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Hepatoprotective Effect of Methanolic Leaf Extract of Vernonia Amygdalina against Acetaminophen-Induced Hepatotoxicity in Wistar Albino Rats

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Abstract

This study investigated the hepatoprotective effect of methanolic leaf extract of Vernonia amygdalina against acetaminophen-induced hepatotoxicity in Wistar albino rats. Albino rats (wistar strain) were randomly divided into groups of five rats each, Group I served as control and was administered normal saline only, Group II were induced 75.35 mg/kg of acetaminophen, Group III were given 200mg/kg of methanolic leaf extract of Vernonia amygdalina, while Group IV and V were administered 75.35mg/kg of acetaminophen along with different doses (the former were administered 200 mg/kg and the latter were administered 400 mg/kg) of methanolic leaf extract of Vernonia amygdalina five minutes after the administration of acetaminophen. Administration of the methanolic leaf extract of Vernonia amygdalina were given orally while intra-peritoneal administration was adopted for acetaminophen induced liver damage rats. All administration was done once daily. There after the rats were sacrificed by cervical dislocation and sera collected was used for various biochemical analyses. Liver enzymes (Alanine aminotransferase, Aspartate aminotransferase and Gammal glutamyl transferase), total proteins, albumin and lipid profiles activities were determined. This study showed that administration of the methanolic extract of Vernonia amydalina reduced the damages to the liver against acetaminophen- induced hepatotoxicity in wistar albino rats.

Keywords: *Methanolic, Hepatoprotective, Vernonia amydalina, Liver enzymes.*

Introduction

Vernonia amygdalina, commonly called bitter leaf, is a perennial shrub of 2-5m in height that grows throughout tropical Africa. It belongs to the family *Asteraceae*, has a rough bark with dense black straits, and elliptic leaves that are about 6 mm in length. The leaves are green and have a characteristic odor and bitter taste (Singha *et al.*, 1966). In many parts of West Africa, the plant has been domesticated (Igile *et al.*, 1994). It is known as 'Ewuro' in Yoruba, 'Etidot' in Ibibio,'Onugbu' in Igbo, 'Ityuna' in Tiv, 'Oriwo' in Edo and 'Chusar-doki' in Hausa (Egedigwe *et al.*, 2010). *Vernonia amygdalina* is drought tolerant (though it grows better in a humid environment).It thrives on a range of ecological zones and is used as an edge plant in some communities (Bonsi *et al.*, 1995).

Vernonia amygdalina has been shown to contain significant quantities of lipids (Ejoh *et al.*, 2007; Eleyinmi *et al.*, 2008), proteins with high essential amino acid score (Igile *et al.*, 1994; Udensi *et al.*, 2002; Ejoh *et al.*,2007; Eleyinmi *et al.*, 2008) that is similar to *Telfairia*

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occidentalis and *Talinum triangulare* (Ijeh *et al*., 1996), carbohydrates (Ejoh *et al*.,2007). The plant has also been shown to contain appreciable quantities of ascorbic acid and caroteinoids (Udensi *et al*., 2002; Ejoh *et al*., 2007). