On the 8th day, all the animals were scarified and blood samples were collected in glass tube from retro-orbital puncture to obtain haemolysis for 30 min at 37°C. Serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, serum bilirubin and alkaline phosphatase and Serum protein were obtained from serum following centrifugation process was used for the biochemical analysis.

**Antioxidant activity of S. grandiflora**

**Liver homogenate preparation**

Liver homogenates were prepared by using a 100mM KCl buffer (pH7.0) containing 0.3mM EDTA and centrifuged at 6000 rpm for 45min at 4°C. After completion of centrifugation process collect the supernatant was used for estimation of antioxidant levels were analyzed Superoxide dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GP).

**Statistical analysis**

The difference of biochemical parameters were measured using the statistical method i.e. Analysis of Variance (ANOVA). Analysis of Variance refers to the examination of differences among the samples and the results are expressed as mean± SEM and p < 0.05, p< 0.01, p< 0.001 was considered to be statistically significant.

**Results and discussion**

The hepatoprotective and antioxidant activity of *S. grandiflora* leaves extracts are shown in Figure. 2-4. the biochemical parameters such as serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, serum bilirubin and alkaline phosphatase were estimated to assess the liver function. The marked increase in SGOT, SGPT and ALP levels were observed in CCl4 treated group II animals are 94.98±0.69, 29.98±0.67 and 299.68±0.12 IU/L, respectively. The increased level of SGOT, SGPT, ALP and bilirubin is conventional indicator of liver injury. However these levels were reversed to near normal levels of group I animals with treatment of aqueous, ethanol and acetone extract of *Sesbania grandiflora*, which are statistically significant. The activities of extracts were comparable to a standard drug. These extracts has restored the all the biochemical parameters levels in serum. And also the standard silymarin has restored the biochemical levels of SGOT, SGPT, and ALP significantly (p<0.01) i.e. 69.42±0.38, 24.42±0.33, and 170.12±0.25 IU/L respectively in serum. In case of bilirubin and total protein there was a noticeable increase i.e. in serum levels treating with CCl4. Treatment with aqueous, ethanol and acetone extract has reversed the serum bilirubin and total protein in serum levels to (0.50±0.07 and 6.85±0.12 mg/dl), (0.45±0.09 and 6.59±0.32 mg/dl), (0.47±1.06 and 6.26±1.07 mg/dl), respectively which are statistically highly significant (p<0.001) when compared with CCl4 treated animals.
Figure 2. Hepatoprotective activity in carbon tetra chloride induced hepatotoxic model shows changes in serum enzymes SGOT, SGPT and ALP.

Figure 3. Hepatoprotective activity in carbon tetra chloride induced hepatotoxic model shows changes in serum bilirubin and total protein.

The restoration of biochemical factors in serum was also noticed in treating with the standard drug silymarin (0.49±0.04 and 6.26±0.16 mg/dl). It is stipulated that the extract treated group was protected from hepatic cell damage caused by CCl₄ induction. The extract at a dose of 300 mg/kg body wt. exhibited orally, significant protective effect by lowering the serum levels of transaminases (SGOT and SGPT), bilirubin and alkaline phosphatase (ALP).

The effects produced were comparable to that of a standard hepatoprotective agent silymarin. In ethanol extract treated animals, the toxicity effect of carbon tetrachloride was controlled significantly by restoration of the levels of serum bilirubin and enzymes as compared to the normal and standard drug silymarin-treated groups. Antioxidant activities of hepatic SOD, CAT and GPx were estimated and shown in the Table 3. Figure 4 SOD, CAT and GPx activities were significantly (p<0.001) enhanced only in the orally received ethanol extract of S. grandiflora leaves. The antioxidant activities of aqueous, ethanol and acetone extract shows significant activity near to the normal group of animals.
Table 1. Hepatoprotective activity in carbon tetra chloride induced hepatotoxic model shows changes serum enzymes sgot, sgpt and alp in serum

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SGOT(IU/L)</th>
<th>SGPT(IU/L)</th>
<th>ALP(IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal)</td>
<td>65.15±0.14***</td>
<td>23.15±0.17***</td>
<td>166.15±0.22***</td>
</tr>
<tr>
<td>Group II (induced)</td>
<td>94.98±0.69*</td>
<td>29.98±0.67*</td>
<td>299.68±0.12*</td>
</tr>
<tr>
<td>Group III (Standard drug)</td>
<td>69.42±0.38**</td>
<td>24.42±0.33***</td>
<td>170.12±0.25***</td>
</tr>
<tr>
<td>Group IV (Aqueous)</td>
<td>67.48±0.39**</td>
<td>23.48±0.38**</td>
<td>178.48±0.28***</td>
</tr>
<tr>
<td>Group V (Ethanol)</td>
<td>59.59±0.76***</td>
<td>21.59±0.74***</td>
<td>164.51±0.17***</td>
</tr>
<tr>
<td>Group VI (acetone)</td>
<td>62.58±0.34***</td>
<td>24.58±0.35**</td>
<td>168.59±0.73***</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001 value are considered statistically significant (BMRT)

Figure 4. Antioxidant levels in carbon tetra chloride induced hepatotoxic model shows changes in the levels of sod, cat and GPx

Table 2. Hepatoprotective activity in carbon tetra chloride induced hepatotoxic model shows changes serum bilirubin and total protein

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Serum bilirubin (mg/dl)</th>
<th>Serum Protein (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I(Normal)</td>
<td>0.44±0.03***</td>
<td>6.58±0.06***</td>
</tr>
<tr>
<td>Group II(induced)</td>
<td>1.76±0.10*</td>
<td>3.32±0.04*</td>
</tr>
<tr>
<td>Group III(Standard drug)</td>
<td>0.49±0.04***</td>
<td>6.26±0.16**</td>
</tr>
<tr>
<td>Group IV (Aqueous)</td>
<td>0.50±0.07**</td>
<td>6.85±0.12**</td>
</tr>
<tr>
<td>Group V (Ethanol)</td>
<td>0.45±0.09***</td>
<td>6.59±0.32***</td>
</tr>
<tr>
<td>Group VI (acetone)</td>
<td>0.47±1.06**</td>
<td>6.26±1.07***</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001 value are considered statistically significant (BMRT)
Table 3: Antioxidant levels in carbon tetra chloride induced hepatotoxic model
Shows changes in the levels of sod, cat and GPX

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SOD (µmol/min/mg protein)</th>
<th>CAT (µmol/min/mg protein)</th>
<th>GPx (µmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal)</td>
<td>18.91 ± 0.31***</td>
<td>58.99 ± 4.80***</td>
<td>5.98 ± 2.46***</td>
</tr>
<tr>
<td>Group II (induced)</td>
<td>04.94± 0.21*</td>
<td>31.89±3.43*</td>
<td>3.98± 0.35*</td>
</tr>
<tr>
<td>Group III (Standard drug)</td>
<td>14.73 ± 0.39**</td>
<td>55.67 ± 3.13**</td>
<td>5.34 ± 2.11**</td>
</tr>
<tr>
<td>Group IV (Aqueous)</td>
<td>20.01 ± 0.17**</td>
<td>49.68 ± 0.55***</td>
<td>4.32 ± 1.92**</td>
</tr>
<tr>
<td>Group V (Ethanol)</td>
<td>23.92 ± 0.27***</td>
<td>54.23 ± 3.17***</td>
<td>4.84 ± 1.70***</td>
</tr>
<tr>
<td>Group VI (acetone)</td>
<td>19.46 ± 0.11***</td>
<td>50.38 ± 0.18***</td>
<td>4.42 ± 1.02***</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001 value are considered statistically significant (BMRT).

Conclusion

In the present report stated that the aqueous, ethanol and acetone extract of commonly available plant *Sesbania grandiflora* leaves was extensively investigated for its hepatoprotective potential against *CCl₄* induced hepatotoxicity. There was a significant increase in serum levels of bilirubin, alanine transaminase, aspartate transaminase and alkaline phosphatase with a decrease in total protein level, in the *CCl₄* treated animals, reflecting liver injury. In the extracts treated animals there was a decrease in serum levels of the markers and significant increase in total protein, indicating the recovery of hepatic cells. A strong conclusion can be drawn that, extract of *Sesbania grandiflora* possess most significant (p<0.001) hepatoprotective activity compared with the standard drug silymarin. So that the development of medicines using the extracts of plant materials or bioactive compounds with standards of safety and efficacy can revitalize treatment of liver disorders and hepatoprotective activity.

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Determination of Molecular Property, Bioactivity Score and Binding Energy of the Phytochemical Compounds Present in Cassia Auriculata by Molinspiration and DFT Method

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Abstract

Phytoconstituent present in Cassia Auriculata were found to obey the Lipinski’s rule (MiLog P <5) α - Tocopherol (2.007) indicated their drug likeness property. Among these compounds, α - Tocopherol exhibited highest score towards GPCR ligand, (0.25) nuclear receptor ligand (0.43) and inhibitory activities towards protease (0.29), enzyme (0.25) and kinase (-0.22) inhibitors compared to others. Insilico determination of binding energy using DFT method proved that α- Tocopherol was found to possess good binding energy (B3LYP and HF method were found to -1228.3913, -1236.9904 & -1243.4557 a.u. and -1220.2810,-1228.4658 &-1234.8000 a.u.) among others hence it was found to be more stable.

Keywords: Cassia auriculata phytochemical compounds, Molinspiration software, DFT methods and Insilico prediction.

Introduction

Cassia auriculata is one of the herbaceous plants that found throughout central and southern India, also cultivated in Punjab, Haryana, Uttar Pradesh and West Bengal. The shrub usually occurs on roadsides, waste line, and railway embankments. Avaram (Cassia auriculata Linn), family Caesalpiniaceae, is also known as Avaram tree. Cassia auriculata Linn (Family: Caesalpiniaceae) commonly known as Tamers senna, is distributed throughout hot deciduous forests of India and holds a very prestigious position in Ayurveda and Siddha systems of medicine. It was profoundly used in Ayurvedic medicine as a tonic, astringent and as a remedy for diabetes, conjunctivitis and ophthalmia [1]. It is one of the principle constituents of ‘Aavaarai panchaga chooranam’- an Indian herbal formulation used in the treatment of diabetes to control the blood sugar level [2].

The plant has been reported to possess antipyretic [3], hepatoprotective [4], antidiabetic, antiperoxidative and antihyperglycemic [5], microbicidal [6] and antihyperlipidaemic activities [7]. The flowers are used to treat urinary discharges, nocturnal emissions, diabetes and throat irritation [8]. They are one of the constituent of polyherbal formulation ‘Diasulin’ in the concentration range of 40 mg/dl which is proven to have antidiabetic activity [9].

It has been found to possess antitumor, oncogenic, and diabeto genic properties [10]. The antioxidant and radical scavenger function of α-tocopherol is essentially dependent on the free state of its hydroxyl group. Spectacular antiallergic and antiinflammatory activities have been attributed to DL-α- tocopheryl-α- D-mannopyranoside and DL-α-tocopheryl-β-D-galactopyranoside [11]. Hexadecanoic acid methyl ester, also known as Methyl palmitate, in the methanol fraction is an aliphatic acid ester reported to cause growth inhibition and apoptosis induction in human gastric cancer cells [12].

The phytoconstituent of a plant will often determine the physiological action on the human body. Cassia species are rich sources of Polyphenols, Anthraquinone derivatives, Flavanoids, Polysaccharides, Saponins, Tannins, and Steroids. Some of the Cassia species are rich in Glycerides with linoleic, oleic, stearic, and palmitic acids. Cassia species are well known for their laxative and purgative constituents and are also used for the treatment of skin diseases. Leaves are anthelmintic and
also used to treat ulcers, skin diseases, and leprosy. An aqueous extract of leaves possesses hypoglycemic activity. The leaves are eaten as a vegetable in times of scarcity, the infusion of leaves possesses a slight purgative activity.

**Molinspiration**

Molinspiration, web based software was used to obtain parameter such as MiLogP, TPSA, drug likeness scores. MiLogP is calculated by the methodology developed by Molinspiration as a sum of fragment based contributions and correction factors. MiLog P parameter is used to check good permeability across the cell membrane. Partition coefficient or Log P is an important parameter used in rational drug design to measure molecular hydrophobicity. Hydrophilic/lipophilic nature of drug molecule affects drug absorption, bioavailability, drug-receptor interactions, metabolism of molecules, as well as their toxicity. Molecular Polar Surface Area TPSA is calculated based as a sum of fragment contributions of O and N- centered polar fragments. Total polar surface area (TPSA) is closely related to the hydrogen bonding potential of a molecule and is a very good predictor of drug transport properties such as intestinal absorption, bioavailability, blood brain barrier penetration etc. Calculation of volume developed at Molinspiration is based on group contributors. Number of rotatable bonds measures molecular flexibility. It is a very good descriptor of absorption and bioavailability of drugs. Through drug likeness datas of molecule, it can be checked molecular properties and structure feature in respect to known drugs.

Bioactivity of the drug can be checked by calculating the activity score of GPCR ligand, ion channel modulator, nuclear receptor legend, kinase inhibitor, protease inhibitor, enzyme inhibitor. All the parameters were checked with the help of software. Calculated drug likeness score of each compounds were compared with the specific activity of other compounds and the results were compared with standard drug. For organic molecules the probability is if the bioactivity score is (>0), then it is active, if (-5.0-0.0) then moderately active, if (< -5.0) then inactive. The drug likeness scores were calculated by considering MiLogP (partition coefficient), molecular weight, number of heavy atoms, number of hydrogen donor, number of hydrogen acceptor and number of violation, number of rotatable bonds and volume [13-18].

i) Lipinski’s rule

Lipinski's rule of five also known as the Pfizer's rule of five or simply the Rule of five (RO5) is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997.

The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion (“ADME”) Components of the Lipinski’s rule:

ii) Lipinski’s rule states

- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms).
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms).
- A molecular mass less than 500 daltons.
- An octanally-water partition coefficient log P not greater than 5.
- No more than one number of violation.

iii) Drug likeness score

Molinspiration, web based software was used to obtain parameter such as MiLogP, TPSA, drug likeness. MiLogP, is calculated by the methodology developed by Molinspiration as a sum of fragment based contributions and correction factors. MiLog P parameter is used to check good permeability across the cell membrane. TPSA is related to hydrogen bonding potential of compound. Calculation of volume developed at Molinspiration is based on group contributors. Number of rotatable bonds measures molecular flexibility. It is a very good descriptor of absorption and bioavailability of drugs.
Through drug likeness datas of molecule, it can be checked molecular properties and structure feature in respect to known drugs.

iv) Bioactivity score

Bioactivity of the drug can be checked by calculating the activity score of GPCR ligand, ion channel modulator, nuclear receptor legend, kinase inhibitor, protease inhibitor, enzyme inhibitor. All the parameters were checked with the help of software Molinspiration drug-likeness score online (www.molinspiration.com). Calculated drug likeness score of each compounds and compared with the specific activity of each compound, and the results were compared with standard drug. For organic molecules the probability is if the bioactivity score is (>0), then it is active, if (-5.0-0.0) then moderately active, if (< -5.0) then inactive.

DFT using gaussian

"Density", because the central quantity being computed and manipulated is not the wave function but the electron density, "Functional" because the central operators in the theory are functional -- mathematic objects that take functions as arguments and return new functions as output. The "right" functional had been proven to exist, but nobody knows what it is. "Theory", because its science. We call things theories.

A variety of computational methods thus exists to reformulate the "exact" QM equations in one way or another, and then solve those equations algorithmically on a computer. Two basic approaches are DFT and something called Hartree-Fock (HF) theory. A variety of methods have also been developed that either extend HF theory (these are usually called post-HF methods), or mix HF and DFT. Nobel prize in chemistry in 1998 was awarded to Kohn and Pople basically for developments in DFT and HF respectively.

The cytochrome P450 enzymes (CYPs) metabolize many drug compounds. They catalyze a wide variety of reactions, and potentially, a large number of different metabolites can be generated. Density functional theory (DFT) has, over the past decade, been shown to be a powerful tool to rationalize and predict the possible metabolites generated by the CYPs as well as other drug-metabolizing enzymes.

DFT is a useful tool for prediction of the site of metabolism. The use of small models of the enzymes work surprisingly well for most CYP isoforms. This is probably due to the fact that the binding of the substrates is not the major determinant. When binding of the substrate plays a significant role, the well-known issue of determining the free energy of binding is the challenge.

Materials and methods

Materials

Then the plant was identified and authenticated by Plant Anatomy Research Centre (PARC/2017/3467). Phytochemical compounds present in Cassia Auriculata like Dodecanoic acid, Ethyl Caprylate, Glycine (trifluoroacetyl) - methyl butyl ester, α – Tocopherol and n – Hexadecanoic acid as given in (Figure - 1 to 6) were selected for insilico prediction.

Figure 1. Dodecanoic acid
Methods

Molinspiration

Structures of six phytochemical compounds selected for our work as given in Figure - 1 to 6 (reported in the literature resources) were drawn using online molinspiration for the calculation of molecular properties like MiLog P, Total polar surface area (TPSA), number of hydrogen bond donors.
and acceptors, molecular weight, number of atoms, number of rotatable bonds etc., and bioactivity scores like GPCR ligands, kinase inhibitors, ion channel modulators, enzymes and nuclear receptors.

- The molecular properties and bio-activity scores predicted by molinspiration were given in Table – I a & b.
Table I (a). Molecular property of phytochemical compounds

<table>
<thead>
<tr>
<th>S.N o</th>
<th>Phytochemical compounds</th>
<th>MiLogP</th>
<th>TPSA</th>
<th>natoms</th>
<th>nON</th>
<th>nOHNH</th>
<th>nviolations</th>
<th>nrotb</th>
<th>volume</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dodecanoic acid</td>
<td>5.038</td>
<td>37.299</td>
<td>14.0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>224.215</td>
<td>200.32</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl caprylate</td>
<td>3.701</td>
<td>26.305</td>
<td>12.0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>191.338</td>
<td>172.26</td>
</tr>
<tr>
<td>3</td>
<td>Glycine (trifluoroacetyl)-methyl butyl ester</td>
<td>2.007</td>
<td>55.405</td>
<td>16.0</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>208.642</td>
<td>241.20</td>
</tr>
<tr>
<td>4</td>
<td>Capric acid ethyl ester</td>
<td>4.711</td>
<td>26.305</td>
<td>14.0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>224.941</td>
<td>200.32</td>
</tr>
<tr>
<td>5</td>
<td>α - Tocopherol</td>
<td>8.847</td>
<td>29.462</td>
<td>30.0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td>457.697</td>
<td>416.69</td>
</tr>
<tr>
<td>6</td>
<td>n- Hexadecanoic acid</td>
<td>7.059</td>
<td>37.299</td>
<td>18.0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>14</td>
<td>291.422</td>
<td>256.43</td>
</tr>
</tbody>
</table>
Table-I (b). Bioactivity score of phytochemical compounds

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phytochemical compounds</th>
<th>GPCR ligand</th>
<th>Ion Channel Modulator</th>
<th>Kinase inhibitor</th>
<th>Nuclear receptor ligand</th>
<th>Protease inhibitor</th>
<th>Enzyme inhibitor</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Dodecanoic acid</td>
<td>-0.27</td>
<td>-0.04</td>
<td>-0.75</td>
<td>-0.24</td>
<td>-0.36</td>
<td>0.04</td>
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<tr>
<td>2</td>
<td>Ethyl caprylate</td>
<td>-0.85</td>
<td>-0.34</td>
<td>-1.25</td>
<td>-0.84</td>
<td>-0.86</td>
<td>-0.40</td>
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<tr>
<td>3</td>
<td>Glycine (trifluoroacetyl)-methyl butyl ester</td>
<td>-0.38</td>
<td>-0.38</td>
<td>-0.88</td>
<td>-0.31</td>
<td>-0.18</td>
<td>-0.12</td>
</tr>
<tr>
<td>4</td>
<td>Capric acid ethyl ester</td>
<td>-0.60</td>
<td>-0.21</td>
<td>-0.93</td>
<td>-0.57</td>
<td>-0.62</td>
<td>-0.23</td>
</tr>
<tr>
<td>5</td>
<td>α - Tocopherol</td>
<td>0.25</td>
<td>0.15</td>
<td>-0.22</td>
<td>-0.22</td>
<td>0.29</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>n- Hexadecanoic acid</td>
<td>0.02</td>
<td>0.06</td>
<td>-0.33</td>
<td>-0.33</td>
<td>-0.04</td>
<td>0.18</td>
</tr>
</tbody>
</table>

>0- active, -5.0-0.0- moderately active, < -5.0- inactive
For the prediction of Molecular property, the following steps were followed:

- Structures were drawn in Molinspiration by opening www.molinspiration.com
- After drawing the structure Click “Go for prediction” button, the Molecular property will appear as given in (Figure-7).

![Figure 7. Molecular property setup window](image)

- For bioactivity score prediction Click “Predict bioactivity” the window will appear as given in (Figure-8).

![Figure 8. Bioactivity score setup window](image)

**DFT calculation using gaussian**

DFT calculations were carried out using Gaussian software 05. Binding energies of the phytochemical constituent present in Cassia auriculata leaves like Dodecanoic acid, Ethyl caprylate, Glycine (trifluoroacetyl)-methyl butyl ester, Capric acid ethyl ester, α–Tocopherol and n-
Hexadecanoic acid were calculated by B3LYP and HF methods using STO-3G, 3-21G, 6-31G basis sets [18-19].

In order to predict the binding energy of the phytoconstituents the following steps were carried out.

To draw the structure

a) Using Gauss View 5.0

   i) To draw the structure of the compounds various steps involved in drawing the chemical structure were
   
   • Open the Gauss view software
   • To start a new blank workspace, go to File New Create molecule group
   • Go to “View” and L-click the “builder” option “builder” window will open.
   • After adding all desired atoms (excluding hydrogens) start the bonding by using the “Modify Bond” option in the “Builder”. The two chosen atoms change color and are marked as 1 and 2. In addition, a new window “Semichem Smart Slide” will open press the “OK” push button after choosing the bond.
   • Add hydrogen atoms by using the “Add valence” option on the “Builder” window.
   • Remove atoms from the structure by using the “Delete Atom” icon on the “Builder” window.
   • To save the structure, in Gaussian input file, File save as in “Gaussian input file” as *gif*. The saved file appeared as given in (Figure-9).

   ![Figure 9. Gauss view of α-tocopherol](image)

   **Figure 9.** Gauss view of α-tocopherol

   • **Click calculation option** in the Gauss view main window menu then open the the Gaussian calculation setup window, which appear as given in (Figure-10).
Figure 10. Calculation setup window

Under Job type, look at the various calculation options – like Optimization, frequency scan and energy calculations were used. Choose optimization to calculate the optimum geometry and the window looks like the window given as appeared as (Figure-11).

Figure 11. Optimization setup window

To calculate the binding energy by B3LYP, Select B3LYP and the corresponding basis set. The windows appear as given in (Figure-12).
On pressing submit button, it sends the calculation from Gauss view to Gaussian. After finishing the calculation press “OK” to save the output file. The binding energy of the above six phytochemical compounds predicted by B3LYP method were given in Tables-II.

Table II. Binding energy of phytochemical compounds in B3LYP method

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compounds</th>
<th>Basis Sets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>STO-3G</td>
</tr>
<tr>
<td>1</td>
<td>Dodecanoic acid</td>
<td>-612.9438</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl caprylate</td>
<td>-534.0572</td>
</tr>
<tr>
<td>3</td>
<td>Glycine(trifluoroacetyl)-methyl butyl ester</td>
<td>-916.3646</td>
</tr>
<tr>
<td>4</td>
<td>Capric acid ethyl ester</td>
<td>-604.5936</td>
</tr>
<tr>
<td>5</td>
<td>$\alpha$ – Tocopherol</td>
<td>-1228.3913</td>
</tr>
<tr>
<td>6</td>
<td>n-Hexadecanoic acid</td>
<td>-769.2641</td>
</tr>
</tbody>
</table>

To carry out energy calculation using an Abinitio Hartree-Fock model. Select ground state, HF and set the basis set for 3-21G, the windows appear as (Figure-13).
The binding energy of the above six phytochemical compounds were predicted by HF method was given in Table-III.

Table III. Binding energy of phytochemical compounds in HF method

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compounds</th>
<th>Basis sets</th>
<th>3-21G</th>
<th>6-31G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>STO-3G</td>
<td>-608.6248</td>
<td>-613.1928</td>
</tr>
<tr>
<td>1</td>
<td>Dodecanoic acid</td>
<td>-608.6248</td>
<td>-613.1928</td>
<td>-616.3251</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl caprylate</td>
<td>-530.3398</td>
<td>-534.3699</td>
<td>-537.1198</td>
</tr>
<tr>
<td>3</td>
<td>Glycine(trifluoroacetyl)-methyl butyl ester</td>
<td>-911.4252</td>
<td>-918.6482</td>
<td>-923.3547</td>
</tr>
<tr>
<td>4</td>
<td>Capric acid ethyl ester</td>
<td>-600.4629</td>
<td>-605.0965</td>
<td>-608.3111</td>
</tr>
<tr>
<td>5</td>
<td>α – Tocopherol</td>
<td>-1220.2810</td>
<td>-1228.4658</td>
<td>-1234.8000</td>
</tr>
<tr>
<td>6</td>
<td>n-Hexadecanoic acid</td>
<td>-764.2778</td>
<td>-769.4424</td>
<td>-773.3822</td>
</tr>
</tbody>
</table>

Results and discussion

Molecular property and bio-activity scores

a) Molecular property of the phytochemical compounds

Among the six phytochemical compounds selected for insilico drug activity prediction by molinspiration showed that compound 2 and 3 obeyed the Lipinski’s rule of five and showed good drug likeness scores. MiLog P values of these compounds were found to be < 5 (range 2.007 to 3.701) indicated their good permeability across the cell membrane.

All the phytochemical compounds were found to have TPSA well below 160Å² (26.305 to 55.403), molecular weight <500, Number of hydrogen bond donors < 5, hydrogen bond acceptors < 4, number of rotatable flexible bonds <5 and n-violations 0. These facts indicated that all the six phytoconstituents were found to have drug likeness property.
b) Bioactivity scores of the compounds

The bioactivity scores of the six compounds observed were as follows:

i) GPCR ligand

\( \alpha \) – Tocopherol (0.25) compound was found to be highly bioactive towards GPCR ligands (>0) and others were found to be moderately active (< -5.0).

ii) Ion channel modulator

The Ion channel modulator property \( \alpha \) – Tocopherol (0.15) was higher (>0) than other compounds (<0).

iii) Kinase inhibitor

Kinase inhibitor activities of Dodecanoic acid (-0.75), Ethyl caprylate (-1.25), Glycine (trifluoroacetyl)-methyl butyl ester (-0.88), Capric acid ethyl ester (-0.21), \( \alpha \)-Tocopherol (-0.22) and n-Hexadecanoic acid (-0.33) were found to moderate as their scores values were found to be less than zero.

iv) Nuclear receptor ligand

Nuclear receptor ligand as the six compounds was observed to be moderate.

v) Protease inhibitor

\( \alpha \)- Tocopherol was found to be active as Protease inhibitor 0.29 (>0). Whereas other compounds were found to be moderately active (<0).

vi) Enzyme inhibitor

\( \alpha \)-Tocopherol (0.25) and n-Hexadecanoic acid (0.18) were exhibited the enzyme inhibitory action higher (>0) than other compounds (<0). Among these two compound \( \alpha \)-Tocopherol the enzyme inhibitor activity was higher.

IV - DFT Calculation

a) B3LYP method

The binding energy calculation by using three basis sets (STO-3G, 3-21G, 6-31G) listed in Table-II showed the following result.

The binding energies for the Dodecanoic acid, Ethyl caprylate, Glycine (trifluoroacetyl) methyl butyl ester, Capric acid ethyl ester, \( \alpha \)-Tocopherol and n-Hexadecanoic acid compounds using STO-3G basis were to be -612.9438, -534.0572, -916.3646, -604.5936, -1228.3913 and -769.2641 a.u. From the above data it was found that \( \alpha \)-Tocopherol was found to have good binding energy as -1228.3913 a.u. among the other bio-active constituents. Using 3-21G basis sets, the binding energies were found to be -617.5225, -538.2141, -923.9007, -609.3381, -1236.9904 and -774.6944 a.u. for the above compounds respectively. From these data it was found that \( \alpha \)-Tocopherol have a good binding energy as -1236.9904 a.u among the other bio-active constituents. The binding energies of the above six compounds by 6-31G basis sets were found to be -620.7334, -541.0285, -928.7137, -612.6145, -1243.4557 and -778.7309 a.u. From the above data it was concluded that \( \alpha \)-Tocopherol was found to have a good binding energy as -1243.4557 a.u. among the other bio-active constituents. It was observed that \( \alpha \)-Tocopherol was found to have -1228.3913, -1236.9904, -1243.4557 a.u. binding energies as per the above three basis sets and hence it was found to be more stable than other bio-active constituents.

b) HF method

The binding energy calculation by HF method were listed in Table-III showed the following Observations. STO-3G basis sets, the binding energy for the selected six compounds were found - 608.6248, -530.3398, -911.4252, -600.4629, -1220.2810 and -764.2778 a.u. and it was found that \( \alpha \)-Tocopherol (-1220.2810 a.u.) have good binding energy.
Using 3-21G basis sets, the binding energies were found to be -613.1928, -534.3699, -918.6482, -605.0965, -1228.4658 and -769.4424 a.u. for the six compounds. From these data, α-Tocopherol (-1228.4658 a.u.) was found to be more stable.

The binding energies observed using 6-31G basis sets for the above six compounds were found to -616.3251, -537.1198, -923.3547, -608.3111, -1224.8000 and -773.3822 a.u. From these data it was found that α- Tocopherol (-1224.8000 a.u.) was found to be more stable among other compounds.

- Phytochemical compounds selected for our work (Figure-1 to 6) were found to obey the Lipinski’s rule (MiLog P <5) and α- Tocopherol (2.007) exhibited higher drug likeness properties compared to others. α- Tocopherol exhibited the highest score towards GPCR ligand, (0.25) nuclear receptor ligand (0.43) and inhibitory activities towards protease (0.29), enzyme (0.25) and kinase (-0.22) inhibitors as higher compared to others.
- DFT method for determination of binding energy of the six phytoconstituent revealed that α- Tocopherol was found to possesses good binding energy among others hence it was found to be more stable.

Conclusion

Phytochemical compounds selected for our work (Figure-1 to 6) were found to obey the Lipinski’s rule (MiLog P <5) and α- Tocopherol (2.007) exhibited higher drug likeness properties compared to others. α- Tocopherol exhibited the highest score towards GPCR ligand, (0.25) nuclear receptor ligand (0.43) and inhibitory activities towards protease (0.29), enzyme (0.25) and kinase (-0.22) inhibitors as higher compared to others. DFT method for determination of binding energy of the six phytoconstituent revealed that α- Tocopherol was found to possesses good binding energy among others hence it was found to be more stable.

References

Nephroprotective Potential Compounds from Leaves Extracts of Andrographis Paniculata

Article by K. Padmalochana¹ and M.S. Dhana Rajan²

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²Registrar, Texila American University, Guyana, South America
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Abstract

Nephrotoxicity is the third most common problem of the renal system. Medicinal plants potentially useful for the treatment of severe renal disorders. In the present study, the effect of aqueous, ethanol and acetone extract of Andrographis paniculata leaves was evaluated by pretreating three groups of rat animals. Renal failure was induced by antibiotic drug gentamicin which was orally administered to group II animals and untreated control model was maintained. After oral administration for 10 days, serum levels of urea, uric acid, creatinine and total protein were assayed. All the biochemical parameters were significantly controlled (p<0.01 and p<0.001) on 10th day after the treatment of aqueous, ethanol and acetone extracts as compared than the gentamicin induced nephrotoxic animals (p<0.05). Maximum nephroprotection was offered by the ethanol extract of A. paniculata leaves (p<0.001). The histological structure of renal was observed by staining with haemotoxylin-eosin viewed using light microscope. Gentamicin treated animals showed acute tubular necrosis due to the injury in kidney. In aqueous, ethanol and acetone extract treated animals showed histopathological changes in renal revealed the nephroprotective activity of A. paniculata leaves. Finally, ethanol extract of A. paniculata was more suitable for nephroprotective action against gentamicin induced renal failure was evidenced by biochemical estimation and by restoration of histological changes of renal system.

Keywords: Kidney, Nephroprotection, Andrographis paniculata, Herbal Medicine.

Introduction

Kidney is the complex and major organ of our body perform several important functions like formation of urine, water and salt metabolism, acid-base balance, regulation of blood calcium level and secretion of hormones. Kidney affected by the diseases are mainly kidney blockage and kidney stones. Major types of stones in kidney are calcium stones, stuvite stones, uric acid stones and cysteine stones. Acute renal failures and chronic renal failure are common and serious problems. Acute renal failure is reversible loss of kidney function whereas chronic renal failure is irreversible loss of kidney function.

Nephrotoxicity is one of the major kidney problems caused by drug or toxin. Drugs, diagnostic agents, chemical reagents and heavy metals are well known to be nephrotoxic. In recent years, development of modern medical, and surgical practices has been followed for the treatment of renal failure like haemodialysis, renal transplantation and chemotherapy. These procedures are complicated and high cost has been utilized to cure the kidney damage. So that the traditional medicine using herbal plant is best method than the conventional method. However, when using these chemotherapy method may induce the side effects to the body. Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. Drugs like gentamicin, cisplatin, cyclosporine, Carbon tetrachloride are common source of acute kidney injury. Gentamicin is an amino glycoside antibiotics used for the treatment of Gram negative bacterial infections. Overdose of gentamicin causes renal damage. It may give serious side effects while continuous consuming at higher concentrations.

Medicinal plants are have curative properties and therapeutic values due to the presence of various complex phytochemical compounds. This traditional medicines are assuming greater important because of very effective, safer, locally available, and no side effects. A. paniculata, a member of the family of Acanthaceae is locally and commonly available plant in India. It is commonly called as “nilavembu or siriyanangai” in Tamil and “King of bitters” in English. It has been shown hepatoprotective, anti-
parasitic, antioxidant anti-inflammatory and antimicrobial activity. And used helps in malaria treatment and treatment of cancer. In this study reported that curative properties of the medicinal plant Andrographis paniculata against nephrotoxicity induced by gentamicin in albino rats. Nephroprotective activity was confirmed by examine biochemical tests for urea, uric acid creatinine and protein level in blood and histopathological studies carried out using light microscopic observations.

Materials and methods

Preparation of plant leaf extracts

Leaves of Andrographis paniculata were collected and shade dried for 3-5 days and grinded into powder. Aqueous extract was prepared by adding dried powder into 100 ml water and incubate overnight. 100 g dry powder was extracted with 80% ethanol at 55°C for 24 hours in soxhlet apparatus. Acetone extract was prepared by mixing of dried leaf powder with the 80% acetone. Solvent elimination was done at room temperature and stored. The resulting aqueous, ethanol and acetone extracts were then used for nephroprotective activity.

Experimental Design for nephroprotective Activity of Andrographis paniculata

Adult male Wister albino rats maintained at the college weighing between 150g-170g were used for the nephroprotective studies. Animals were divided into six groups in six rats each. Group I (Normal): Orally received distilled water for 10 days.

Group II (Induced): Orally received Gentamicin (80 mg/kg body weight) only for 10 days. Group III (Standard): Orally received Cystone (20 mg/kg body weight) along with gentamicin (80 mg/kg body weight) for 10 days.

Group IV (Treatment): Orally received aqueous leaf extract (300mg/kg body weight) along with Gentamicin (80mg/kg body weight) for 10 days.

Group V (Treatment): Orally received ethanol leaf extract (300mg/kg body weight) along with Gentamicin (80 mg/kg body weight) for 10 days.

Group VI (Treatment): Orally received acetone leaf extract (300mg/kg body weight) along with Gentamicin (80 mg/kg body weight) for 10 days.

Cystone was used as positive control for comparing nephroprotective potential of different leaves extract of A. paniculata. Gentamicin is act as nephrotoxin which induces the kidney damage.

Histopathological and biochemical study

After 10 days, all animals from every group were sacrificed and separated the kidneys by dissection procedure. Pieces of kidneys obtained from each group were immediately fixed in 10% formalin solution. The fixed formalin fixed kidneys were embedded in paraffin and serial section were made and stained with haemotoxylin and eosin. The stained sections were examined under light microscope. Blood samples were collected from jugular vein. Serum was separated from the blood for the analysis of the parameters like Blood Urea, Uric Acid, Creatinine and Total Protein.

Statistical analysis

Data were analyzed using one way Analysis of Variance (ANOVA) and expressed as mean± S.E.M. Statistical significance was fixed p< 0.05.

Results and discussion

Biochemical studies

The nephroprotective activity of aqueous, ethanol and acetone extract of A. paniculata leaves was assessed against nephrotoxicity induced using gentamicin in albino rats. The nephroprotective activity was determined by biochemical tests and histopathological studies. Table 1 shows the changes of urea and uric acid level in blood. Blood urea and blood uric acid in the control (group I) was estimated to be 30.16±1.72mg/dl and 5.08±0.21 mg/dl, respectively. In the negative control i.e. group II animals received only gentamicin which shows level of urea and uric acid in blood to be 59.00±2.19 mg/dl and 8.25±0.54mg/dl, respectively. Group IV, V and VI animals received gentamicin along with aqueous leaf extract, ethanol and acetone extract demonstrated a significant increase (p<0.05 to p<0.001) in
blood urea and uric acid as compared with negative control group. In the ethanol treated groups shows most significant changes (p<0.001) in urea and uric acid recorded as 33.28±0.54 mg/dl and 5.21±0.12mg/dl as compared with aqueous and acetone extract (Figure 1).

Creatinine concentrations in blood was significantly increased (p<0.05) in the gentamicin treated negative control group of animals (2.88±0.11mg/dl) compared to the normal animals indicating the induction of severe nephrotoxicity. Treatment with plant extracts of A. paniculata showed significant (p<0.01 and p<0.001) increase in creatinine concentrations. Ethanol extract treated animals showed increased significant changes (p<0.001) recorded as 0.82±0.54 mg/dl concentrations of creatinine, indicates that nephrotoxicity curative properties of A. paniculata leaves (Table 2).

Normal total protein level was observed in group I animals. Gentamicin treated group II animals showed low amount of secretion of total proteins (p<0.05) as compared to normal animals. This low protein level in serum is probably due to an inhibitory action of protein synthesis induction of tissue damage and may leads to increased excretion of protein in urine (Ramesh et al 2014). Treatment with the plant extracts of A. paniculata (group IV, V and VI) showed (p<0.01 and p<0.001) increase in concentrations of total protein compared to the gentamicin treated groups (group II) (Table 2, Figure 2).

This inhibitory action of A. paniculata leaves extract against nephrotoxin was confirmed through biochemical and histopathological studies. This activity may due to the presence of secondary metabolites like flavonoid and polyphenolic compounds which may be responsible for the kidney protective activity.

**Figure captions**

**Table 1.** Effect of aqueous, ethanol and acetone extract of A. paniculata leaves on blood urea and uric acid in gentamicin induced nephrotoxic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Blood Urea(mg/dl)</th>
<th>Blood Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal)</td>
<td>30.16 ± 1.72</td>
<td>5.08 ± 0.21</td>
</tr>
<tr>
<td>Group II (induced)</td>
<td>59.00 ± 2.19*</td>
<td>8.28 ± 0.54*</td>
</tr>
<tr>
<td>Group III (Standard drug)</td>
<td>34.83 ± 3.06**</td>
<td>5.95 ± 0.21**</td>
</tr>
<tr>
<td>Group IV (Aqueous)</td>
<td>36.16 ± 2.48**</td>
<td>6.12 ± 0.33**</td>
</tr>
<tr>
<td>Group V (Ethanol)</td>
<td>33.28 ± 0.54***</td>
<td>5.21 ± 0.12***</td>
</tr>
<tr>
<td>Group VI (acetone)</td>
<td>39.08 ± 0.21***</td>
<td>6.36 ± 0.13***</td>
</tr>
</tbody>
</table>

*p< 0.05, **p < 0.01, ***p < 0.001 value are considered statistically significant (BMRT)

**Table 2.** Effect of aqueous, ethanol and acetone extract of A. paniculata leaves on creatinine and total protein levels in gentamicin induced nephrotoxic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Creatinine (mg/dl)</th>
<th>Total protein (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal)</td>
<td>0.79 ± 0.02</td>
<td>6.91 ± 0.59</td>
</tr>
<tr>
<td>Group II (induced)</td>
<td>2.88 ± 0.11*</td>
<td>3.02 ± 0.84*</td>
</tr>
<tr>
<td>Group III (Standard drug)</td>
<td>1.21 ± 0.12**</td>
<td>7.55 ± 0.70**</td>
</tr>
<tr>
<td>Group IV (Aqueous)</td>
<td>1.11 ± 0.13***</td>
<td>7.12 ± 0.77**</td>
</tr>
<tr>
<td>Group V (Ethanol)</td>
<td>0.82 + 0.54***</td>
<td>7.91 + 0.12***</td>
</tr>
<tr>
<td>Group VI (acetone)</td>
<td>1.38 + 0.21**</td>
<td>7.56 + 0.13***</td>
</tr>
</tbody>
</table>

*p< 0.05, **p < 0.01, ***p < 0.001 value are considered statistically significant (BMRT)
Figure 1. Effect of aqueous, ethanol and acetone A. paniculata leaves extract on the alterations of urea, and uric acid level in blood

Figure 2. Effect of aqueous, ethanol and acetone A. paniculata leaves extract on the alterations of creatinine, and total protein level in blood
Figure 3. Sectioning of (A) normal kidney showing tubular brush borders and intact glomeruli in renal tissues without alterations (B) Representing the tubular necrosis in gentamicin treated animals (C) shows microscopic observation of normalized kidney structure on treated with cystone is a positive control
Figure 4. Shows histological structure of kidney treated with (A) aqueous extract (B) ethanol extract (C) acetone extract of A. paniculata leaves on gentamicin induced nephrotoxicity rat animals revealed normalized and restored function of renal system

Conclusion

Herbal medicine have useful for the development of effective therapy to treat variety of diseases. Nephrotoxicity induced by gentamicin in rats developed significant kidney injuries was estimated from increased levels of urea, uric acid, and creatinine(p<0.05) and decreased levels of total protein in blood serum. These parameters were more significantly (p<0.001) altered and restored by oral supplementation of aqueous, ethanol and acetone extract of A. paniculata leaves to gentamicin intoxicated rats. Our present study clearly indicated a significant nephroprotective activity by normalize the elevated biochemical and restoration of renal system using with the extract of A. paniculata leaves and supported the traditional usage of the plant in the medicinal system.

References


Pharmacological Activities of Compound Present in Cassia Auriculata by Pass Prediction Method

Article by Chandra Mohan. A¹, Geetha. S², Gajalakshmi. R³, Divya. S. R⁴, and Dhanaranjan M. S⁵

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Abstract

PASS Prediction of pharmacological activity for the above compound indicated that these compounds were found to possess various pharmacological activities in the range 69.3 - 97.8%. Both Dodecanoic Acid and n-hexadecanoic Acid were found to exhibit similar pharmacological activities as Acylcarnitine hydrolyse inhibitor (97.3%). α-Tocopherol exhibit the highest pharmacological activity as Lipid peroxidase inhibitor (97.8%) among the six phytoconstituent selected for PASS prediction.

Keywords: Cassia auriculata, Phytochemical compounds, Pharmacological activity and PASS prediction.

Introduction

Cassia auriculata is one of the herbaceous plants that found throughout central and southern India, also cultivated in Punjab, Haryana, Uttar Pradesh and West Bengal. The shrub usually occurs on roadsides, waste line, and railway embankments. Avaram (Cassia auriculata Linn), family Caesalpiniaceae, is also known as Avaram tree. Cassia auriculata Linn (Family: Caesalpiniaceae) commonly known as Tanners senna, is distributed throughout hot deciduous forests of India and holds a very prestigious position in Ayurveda and Siddha systems of medicine. It was profoundly used in Ayurvedic medicine as a tonic, astringent and as a remedy for diabetes, conjunctivitis and opthalmia [1]. It is one of the principle constituents of ‘Avaarai panchaga chooranam’ - an Indian herbal formulation used in the treatment of diabetes to control the blood sugar level [2].

The plant has been reported to possess antipyretic [3], hepatoprotective [4], antidiabetic, antiperoxidative and anti hyperglyceamic [5], microbicidal [6] and antihyperlipidaemic activities [7]. They are one of the constituent of polyherbal formulation ‘Diasulin’ in the concentration range of 40 mg/dl which is proven to have antidiabetic activity [9].

It has been found to possess antitumor, oncogenic, and diabeto genic properties [10]. The antioxidant and radical scavenger function of α-tocopherol is essentially dependent on the free state of its hydroxyl group. Spectacular antiallergic and anti inflammatory activities have been attributed to DL-α-tocopheryl-α-D-mannopyranoside and DL-α-tocopheryl-β-D-galactopyranoside [11]. Hexadecanoic acid methyl ester, also known as Methyl palmitate, in the methanol fraction is an aliphatic acid ester reported to cause growth inhibition and apoptosis induction in human gastric cancer cells [12].

The phytoconstituent of a plant will often determine the physiological action on the human body. Cassia species are rich sources of Polyphenols, Anthraquinone derivatives, Flavanoids, Polysaccharides, Saponins, Tannins, and Steroids. Some of the Cassia species are rich in Glycerides with linoleic, oleic, stearic, and palmitic acids . Cassia species are well known for their laxative and purgative constituents and are also used for the treatment of skin diseases. Leaves are anthelmintic and also used to treat ulcers, skin diseases, and leprosy. An aqueous extract of leaves possesses hypoglycemic activity. The leaves are eaten as a vegetable in times of scarcity, the infusion of leaves possesses a slight purgative activity.
PASS prediction

PASS provides simultaneous predictions of many types of biological activity based on the structure of organic compounds. It can predict more than 1500 pharmacological effects, molecular mechanism of action and toxicities on basis of structural descriptors of compounds. Thus, PASS can be used to estimate the biological activity profiles for virtual molecules, prior to their chemical synthesis and biological testing. Pa (probability to be active) estimates the chance that the studied compound is belonging to the sub-class of active compounds resembles the structures of molecules, which are the most typical in a sub-set of actives in PASS training set.

Pi (probability to be inactive) estimates the chance that the studied compound is belonging to the sub-class of inactive compounds resembles the structures of molecules, which are the most typical in a sub-set of inactive in PASS training set. PASS (Prediction of Activity Spectra for Substance) which is commonly used technique in drug discovery and development. PASS predict the biological activity spectrum for a compound on the basis of its structural formula [13-15].

Materials and methods

Materials

Then the plant was identified and authenticated by Plant Anatomy Research Centre (PARC/2017/3467). Phytochemical compounds present in Cassia Auriculata like Dodecanoic acid, Ethyl Caprylate, Glycine (trifluoroacetyl) - methyl butyl ester, α – Tocopherol and n – Hexadecanoic acid as given in (Figure - 1 to 6) were selected for insilico prediction.

Figure 1. Dodecanoic acid

Figure 2. Ethyl caprylate

Figure 3. Capric acid ethyl ester

Figure 4. Glycine (trifluoroacetyl)-methyl buty l ester
Methods

**Pass prediction of pharmacological activity**

Various constituents of Cassia auriculata leaves extract reported were selected for predicting pharmacological activity using PASS [16, 17]. Phytochemical compounds like a) Dodecanoic Acid, b) n-Hexadecanoic acid, c) Ethyl Caprylate, d) Capric acid ethyl ester, e) Glycine (trifluoroacetyl)-methyl butyl ester and f) α-Tocopherol were selected. The structures of phytochemical compounds were drawn in Molinspiration online software and appear as given in (Figure-7) and their structures were saved in mol file with *.mol*.

![Figure 7. Molinspiration structure](image)

PASS prediction window for prediction of pharmacological activity appeared as given in Figure-8 & 9.
Result and discussion

PASS prediction

All the phytochemical compounds were found to exhibit various Pharmacological activities in the range (69.3-97.8%) as given in Table-I (a, b & c).
<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the compound</th>
<th>Activity</th>
<th>$P_a$</th>
<th>$P_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dodecanoic acid</td>
<td>Acylcarnitine hydrolyse inhibitor</td>
<td>0.973</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alkylacylglcerophosphatase inhibitor</td>
<td>0.966</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alkynylglycerophosphocholine hydrolase inhibitor</td>
<td>0.963</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CYP2J substrate</td>
<td>0.962</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CYP2J2 substrate</td>
<td>0.961</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acrocyldropepsin inhibitor</td>
<td>0.961</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chymosin inhibitor</td>
<td>0.961</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saccharopepsin inhibitor</td>
<td>0.957</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dextranase inhibitor</td>
<td>0.954</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CarboxypeptidaseTag inhibitor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ethyl caprylate</td>
<td>All-trans-retinyl-paluitate hydrolase inhibitor</td>
<td>0.953</td>
<td>0.001</td>
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<td></td>
<td>Cutinase inhibitor</td>
<td>0.946</td>
<td>0.001</td>
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<td></td>
<td></td>
<td>Acylcarnitine hydrolase inhibitor</td>
<td>0.934</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alkanal monooxygenase (FMN- linked) inhibitor</td>
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<td>0.002</td>
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<td></td>
<td></td>
<td>Sugar-phosphatase inhibitor</td>
<td>0.924</td>
<td>0.003</td>
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<td></td>
<td>Alkenylglycerophosphocholine hydrolase inhibitor</td>
<td>0.922</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acrocyldropepsin inhibitor</td>
<td>0.919</td>
<td>0.004</td>
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<tr>
<td></td>
<td></td>
<td>Chymosin inhibitor</td>
<td>0.919</td>
<td>0.004</td>
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<tr>
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<td></td>
<td>Saccharopepsin inhibitor</td>
<td>0.919</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antieczematic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Glycine(trifluoroacetyl)-methyl butyl ester</td>
<td>Acrocyldropepsin inhibitor</td>
<td>0.839</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chymosin inhibitor</td>
<td>0.839</td>
<td>0.013</td>
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<tr>
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<td></td>
<td>Saccharopepsin inhibitor</td>
<td>0.839</td>
<td>0.013</td>
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<td>Acetylemesterase inhibitor</td>
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<td>0.788</td>
<td>0.015</td>
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<tr>
<td></td>
<td></td>
<td>Fucosterol-epoxide lyase inhibitor</td>
<td>0.745</td>
<td>0.011</td>
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<tr>
<td></td>
<td></td>
<td>Pro-opiomelanocartin converting</td>
<td>0.733</td>
<td>0.023</td>
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<tr>
<td></td>
<td></td>
<td>enzyme inhibitor</td>
<td>0.719</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Polyporopepsin inhibitor</td>
<td>0.695</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Macrophage colony stimulating factor agonist</td>
<td>0.693</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cutinase inhibitor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Capric acid ethyl ester</td>
<td>All-trans-retinyl-paluitate hydrolase inhibitor</td>
<td>0.953</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
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<td>Cutinase inhibitor</td>
<td>0.946</td>
<td>0.001</td>
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<tr>
<td></td>
<td></td>
<td>Acylcarnitine hydrolase inhibitor</td>
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<tr>
<td></td>
<td></td>
<td>Saccharopepsin inhibitor</td>
<td>0.919</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Dodecanoic acids various pharmacological activities as given in Table I (a) showed that this exhibited very good inhibitors as Acylcarnitine hydrolyse inhibitor (97.3%), Alkylacetyl glycerophosphocholine hydrolyse inhibitor (96.6%), Alknylglycerophosphocholine Ethyl caprylate was also observed to exhibit various pharmacological activities in the range 91.9 – 95.3% as All-trans-retinyl-paluitate hydrolase inhibitor (95.3%), Cutinase inhibitor (94.6%), Acylcarnitine hydrolyse inhibitor (93.4%), Alkanal monooxygenase (FMN-linked) inhibitor (93.0%), Sugar-phosphatase inhibitor (92.4%), Alkenylglycerophosphocholine hydrolase inhibitor (92.2%), Acrocylindropepsin inhibitor (91.9%), Chymosin inhibitor (91.9%), Saccharopepsin inhibitor (91.9%) and CarboxypeptidaseTag inhibitor (95.4%) respectively.

Glycine (trifluoroacetyl) - methyl butyl ester exhibited various pharmacological activities as Capric acid ethyl ester exhibited various pharmacological activities as Acylcarnitine hydrolyse inhibitor (95.3%), Cutinase inhibitor (94.6%), Acylcarnitine hydrolyse α-Tocopherol was also observed to exhibit various pharmacological activities in the range 85.1 – 97.8% as Lipid peroxidase inhibitor (97.8%), Peroxidase inhibitor (97.1%), TP53 expression inhibitor (95.9%) and AR expression inhibitor (85.1%). n-Hexadecanoic acids various pharmacological activities as given in Table I (b) showed that this exhibited very good inhibitors as Acylcarnitine hydrolyse inhibitor (97.3%), Alkylacetyl glycerophosphocholine hydrolyse inhibitor (96.6%), Alknylglycerophosphocholine hydrolyse inhibitor (96.3%), CYP2J substrate (96.1), CYP2J2 substrate (96.1), Acrocylindropepsin inhibitor (96.1%), Chymosin inhibitor (96.1%), Saccharopepsin inhibitor (96.1%), Dextranase inhibitor (95.7%) and CarboxypeptidaseTag inhibitor (95.4%) respectively.

Conclusion

PASS Prediction of pharmacological activity for the above compound indicated that these compounds were found to possess various pharmacological activities in the range 69.3 - 97.8%. Both Dodecanoic Acid and n-hexadecanoic Acid were found to exhibit similar pharmaceutical activities as Acylcarnitine hydrolyse inhibitor. α-Tocopherol exhibit the highest pharmaceutical activity as Lipid peroxidase inhibitor (97.8%) among the six phytoconstituent selected for PASS prediction.
Reference

Analysis of Total Phenol, Cellulose and Tannin Content by Using Different Parameters in Ethanol Extract of Pomegranate Peel

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E-mail: dhanarajan.m@tau.edu.gy2

Abstract

Pomegranate (Punica granatum L.) is considered one of the oldest known edible fruits. Pomegranate peels are characterized by an interior network of membranes comprising almost 26–30% of total fruit weight and are characterized by substantial amounts of phenolic compounds, including flavonoids and tannins. Pomegranate fruit and its peel exhibit a high antioxidant potential. Pomegranate peel attracts attention due to its apparent wound healing properties. Antioxidative activity has often been associated with a decreased risk of various diseases. The purpose of the present study was to evaluate the effect of using different parameters with ethanol extract and estimate the efficiency of effective compounds, such as polyphenolic, cellulose and tannin compounds from the pomegranate peel extracts.

Keywords: Pomegranate peel extracts, polyphenolic, cellulose and tannin.

Introduction

Pomegranate (Punica granatum L. Punicaceae; the common name is derived from Latin words ponusandgranatus), a seeded or granular apple, is a delicious fruit consumed worldwide. The fruit is native to Afghanistan, Iran, China and the Indian sub-continent. The ancient sources of pomegranate linked Iran to Pakistan, China and eastern India, where pomegranate had been under cultivation for thousands of years.

Pomegranate peels are characterized by an interior network of membranes comprising almost 26–30% of total fruit weight and are characterized by substantial amounts of phenolic compounds, including flavonoids (anthocyanins, catechins and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid). These compound sareconcentrated in pomegranate peel (PoP) and juice, which account for 92% of the antioxidant activity associated with the fruit [1], [11] and [17].

The mechanism of antimicrobial activity of Pomegranate peel phenolics involves precipitation of membrane proteins resulting in microbial cell lysis. The ethno pharmacological profile of PoP makes it a valuable traditional asset due to its antimicrobial and anti-mutagenic properties. Moreover, the phyto-chemical concentration of PoP is high enough to be effective without further enrichment with the extracts of any other fraction of the fruit [13].

Traditional medicinal uses

A variety of cultures and traditions in both the developing and developed worlds recommend pomegranate peel to treat common health problems. Traditionally, aqueous PoP extract is obtained by boiling for 10–40min. The extract has been used to treat diarrhoea, dysentery, and dental plaque, in addition to being used as adouche and enemaagent [8]. Similarly, diarrhoea, intestinal worms, bleeding noses and ulcers have been treated in Indian Subcontinent with dried PoP.

Pomegranate peel attracts attention due to its apparent wound healing properties [3], immune modulatory activity [5] and antibacterial activity [10] antiatherosclerotic and antioxidative capacities [15]. Antioxidative activity has often been associated with a decreased risk of various diseases [16]. The peel packs some of the weight boosting and health enhancing effects of antibiotics and hormones.
without the detrimental effects and it may yield meat with higher level of beneficial antioxidants [14]. Pomegranate Ellagitannin have been identified as the active antioxidant compound and anticancer activities responsible for protecting low density lipoprotein, cholesterol from in the last few years the identification and development of phenolic compounds or extracts from different plants has become a major area of health and medical related research [4]. The present study is undertaken to know the nutritional importance and suitability of de-tanninated pomegranate peel, a by-product of pomegranate juice industry, as a cattle feed supplement.

Materials and methods

A. Collection of pomegranate peel

Collection, separation and drying of pomegranate peel. The orange fruits were purchased. The peels were manually separated from the fruit. The peels were shade dried. The dried peels were collected and ground well to form a powder. The powdered orange peel was stored in an airtight container and used for various tests.

B. Preparation of the peel extract

Preparation of the extracts was assessed by the following methods. One gram of dried bitter orange peel powder was extracted with 20 ml of aqueous ethanol was soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel.

The filtered solution was evaporated under vacuum in a rota-vator at 40°C to a constant weight and then dissolved in respective solvents. The dissolving rate of the crude extract was approximately 100%. The extracts were used for further tests.

Qualitative phytochemical analysis: The phytochemical tests were carried out using standard methods of analysis of tannins, saponins, quinones, flavonoids, glycosides, cardiac-glycosides, terpenoids, phenols, coumarins, steroids, alkaloids, anthocyanin and betacyanin.

C. Materials required

1) Acetic/Nitric Reagent: Mix 150ml of 80% acetic acid and 15mL of concentrated nitric acid.
2) Anthrone: Dissolve 200mg anthrone in 100mL of ice-cold 95% sulphuric acid. Prepare fresh and chill for 2h before use.
3) 67% sulphuric acid
4) Folin-Denis Method: This is based on the non-stoichiometric oxidation of the molecules containing a phenolic hydroxyl group.
5) Sodium carbonate
6) Tannic acid
7) Folin-ciocalteau reagent
8) Distilled water

Methods

D. Sonication

Sonication is the process of converting an electrical signal into a physical vibration that can be directed toward a substance. Sonicators are vital lab equipment and are used for a number of purposes. Sonication is usually performed to break apart compounds or cells for further examination. The vibration has a very powerful effect on solutions, causing their molecules to break apart and cells to rupture. A prime example is in DNA testing, where the cells that may contain DNA information are subjected to sonication to break them apart and release the DNA proteins so they can be tested.

The primary part of a sonication device is the ultrasonic electric generator. This device creates a signal (usually around 20 KHz) that powers a transducer. This transducer converts the electric signal by using piezoelectric crystals, or crystals that respond directly to the electricity by creating a mechanical vibration. This vibration, molecular in origin, is carefully preserved and amplified by the sonicator, until it is passed through to the probe.

The sonication probe transmits the vibration to the solution being sonicated. This probe is a carefully constructed tip that moves in time with the vibration, transmitting it into the solution. The
probe moves up and down at a very high rate of speed, although the amplitude can be controlled by the operator and is chosen based on the qualities of the solution being sonicated.

E. Magnetic stirrer

A magnetic stirrer or magnetic mixer is a laboratory device that employs a rotating magnetic field to cause a stir bar (also called "flea") immersed in a liquid to spin very quickly, thus stirring it. The rotating field may be created either by a rotating magnet or a set of stationary electromagnets, placed beneath the vessel with the liquid.

Magnetic stirrers are often used in chemistry and biology, where they can be used inside hermetically closed vessels or systems, without the need for complicated rotary seals. They are preferred over gear-driven motorized stirrers because they are quieter, more efficient, and have no moving external parts to break or wear out (other than the simple bar magnet itself). Magnetic stir bars work well in glass vessels commonly used for chemical reactions, as glass does not appreciably affect a magnetic field.

The limited size of the bar means that magnetic stirrers can only be used for relatively small experiments, of 4 liters or less. Stir bars also have difficulty in dealing with viscous liquids or thick suspensions. For larger volumes or more viscous liquids, some sort of mechanical stirring is typically needed. Because of its small size, a stirring bar is more easily cleaned and sterilized than other stirring devices. They do not require lubricants which could contaminate the reaction vessel and the product. Magnetic stirrers may also include a hot plate or some other means for heating the liquid.

F. Quantitative phytochemical analysis

1) Estimation of Total phenolic content: Total Phenolic content (TPC) in the ethanol extracts was determined using the Folin-Ciocalteu reagent method [9]. This method depends on the reduction of FCR by phenols to a mixture of blue oxides which have a maximal absorption in the region of 765 nm using spectrophotometer. Stock solution of leaf extracts was prepared to the concentration of 1mg/ml. To 0.1ml of each extract, 5ml of Folin-Ciocalteu Reagent were added. The mixture solution was vortexed and incubated in the dark for 3 minutes, respectively. To the incubated content 5 ml of sodium carbonate (75g/L) solution was added to the above content and mixed thoroughly. The reaction content was incubated in the dark for 1 hour. The absorbance was read at 765 nm. Blank was maintained with 5 ml Folin-Ciocalteu reagent, 1 ml ethanol and 4 ml sodium carbonate solution. The concentration of total phenolic content in the extract was expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g) of extract. Gallic acid stock solution was prepared to the concentration of 1 mg/ml. Serial dilution was carried out; gallic acid solution was dissolved in ethanol. A linear dose-response regression curve was generated using absorbance reading of gallic acid at the wavelength of 765 nm.

2) Estimation of Total cellulose content: Add 3mL acetic/nitric reagent to a known amount (0.5g or 1g) of the sample in a test tube and mix in a vortex mixture. Place the tube in a water bath at 100°C for 30 min. Cool and then centrifuge the contents for 15-20min. Discard the supernatant and Wash the residue with distilled water. Add 10mL of 67% sulphuric acid and allow it to stand for 1h. Dilute 1mL of the above solution to 100mL. To 1mL of this diluted solution, add 10mL of anthrone reagent and mix well. Heat the tubes in boiling water bath for 10min. Cool and measure the color at 630nm. Set a blank with anthrone reagent and distilled water. Take 100mg cellulose in a test tube and proceed from step No. 6 for standard. Instead of just taking 1mL of the diluted solution (Step 7) take a series of volumes (say 0.4 to 2mL corresponding to 40-200mg of cellulose) and develop the color.

3) Estimation of total tannins content: Total Tannin content in the ethanol extract was determined by Folin–Denis method [12] with minor modifications. Stock solution of leaf extracts was prepared to the concentration of 1mg/ml. To 0.1ml of each extract, 1ml of distilled water was added and then mixed with 0.5 ml of Folin–Denis reagent. The reaction mixture was alkalinized by the addition of 1 ml of 15% (w/v) sodium carbonate solution and kept in dark for 30 min at room temperature. The absorbance of the solution was read at 700 nm using spectrophotometer, and the concentration of tannin in the extract was determined using pure tannic acid as standard (1mg/ml). A calibration curve was generated using various concentrations of Tannic acid (20 - 120µg) was
obtained. Blank consist of all the reagents, except for the extract or standard solution is substituted with 0.1 ml of water. Results were expressed as mg of Tannic acid equivalent/g of dry weight (mg TE/g) of extracts.

**Result and discussion**

The preliminary Phytochemical screening and analysis carryout with standard procedures of ethanol solvent in orange peel extracted and with different parameter a) ultrasonicator b) magnetic stirrer at various temperatures (37°C and 50°C) evaluated the presence of phytochemicals such as Phenol, cellulose and tannins, [6].

The peel samples shows the strong presence of Carbohydrates, Tannins, and Phenols in ethanol extracts. Saponin, coumarins and steroids are present in the methanol extract. Based on the presence of phytochemicals the further estimation will carried out phenol, cellulose and tannin.

As phytochemicals often play an important role in plant defence against prey, microorganism, stress as well as interspecies protection, these plant components have been used as drugs for millennia and hence, screening of phytochemicals serves as the initial step in predicting the types of potential active compounds from plants [2].

These compounds present in a variety of medicinal plants and fruits have significant application against human. Pathogens, including those that cause enteric infections and are reported to have curative properties against several pathogens and therefore could suggest their use in the treatment of various diseases [7].

**A. Estimation of total phenolic content (TPC)**

The concentrations of total phenolic content in the extracts were expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g) of extract the total phenolic content of the pomegranate of peel extract samples was determined using the Folin-Ciocalteu reagent method. The reduction of FCR by phenols to a mixture of blue oxides which have a maximal absorption in the region of 765 nm was measured spectro-photometrically.

The results revealed the presence of highest total phenol content in the ethanol extract of pomegranate peel in different parameter at various temperature in 37°C and 50°C is shown in Fig. 1. The increasing concentration of ethanolic extract 2mg, 4mg, 6mg, 8mg and 10mg showed increasing OD value respectively. The highest OD value for ultra sonicator at 37°C in 10mg is (1.1791), where as in magnetic stirrer the highest OD value is seen at 50c in 10 mg is (1.4647). The highest total phenolic content were obtained in Ultra sonicator at 37 in 10mg. Phenolic compounds possess different biological activities, but most important are antioxidant activities. Phenols are able to scavenge reactive oxygen species due to their electron donating properties.

![Figure 1. Estimation of total phenolic content from ethanol extracts](image)

**B. Estimation of total cellulose content (TCC)**

The total cellulose content of the pomegranate peel extract samples were determined using the anthrone method. The reduction of AR by cellulose to a mixture of blue oxides which have maximal absorption at 630nm was measured spectrophotometrically. The result revealed the presence of
highest total cellulose content in the ethanol extract of pomegranate peel in different parameters at various temperature in 37˚c and 50˚c is shown in the Fig. 2.

The increasing concentration of ethanolic extract 2mg, 4mg, 6mg, 8mg and 10mg showed increasing increasing OD value respectively. The highest OD value of ultrasonicator at 37˚c in 10mg is (1.5519), where as in magnetic stirrer the highest OD value seen at 50˚c in 8mg (1.1295). The highest total cellulose content were obtained in ultra sonicator at 37˚c in 10mg.

![Figure 2. Estimation of total cellulose content from ethanol extracts](image)

C. Estimation of total Tannin (TTC)

The concentration of total tannin content was expressed as mg of Tannic acid equivalent/g of dry weight (mg E/g) of extracts. The total Tannin content of the pomegranate peel extract samples were determined using by Folin–Denis method. The reduction of FDR by tannin to a mixture of blue oxides which have a maximal absorption in the region at 700nm was measured spectrophotometrically.

The result revealed the presence of highest total-Tannin content in the ethanol extract of pomegranate peel in different parameters at various temperature in 37˚c and 50˚c in shown in Fig. 3. The increasing concentration of ethanolic extract, 2mg, 4mg, 6mg, 8mg and 10mg showed increasing OD value respectively. The highest OD value of ultrasonicator at 37˚c in 10mg is (0.6507), where as in magnetic stirrer the highest OD value is seen at 50˚c in 10mg (0.7009). The highest total tannin content were obtained in magnetic stirrer at 50˚c in 10mg.
Conclusion

Recycling of fruit waste is one of the most important means of utilizing it in a number of innovative ways yielding new products and meeting the requirements of essential products required in human, animal and plant nutrition as well as in the pharmaceutical industry. Pomegranate fruit and its peel exhibit a high antioxidant potential.

References

oxidatively injured mammalian cells in comparison with their antioxidant capacity in cell free systems. 


Reflective Assessment of Learning Outcomes [RALO] in Basic Medical Sciences Subjects - [TAU MODEL]

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Abstract
The assessment of learning outcomes at the program level has been a topic of international interest as a method for quality assessment and ongoing program quality enhancement. According to UNESCO report, increasing global integration and exchange of both students and instructors has been an important international objective in higher education in recent years. This trend requires institutions to identify standards of quality, resulting in an increased emphasis on both learning outcomes and evidence from course assessments to demonstrate that students have mastered the expected learning.

Medical educators are increasingly laying emphasis in the assessment and learning outcome, and over a period of time standardized assessment methods like, OSPE and OSCE came into existence and they are widely used in many institutions and also by the international examining bodies worldwide.

However, there aren’t many assessment methods devised, wherein the medical students themselves can assess their level of understanding in basic medical sciences. Continuous methods of understanding the level of knowledge gained by a student will give him/her insight into his learning outcomes and as well as to the teachers.

This concept paper provides an overview of experience gained in developing a self-assessment methods of learning outcome of the medical students.

In 2014 Texila American University created the concept of Reflective Assessment of Learning Outcome, initially this method of assessment was experimented on the distance and blended learning programs, having found it to be very effective in terms of understanding the learning outcomes, this model was implemented for the Doctor of Medicine [MD] students in the year 2016.

Keywords: RALO – Reflective Assessment of Learning Outcome.

Introduction

History of reflective learning

The origins of thinking and writing about reflection started in the last century when John Dewey (1933) first described the concept and how it could help an individual to develop thinking and learning skills.

Dewey defined the concept of reflection as “the active, persistent and careful consideration of any belief or supposed form of knowledge in the light of the grounds that support it and the further consideration to which it tends” (Dewey, 1933).

A wider recognition of the importance of reflection for learning emerged in the mid-1980s in the work of David Kolb (1984) who suggested that learning can happen as result of reflection on experience- experiential learning. He suggested that reflection enables the experiential learner to move through steps from concrete experience to sense-making through reflection. Learners can explore abstract conceptualization- the application of theory- which informs further action and new experiences.

At the same time in the early 1980s the concept of reflection was developed further by Donald Schöen (1982), a social scientist. He developed the idea of reflecting on experience to gain professional knowledge and develop professional skills in his seminal book The Reflective Practitioner: How Professionals think in action.
Reflective learning

Reflective learning enables one

- To accept responsibility for his own personal growth
- To see a clear link between the effort one has put into his/her development activity and the benefits he/she get out of it
- To help see more value in each learning experience, by knowing why he/she is doing it and what’s in it for him/her
- Learn how to ‘learn’ and add new skills over time.

Reflecting on learning

Reflecting on learning enables an individual to link his professional development to practical outcomes and widens the definition of what counts as useful activity. Quite simply, one need to keep asking ‘what did I get out of this?’

As a reflective learner, an individual will think about how he will use new knowledge and skills in his future activities – so learning is always linked to action, and theory to practice. It’s also useful to reflect on how he learn best.

How often one should reflect on his learning?

Reflection should become a routine part of college life that is more or less instinctive. People who routinely plan, record and reflect on their learning tend to see more opportunities for personal development.

Reflection also involves drawing forth cognitive and emotional information from several sources: visual, auditory, kinesthetic, and tactile. To reflect, one must act upon and process the information, synthesizing and evaluating the data. In the end, reflecting also means applying what one has learned to contexts beyond the original situations in which he learned something.

What is Reflective Assessment of Learning Outcome [RALO]

This is a self-assessment done by the students on the learning outcomes. Student learning outcomes articulate what a student should know or can do after completing a course or program. Reflective assessment of learning outcome helps the students to check their mastery level obtained based on the learning outcome determined in the syllabus. Students determines his/her level of understanding of the learning outcomes

Why student self-assessment?

“Self-assessment by pupils, far from being a luxury, is in fact an essential component of formative assessment. When anyone is trying to learn, feedback about the effort has three elements: recognition of the desired goal, evidence about present position, and some understanding of a way to close the gap between the two.

Benefits for students

- Development of metacognitive skills – students become more skilled at adjusting what they are doing to improve the quality of their work (Cooper, 2006).
- Increased responsibility for students’ own learning as a result of more opportunities for self-reflection (Cyboran, 2006).
- Positive effects for low achievers – reducing achievement gaps (Black & Wiliam, 1998; Chappuis, & Stiggins, 2002).
- Development and refinement of students’ capacity for critical thinking (Cooper, 2006)

RALO feedback and growth continuum

RALO provides a concrete feedback on the learning outcomes and as well the comparison between the objective and subjective assessment. RALO can also prove to be a feedback system for the teachers to know how effectively they had imparted their classes. This
feedback will act as benchmark for the curriculum committee to make necessary modifications in determining the outcomes

Ralo feedback and growth continuum

<table>
<thead>
<tr>
<th>Learning Outcomes</th>
<th>Students</th>
<th>Faculty</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focus on CORE Competencies. Learning outcomes are determined based on knowledge, Skills and Attitude and competencies</td>
<td>What is expected to be learned by the students by the end of the course</td>
<td>Update the learning outcomes for an appropriate mastery</td>
<td></td>
</tr>
<tr>
<td>Teaching Learning Activities [TLA]</td>
<td>Effectively participate in the TLA</td>
<td>Application of identified strategies for achieving the expected learning outcome</td>
<td>Modify the teaching learning methods for a better outcome</td>
</tr>
<tr>
<td>Continuous and Summative Assessment</td>
<td>Participates in Objective Assessment</td>
<td>Assessment developed to assess the learning outcome objectively</td>
<td>Refining the Assessment pattern</td>
</tr>
<tr>
<td>RALO Assessment</td>
<td>Self-Assessment by the Students on the Learning Outcomes [Subjective]</td>
<td>Comparison between Objective and Subjective Assessment</td>
<td>Feedback to curriculum committee</td>
</tr>
</tbody>
</table>

Figure 1. RALO feedback and growth continuum chart, shows the connection to the learning outcome, its assessment and feedback from the assessment leading to the modification of the teaching learning activities and eventually refinement of the learning outcomes.

Reflective assessment of learning outcomes [RALO] in basic medical sciences subjects - [TAU MODEL]

Objectives of RALO

Students need to learn how to assess their own progress by asking themselves some key questions about where they are in their learning: Where am I now? Where am I trying to go? What do I need to get there? How will I know I have accomplished what I set out to do?

To help students determine where they are now, teachers can...

- Ensure that students understand the criteria for quality work, so that they are able to assess themselves as fairly and accurately as possible
- Help students gradually assume more responsibility for their own learning, as they practice using self-assessment tools such as RALO
- Provide students with opportunities to discuss their self-assessments in light of peer and teacher assessments
- Ensure that all stakeholders provide specific anecdotal feedback rather than scores or grades to identify explicit next steps for student learning

To help students determine where they intend to go, teachers can ...

- Develop with students clearly articulated learning targets and provide concrete exemplars of student work; students need to understand what they’re “aiming for”.
- Model goal-setting for students.
To help students determine what they need to do to get there ...
- Collaboratively identify strengths and gaps in student learning through the analysis of a variety of data.
- Help students to develop realistic action plans that are practical and directly linked to the learning goals that have been selected.
- Monitor students’ progress as they implement action plans.

To help students determine whether they have accomplished what they had set out to do...
- have students revisit long-term learning goals periodically to reflect on their relevance and to make any necessary adjustments
- talk with each student about his/her learning goal(s)
- have students write a specific reflection about their learning outcome and what they did to achieve them – students may need guidance to identify their strengths and areas for improvement

Methods
- All basic medical science teachers were informed about the RALO process and they were advised to check the appropriateness of the learning outcome of their respective subjects.
- The learning outcome were made known to the students and it was published in the learning management system.
- Teachers deliver the courses through various teaching learning methodologies
- At the end of the course the students were advised to do a self-assessment and rate themselves their level of achieving competencies over the subject matter. This was done through the learning management system

Results
The results of the RALO of all the four levels of classes are given below

Discussion

Mapping the learning outcome with peers
Through RALO assessment students can compare their understanding of the learning outcomes with their classmates. This provides more insight into their learning and understanding. The following gives the overall RALO scores of a class, an individual student can rate himself against the given learning outcome and compare it with the overall class score

The following is the overall RALO Score of MD-1[2016] class in Medical Embryology. An individual student can compare his level of learning with the overall learning outcome of his class
RALO assessment

Students to rate the following learning outcome based on their level of competency as 5: Outstanding 4: Competent 3: Satisfactory 2: Need to improve 1: Inadequate Upon completion of the course, students will be able to:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Responses</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Understand and describe human gamete biology, embryology and developmental biology from a cellular and genetic perspective</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Describe the key events in early and systematic embryological development</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Apply developmental theory of anatomical development</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Apply developmental theory to abnormalities of development and current medical research techniques</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Students are able to broadly understand the abnormalities in the development and current applications in medical research</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Identify and define key structural and molecular elements involved in each stage of human development, the precursors of each structure, and the functional significance of each structure</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Construct a temporal sequence of key events in each developmental period</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Explain and identify the normal embryological anatomy and identify anomalies in the development of various tissues through a comparison of normal and abnormal development</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Understand, using a comparative approach, the key differences in embryological development across animals</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Accurately and effectively communicate scientific ideas through written assignments and reports within the lab and a case study presentation</td>
<td></td>
</tr>
</tbody>
</table>
An individual can score himself against the learning outcome for the subject Medical embryology and total his score and percentage in the table given below and compare it with his peers.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Score and Level of Understanding</th>
<th>Total Score</th>
<th>Percentage</th>
<th>Class %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5: Outstanding</td>
<td></td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4: Competent</td>
<td></td>
<td>34%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3: Satisfactory</td>
<td></td>
<td>23%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2: Need to improve</td>
<td></td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1: Inadequate</td>
<td></td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

Summary

After the assessment is complete, those faculty and/or other parties most involved in the RALO, analyze the results and determine how to use them to improve students' success in achieving Learning Outcomes. For example, they might decide to change or augment instruction in a particular way, change curriculum, or improve future RALO assessments in a specific way.

References


The Human Inter Vertebral Disc - A Histological Approach

Article by Anuradha K1, Sujatha Kiran P2

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Abstract

The human intervertebral disc (IVD) is a very complex joint structure that can be made up of highly organized matrix laid down by relatively few cells in a specific manner. Macroscopically it can be separated into three distinct components: 1) the nucleus pulposus (NP) representing a centrally located gelatinous homogenous mass and of mucoid material with a few multinucleated notochordal cells 2) the anulus fibrosus (AF) consisting of concentrically organized layers of collagen fibrils 3) the cartilaginous endplates (EP), which separate the nucleus pulposus and anulus fibrosus from the adjacent vertebral bone. Any disturbance of the integrity and interplay of one of the three structures can result in a compromised function of the intervertebral disc (3).

According to Buckwalter JA et al like no other musculoskeletal tissue, the lumbar intervertebral disc undergoes very extensive destructive changes with age and degeneration (4). The degree of this tissue destruction is closely linked to age, but different components of the disc undergo more extensive alterations than others (5).

The present study focuses on the extent of cellularity, structural changes of granular matrix degeneration, the formation of clefts and tears and mucoid matrix changes in the nucleus pulposus and anulus fibrosus of the intervertebral disc by the histochemical approach.
Materials and methods

The material for present study consists of 25 fully formed dead foetuses (14 male, 9 female). They were collected from Meenaz maternity hospital, Gulbarga, Karnataka, MNR medical college & hospital, Sangareddy, Telangana India.

The dead foetuses with anomalies like spina bifida, cervical spondylosis, degenerative disc diseases and other skeletal & vertebral deformities were excluded from the study. Normal dead foetuses were considered for this study.

All the lumbar vertebral discs are harvested during routine autopsy under anterior approach. Lumbar intervertebral discs are choose because of bigger and convenient than other region. After dissection Length and Breadth of inter vertebral discs were measured with the standard sliding calipers.

All the slices were fixed in buffered 10% formaldehyde for 24 hours and subsequently decalcified depending on the calcification of the osseous matrix of the vertebral bone. The decalcified disc slices were then embedded into paraffin as routinely performed. From the resulting blocks, paraffin sections were cut and placed on salinized glass slides for routine staining using standard histochemical protocols. The following staining methods are used

i. Haematoxylin and eosin to identify cells and fibres.

ii. Vangieson method to identify collagen fibers.

A histomorphological distinction between anular and nuclear disc tissue was performed by use of light microscopic criteria particularly under polarized light, allowing the evaluation of the organization of the collagen network.

Result & observations

Morphological observation:

The length and breadth of intervertebral disc were calculated by standard sliding callipers.

Table 1. Mean and SD of intervertebral disc in male & female

<table>
<thead>
<tr>
<th>Sex</th>
<th>Average length in mm</th>
<th>Average breadth in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
</tr>
<tr>
<td>Male</td>
<td>11.21</td>
<td>1.01</td>
</tr>
<tr>
<td>Female</td>
<td>11.27</td>
<td>1.05</td>
</tr>
</tbody>
</table>

Histological observations

For descriptive purpose, the disc was analyzed in 2 parts – anulus fibrosus and nucleus pulposus. The collagen fibers in anulus are circularly, concentrically arranged and centrally placed nucleus pulposus shows multi nucleated degenerated notochordal cells seen in the form of mucoid material.
### Table 2. Histological observations of intervertebral disc

<table>
<thead>
<tr>
<th>Staining Method</th>
<th>Anulus fibrosus</th>
<th>Fibers</th>
<th>Nucleus Pulposus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chondroblast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haematoxylin &amp; Eosin</td>
<td>Seen in lacunae more in number then fibers</td>
<td>Circularly &amp; Concentrically arranged</td>
<td>Degenerated multinucleated notochordal cells are seen in the form of mucoid material</td>
</tr>
<tr>
<td>Vangieson</td>
<td>Seen in lacunae more in number then fibers</td>
<td>Circularly &amp; Concentrically arranged</td>
<td>Degenerated multinucleated notochordal cells are seen in the form of mucoid material</td>
</tr>
</tbody>
</table>

**H & E STAIN TOTAL MAGNIFICATION 10X10=100**

**Figure 1.** Intervertebral disc showing specialized embryonic fibro cartilage
Figure 2. Intervertebral disc showing mucoid material

Figure 3. Intervertebral disc showing degenerated notochordal cell
**VANGIESON STAIN TOTAL MAGNIFICATION 4X10=40X**

**Figure 4.** Intervertebral disc showing specialized embryonic fibro cartilage

**Figure 5.** Diagrammatic representation of intervertebral disc showing anulus fibrosus and nucleus pulposus
VANGIESON STAIN TOTAL MAGNIFICATION 4X10=40X

Figure 6. Diagrammatic representation of intervertebral disc showing anulus fibrosus and nucleus pulposus

Discussion

In prenatal development, the anulus fibrosus has collagen fibers which are circularly arranged and centrally placed nucleus pulposus show degenerated notochordal cells.

The intervertebral discs are subject to continuous and progressive changes throughout life so marked that it is difficult to determine what is normal and what is pathologic. (6)

Annular changes are characterized by a gradual loss of fine fibrous connective tissue meshwork and its replacement by increasingly hyalinized collagen fibers, the occurrence of fissures beginning in the third decade, cellular proliferation and enhanced cell death in the fourth decade, and finally the invasion of blood vessels along tears and clefts. In the nucleus pulposus of infants, residues of notochordal cell aggregates are replaced by proliferating chondrocytes, beginning in the second life decade. This is followed by the occurrence of tissue clefts, beginning in the fourth decade, and the progressive replacement of the nucleus by fibrous tissue from the fifth decade onward. (7).

In the morphological observations the average length of disc in males is 11.21 and SD is 1.01 mm but in females average length is 11.27 and SD is 1.05. The values showing length of the disc is minutely higher in males than females (Table 1).

The average breadth of disc in males is 18.9 mm and SD is 0.85 and in female average breadth is 18.54 and SD is 0.78. These values showing breadth of the disc is slightly higher in males than females (Table 1).

In the histological observation of present study, anulus fibrosus shows circularly, concentrically arranged collagen fibers on peripheral part and centrally placed nucleus pulposus shows multi nucleated degenerated notochordal cells seen in the form of mucoid material (figure 1-6).
Conclusion

It is shown by previous authors that the intervertebral disc development starts by 4th week of intrauterine life as Mesenchymal condensation. By 10th week, this Mesenchymal condensation differentiates into anulus and nucleus pulposus.

The present extensive study by different special staining techniques on full term human intervertebral discs show the definite circular, concentrically arranged collagen fibers in anulus fibrosus and chondrocytes and degenerated notochordal cells in nucleus pulposus. Notochordal cells disappear after birth in the first decade of life, followed by gradual replacement of mucoid material by fibro cartilage.

References

Assess the Pre Test Knowledge and Practice of Post-Operative Exercises among Abdominal Surgery Patients Before Video Assisted Teaching

Article by Chakrapani Cheekavolu¹, Vinod kumar Gurram², P.Leela³, Jagan Nadipelly⁴, G. Obulesu⁵

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Abstract

Background: A study to determine the effectiveness of video assisted teaching in knowledge and practice on post-operative exercises among abdominal surgery patients in selected hospitals at Kerala.

Methodology: A pre experimental research with group pre-test and post-test design and chosen 40 patients was chosen to assess the effectiveness of video assisted teaching programme on post-operative exercises among abdominal surgery patients in tertiary care hospital in Kerala. The study was conducted in tertiary care hospital after obtained the ethical committee approval during the period of July to Dec 2017.

Results: The pre-test knowledge, out of 40 patients all of them 40 (100%) had inadequate knowledge on post-operative exercises. In post-test knowledge, out of 40 patients one (2.5%) of them had moderately adequate knowledge, 39 (97.5%) of them had adequate knowledge and none of them had inadequate knowledge in post-test on post-operative exercises.

Conclusion: The pre-test knowledge had inadequate knowledge on post-operative exercises among abdominal surgery patients in tertiary care hospital in Kerala.

Keywords: knowledge and practice, post-operative exercises, abdominal surgery.

Introduction

Surgery is the art and science of treating diseases, injuries and deformities by operation and instrumentation”. Surgery may be performed for the purpose of diagnosis, cure, palliation, cosmetic improvement and prevention. Surgery may be elective in which it is carefully planned and anticipated. The need for surgery may sometimes arise with sudden and unanticipated surgery and is called emergency surgery. The term abdominal surgery broadly covers surgical procedures that involve opening the abdomen. Exercise is important to keep both your body and mind "in shape". Physical exercise is important for maintaining physical fitness and can contribute positively for maintaining a healthy weight, building and maintaining healthy bone density, muscle strength, and joint mobility, promoting physiological well-being, reducing surgical risks, and strengthening the immune system.

Exercising plays an important role in regaining function and strength after undergoing an operation. The goal of post-operative care is to prevent complications such as infection, to promote healing of the surgical incision, and to return the patient to a state of health. Particular patient groups susceptible to fluid or electrolyte disturbances include the elderly, those with pre-existing
cardiovascular/cerebrovascular/renal disease and patients who have suffered a peri-operative myocardial ischemic event [1]. In cases of common conditions can affect peri-operative care include ischemic heart disease, congestive cardiac failure, chronic respiratory disease, diabetes mellitus and liver or renal dysfunction [2]. Hypotension is also common post-operatively and has been defined as a systolic blood pressure below 90 mmHg [3]. Causes include hypovolemic due to bleeding or dehydra, or drug therapy. These variables should be measured multiple times during the day, depending on the type of surgery involved. Other examples of monitoring include ECGs, arterial blood gas analysis (ABGs) and central venous pressure (CVP) monitoring [4]. In addition, assessment of drainage and bleeding should also be performed routinely [5].

Having surgery is a major event in any person’s life. Some of the patient may respond with expression of helplessness, security and isolation due to discomfort, pain and fear of breaking stitches. These feeling can be minimized with pre-operative teaching about postoperative practice related to activities, nutrition, medication and ambulation. [6]

A study was conducted regarding chest physiotherapy during immediate post-operative period among patients undergoing abdominal surgery. Chest physiotherapy during immediate post-operative period following laparotomy was effective for improving oxygen haemoglobin saturation without increased abdominal pain. Breathing exercises could be adopted post anaesthesia care units with benefits for patients. [7]. The present assessment to determine the effectiveness of video assisted teaching in knowledge and practice on post-operative exercises among abdominal surgery patients in selected hospitals at Kerala.

Methodology

Research design

A pre experimental research with group pre-test and post-test design and chosen 40 patients was chosen to assess the effectiveness of video assisted teaching programme on post-operative exercises among abdominal surgery patients in tertiary care hospital in Kerala. The study was conducted in tertiary care hospital after obtained the ethical committee approval during the period of July to Dec 2017. It is a 250 bedded multi-speciality hospital and conducted study in general surgery department.

Inclusion criteria

Who are in age group between 20-50 years?
Who undergoing abdomen surgery for first time?
Who undergoing abdomen surgery through open laparotomy?
Who are willing to participate?
Who are planning for surgery electively?

Exclusion criteria

Who are undergoing emergency surgery?
Who are affected with sensory and motor disabled persons?
Results

Table 1. Distribution of demographic variables among abdominal surgery patients (n=40)

<table>
<thead>
<tr>
<th>Demographic Variables</th>
<th>Abdominal Patients</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age (in years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. 20 - 30</td>
<td>9</td>
<td>22.5</td>
<td></td>
</tr>
<tr>
<td>b. 31 - 40</td>
<td>17</td>
<td>42.5</td>
<td></td>
</tr>
<tr>
<td>c. 41 – 50</td>
<td>14</td>
<td>35.0</td>
<td></td>
</tr>
<tr>
<td>2. Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Male</td>
<td>25</td>
<td>62.5</td>
<td></td>
</tr>
<tr>
<td>b. Female</td>
<td>15</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td>3. Educational Qualification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Non formal education</td>
<td>0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>b. Primary</td>
<td>2</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>c. Secondary</td>
<td>12</td>
<td>30.0</td>
<td></td>
</tr>
<tr>
<td>d. Higher Secondary</td>
<td>17</td>
<td>42.5</td>
<td></td>
</tr>
<tr>
<td>e. Graduate</td>
<td>9</td>
<td>22.5</td>
<td></td>
</tr>
<tr>
<td>4. Occupation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Farmer</td>
<td>4</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>b. Driver</td>
<td>8</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>c. Worker in a company</td>
<td>11</td>
<td>27.5</td>
<td></td>
</tr>
<tr>
<td>d. Student</td>
<td>4</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>e. House wife</td>
<td>13</td>
<td>32.5</td>
<td></td>
</tr>
<tr>
<td>5. Monthly Income</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Less than Rs. 10,000</td>
<td>4</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>b. Rs. 10,000 – 15,000</td>
<td>13</td>
<td>32.5</td>
<td></td>
</tr>
<tr>
<td>c. Rs. 15,001 – 20,000</td>
<td>18</td>
<td>45.0</td>
<td></td>
</tr>
<tr>
<td>d. Above Rs. 20,001</td>
<td>5</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>6. Family Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Joint</td>
<td>29</td>
<td>72.5</td>
<td></td>
</tr>
<tr>
<td>b. Nuclear</td>
<td>11</td>
<td>27.5</td>
<td></td>
</tr>
<tr>
<td>7. Personal Habits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Smoking</td>
<td>8</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>b. Tobacco and betel leaves</td>
<td>1</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>c. Alcohol</td>
<td>6</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>d. Nil</td>
<td>25</td>
<td>62.5</td>
<td></td>
</tr>
<tr>
<td>8. Chronic Diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Diabetes Mellitus</td>
<td>5</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>b. Hypertension</td>
<td>4</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>c. Asthma</td>
<td>3</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>d. Nil</td>
<td>28</td>
<td>70.0</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Distribution of level of knowledge on post-operative exercises in pre-test among abdominal surgery patients (n=40)

<table>
<thead>
<tr>
<th>Level of Knowledge</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inadequate Knowledge</td>
<td>40</td>
<td>100.0</td>
</tr>
<tr>
<td>Moderate Knowledge</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adequate Knowledge</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 3. Distribution of level of knowledge on post-operative exercises in post-test among abdominal surgery patients (n=40)

<table>
<thead>
<tr>
<th>Level of Knowledge</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inadequate Knowledge</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Moderate Knowledge</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Adequate Knowledge</td>
<td>39</td>
<td>97.5</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 4. Mean and standard deviation for knowledge on post-operative exercises in pre-test and post-test (n=40)

<table>
<thead>
<tr>
<th>Knowledge on post-operative exercises</th>
<th>Pretest</th>
<th>Post test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>4.55</td>
<td>18.00</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.39</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Distribution of samples in age 20-30 years were 9 (22.5%), 17 (42.5%) were in age 31-40 years and 14 (35%) were in age 41-50 years. Distribution of samples with gender were male 25 (62.5%) and female 15 (37.5%). According to their educational qualification 9 (22.5%) were graduates, 17 (42.5%) were higher secondary, 2 (5%) were primary education (Table 1).

Distribution of samples with their occupation as 4 (10%) were farmers, 8 (20%) were drivers, 11 (27.5%) were worker in a company, students were 4 (10%) and 13 (32.5%) were housewife. Distribution regarding their monthly income 4 (10%) was less than 10,000/month, 13 (32.5%) were 10,000-15,000 rupees/month, 18 (45%) were around 15000-20000 rupees/month and 5 (12.5%) were above 20000 rupees of monthly income. Out of 40 samples 29 (72.5%) were from nuclear family and 11 (27.5%) were from joint family (Table 1).

According to personal habits 8 (20%) having habit of smoking, 1 (2.5%) has chewing tobacco and betel leaves, 6 (15%) were alcoholic and 25 (62.5%) were having none of these habits. Distribution of samples with chronic disease 5 (12.5%) having diabetes mellitus, hypertension for 4 (10%) samples, 3 (7.5%) having asthma and 28 (70%) were not having such associated disease.

Out of 40 patients all of them 40 (100%) had inadequate knowledge on post-operative exercises. (Table 2). And out of 40 patients one (2.5%) had moderately adequate Knowledge, 39 (97.5%) had adequate knowledge (Table-3). The pre-test mean for the samples was 4.55 with standard deviation 1.39 and in post-test samples mean was 18.00 with standard deviation 1.13. It shows that there is an increase in mean and standard deviation between pre-test and post-test (Table 4).

Discussion

The aim of the present study was to determine the effectiveness of video assisted teaching in knowledge and practice on post-operative exercises among abdominal surgery patients in selected hospitals at Kerala. Out of 40 patients in pretest knowledge level, all of 40 (100%) had inadequate knowledge on post-operative exercises which was explained in table 2. The previous studies reported that,
awareness and knowledge regarding post-operative exercise is still inadequate among the abdomen surgery patients. The study was conducted with 100 samples were 50 for control group and 50 for experimental group. In that structured teaching was given for experimental group and the scores were compared for both groups, the paired’t’ test value for experimental group was t=36.686 which is highly significant at the level P=0.000 and for control group was t= 0.829 which is not significant at the level P=0.411.

There is no difference in measured pain during the preoperative and postoperative periods for either group, or after physiotherapy. These findings are at odds with the reasoning that mobilization may increase pain intensity after abdominal surgery Nonetheless; these same findings are in line with the notion that not only analgesic treatment but also physiotherapy for abdominal and thoracic surgery can reduce the hospital stay and improve recovery. [8]. There was no difference in measured pain during the preoperative and postoperative periods for either group, or after physiotherapy. Some patients in the chest physiotherapy group even reported some pain reduction after the exercises. These findings are at odds with the reasoning that mobilization may increase pain intensity after abdominal surgery [9]. The effects of different chest physiotherapy regimens have been evaluated among high-risk postoperative patients and none of them could be considered highly satisfactory with regard to preventing such complications. [10] Nonetheless, these same findings are in line with the notion that not only analgesic treatment but also physiotherapy for abdominal and thoracic surgery can reduce the hospital stay and improve recovery. [11]. the postoperative spirometry results presented in this study by the two groups did not show any significant differences. Spirometry as a means of quantifying lung function is controversial. Its best results may not be achieved after abdominal surgery, since patients are un-able to perform at their best or even to make a moderate effort to reach total pulmonary capacity or produce maximal forced expirations [12] Intraoperative, bronchospasm occurs most commonly during the induction and maintenance stages of anesthesia and is less often encountered in the emergence and recovery stages [13].

**Conclusion**

The pre-test knowledge had inadequate knowledge on post-operative exercises among abdominal surgery patients in tertiary care hospital in Kerala.

**References**


Role of Artificial Sweeteners in Development of Type 2 Diabetes Mellitus (DM): A Review

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Abstract

A number of lifestyle factors are known to be important to the development of type 2 Diabetes mellitus (DM). These are physical inactivity, sedentary lifestyle, cigarette smoking, dietary habits and generous consumption of alcohol. Recently, it has been reported that 385 million people had diabetes and the number of people with type 2 DM is increasing in every year. obesity has been found to contribute to approximately 55% of cases of type 2 DM. Consumption of sugar-sweetened beverages has been increasingly associated with obesity and type 2 DM. Hence, many people have turned to high-intensity sugar substitute sweeteners like aspartame, sucralose and saccharin as a way to reduce the risk of these consequences. However, accumulating evidence suggests that frequent consumers of these sugar substitutes may also be at increased risk of excessive weight gain, metabolic syndrome, type 2 diabetes and cardiovascular disease. A rise in the percent of the population who are obese coincides with an increase in the widespread use of noncaloric artificial sweeteners, such as aspartame (e.g., Diet Coke) and sucralose (e.g., Pepsi), in food products. This paper discusses these findings and considers the hypothesis that consuming sweet-tasting but noncaloric or reduced-calorie food and beverages interferes with learned responses that normally contribute to glucose and energy homeostasis. Because of this interference, frequent consumption of high-intensity sweeteners may have the counterintuitive effect of inducing metabolic derangements. This review is based on a search of articles published in PUBMED, Medline, the Cochrane Database of Systemic Reviews, and mainly focused on type 2 diabetes mellitus, current diagnosis, treatment and role artificial sweeteners in development of diabetes.

Keywords: Type 2 diabetes mellitus; Artificial Sweeteners; Obesity

Type 2 diabetes mellitus (T2DM)

Type 2 diabetes mellitus is a metabolic disorder characterized by glucose intolerance and insulin resistance leading to hyperglycemia. Type 2 diabetes mellitus (T2DM) is a global epidemic with an estimated worldwide prevalence of 6% (246 million people) in 2007, and forecast to rise to 7.3% (380 million) by 2025. The health, social, and economic burden is great [1, 2]; consequently, T2DM presents a major challenge to healthcare systems around the world. T2DM is a complex disorder in which the interaction between environmental and genetic factors results in the development of insulin resistance and β-cell dysfunction [3, 4]. The development of insulin resistance precedes the onset of T2DM by many years [5] and is influenced by many factors including puberty, ageing, pregnancy, physical activity and oral intake [5 – 9]. Obesity is the single most important contributor to insulin resistance [9] modulating insulin sensitivity via multiple factors including imbalance of hormones (leptin and adiponectin), cytokines (tumour necrosis factor-α, interleukin-6), suppressors of cytokine signalling (SOCS), inflammatory signalling pathways (nuclear factor-KB and IKB Kinase) and retinol binding protein-4 [10 – 13]. The most crucial factor relating obesity to insulin resistance is thought to be the release of non-esterified fatty acids (NEFAs) particularly from intra-abdominal fat. Increased NEFAs result in increased
intracellular diacylglycerol and fatty acyl-co A., which result in phosphorylation of insulin-receptor substrate -1 (IRS-1) and insulin-receptor substrate (IRS-2); this in turn diminishes downstream events of the insulin receptor signalling resulting in insulin resistance [14]. Despite obesity being the single most important contributor to IR; most obese insulin-resistant individuals do not develop T2DM [15] because their β-cells are capable of producing significantly elevated levels of insulin to maintain glycaemic control [16 – 18]. Hence, the failure of β-cells to secrete sufficient insulin to overcome insulin resistance (i.e., β-cell dysfunction) is the crucial step in the development and progression of T2DM [19, 20]. The reason for the decline in β-cell function is not entirely clear, but appears to involve hyperglycaemia per se, together with excessive production of NEFAs, amyloid formation and genetic factors [21 – 24]. In addition to β-cell dysfunction, patients with T2DM have pancreatic α-cell dysfunction manifesting as elevated (or non-suppression of) glucagon secretion in the presence of hyperglycaemia [25].

Obesity has been strongly linked with type 2 DM, and nearly 90% of individuals with diabetes have overweight or obesity [26]. Sugar consumption is one of the common suspected culprits for the rise in obesity and may contribute to the development of type 2 DM [27]. Consequently, artificial sweeteners are commonly consumed by individuals with obesity and type 2 diabetes as they are thought to lower the caloric content and blood glucose response. However, recent studies have proven that, use of artificial sweeteners may also increase weight gain over time [28], and may promote glucose intolerance through the altered function and composition of intestinal microbiota [29].

Complications of diabetes

Chronic hyperglycemia causes many of the major complications of diabetes, including nephropathy, retinopathy, neuropathy, macro and microvascular damage. The risk for microvascular and neuropathic complications is related to both duration of diabetes and the severity of hyperglycemia [30]. In particular, diabetes increases the risk of microvessel disease [31, 32]. As a result, serious conditions such as retinopathy, neuropathy and nephropathy are frequently encountered among patients with diabetes. Diabetic retinopathy is estimated to account for 5% of all cases of blindness globally [33] and up to 50% of patients receiving renal replacement therapy have diabetic nephropathy [34]. Diabetic peripheral neuropathy (DPN) is associated with considerable morbidity, mortality and diminished quality of life and affects up to 50% of people with diabetes [35]. Hyperglycemia is a pre-requisite for the development of diabetic complications and in chronic diabetes, hyperglycemia instigates activations of hexosamine biosynthetic pathway, sorbitol–aldose reductase pathway [36], mitogen activated protein kinases (MAPKs) [37] and protein kinase C [38]. Further, hyperglycemia increases the expression of growth factors and cytokines such as transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), platelet-derived growth factor, insulin-like growth factor (IGF) and tumor necrosis factor-α (TNF-α). Reactive oxygen species (ROS) are important arbitrator factors involved in all these events [39, 40] and activate intracellular signal transduction and transcription cascades, in which MAPKs and nuclear factor kappa B (NF-kB) play the most significant roles [41, 42] and damage proteins, lipids, and nucleic acids by oxidation (Fig. 1). Clinical studies have demonstrated that chronic diabetic complications occur late after disease onset, reflecting structural abnormalities in nerves, kidney, retina and blood vessels, with the appearance strongly correlated with the duration of the diabetes and the level of glycemic control [43]. Large clinical trials have demonstrated that normalization of glycemia can greatly reduce the incidence of diabetic complications. However, in clinical practice, normalizing blood glucose is not a trivial task and almost 50% of diabetic subjects fail to reach the recommended target of an HbA1c lower than 7% [44]. Based on the present perspective of pathophysiology of diabetes mellitus, plentiful pharmacological and non pharmacological interventions have been employed in the previous 50 years in order to treat hyperglycemia and interrupt the progression of disease. However, most of the observed initial improvements in hyperglycemia are not constant because of the progressive nature of disease [45]. These pharmacotherapies also have undesired side effects, such as hypoglycemia, weight gain, gastrointestinal symptoms and peripheral edema, variable effects on β-cell function and decline [46, 47].
Figure 1. Complications of type 2 diabetes mellitus

Current treatment options for diabetes

Based on the current understanding of the pathophysiology of T2DM, multiple pharmacological and non-pharmacological interventions have been developed over the past five decades with the aim of improving glycaemic control and hopefully slowing disease progression. Treatment modalities include lifestyle modifications, treatment of obesity, oral hypoglycemic agents, and insulin sensitizers like metformin, a biguanide that reduces insulin resistance, is still the recommended first line medication especially for obese patients. Other effective medications include non-sulfonylurea secretagogues, thiazolidinediones, alpha glucosidase inhibitors, and insulin. Recent research into the pathophysiology of type 2 DM has led to the introduction of new medications like glucagon like peptide 1 analogues: dipeptidyl peptidase-IV inhibitors, inhibitors of the sodium-glucose cotransporter 2 and 11s-hydroxysteroid dehydrogenase 1, insulin-releasing glucokinase activators and pancreatic-G-protein-coupled fatty-acid-receptor agonists, glucagon-receptor antagonists, metabolic inhibitors of hepatic glucose output and quick-release bromocriptine. Inhaled insulin was licensed for use in 2006 but has been withdrawn from the market because of low patronage. These treatments may also have undesired side effects, such as hypoglycemia, weight gain, gastrointestinal symptoms and peripheral oedema, in addition to variable effects on β-cell function and decline [46, 47]. Hence, interventions that can slow and/or reverse β-cell decline, which result in weight loss (or at least cause no weight gain) and have low risk of hypoglycemia, might be expected to have an important impact in patients with T2DM. Incretin-based therapies are a new class of antidiabetic medication that may address some of the abovementioned shortfalls of current treatments. In addition, other therapies are in development with the potential to address some of the disadvantages of currently available treatments.
Figure 2. Strategies for treatment of type 2 diabetes mellitus (DM).

Source: Silvio inzucchi et al., 2015, diabetes care 38(1): 140-149.

Role of artificial sweeteners in development of type 2 DM

There are five artificial sweeteners currently ruling the arena of food processing, which include saccharin, neotame, acesulfame potassium, aspartame, and sucralose. Of the five main artificial sweeteners, sucralose and aspartame are the most pervasive and dangerous substitutes found in products on store shelves today. All these artificial sweeteners are marketed under the names of Splenda, Equal and NutraSweet and there are many everyday eatable products such as yogurt, sodas, pudding, tablets, chewing gum, bread, etc consist of these artificial sweeteners.

Many accumulating evidence suggests that frequent consumption of these sugar substitutes may increase risk of excessive weight gain, metabolic syndrome, type 2 diabetes, and cardiovascular disease. [48, 49]. Significant risk of weight gain, obesity, increased body mass index (BMI) and increased body fat percentage in males and females after consumption of artificial sweeteners containing food products was observed in few recent studies [50, 51]. Several large-scale studies, including the National Health and Nutrition Examination Survey (NHANES) and the San Antonio Heart study, have shown a associations between artificial sweetener intake and incidence of the metabolic syndrome and its components, including waist circumference, blood pressure, and fasting blood glucose [52, 53]. Recent studies conducted in adults have demonstrated the link between artificial sweetener consumption and insulin
resistance, incidence of type 2 diabetes, and poor glucose control in patients with pre-existing diabetes [54]. In the European E3N study and the Health Professionals Follow-up (HPFS) [55] risk for type two diabetes was more than doubled for participants in the highest quartile of artificial sweeteners consumption compared with non-consumers. Most recently, data from the European Prospective Investigation into Cancer and Nutrition (EPIC) has also indicated that risk for type two diabetes was elevated in those consuming at least one artificial sweetener per day [56]. A study conducted by Pepino et al. (2013) [57] and Suez et al., (2014) [58] demonstrates a deleterious effect increasing glucose concentrations in subjects with a high degree of obesity after an acute and a 7-day exposure to sucralfose and saccharin, respectively.

**Mechanism of artificial sweeteners in development of diabetes**

Several mechanisms have been proposed to account for the association between artificial sweetener use and diabetes. Recent research have provided convincing evidence that artificial sweeteners play an active role in the gastrointestinal tract, thus providing a mechanistic explanation for observed metabolic effects. Sweet-taste receptors, including the taste receptor T1R family and α-gustducin, respond to artificial sweeteners [59, 60]. In both humans and animals, these receptors have been situated in gastrointestinal tract and glucagon-like peptide-1 (GLP-1) [61]. Consumption of artificial sweeteners containing products along with normal day to day food products resulting in stimulation of taste receptors in GI tract and secretion of more amount of GLP-1 [62]. This could lead to more and rapid absorption food materials from intestine into the blood stream and as well as increase GLP-1 stimulates insulin, which enhances the glucose absorption by cells and eventually it lead to weight gain and central visceral adiposity. Many previous studies revealed the crucial role of adiposity in the pathogenesis of type 2 diabetes.

**References**

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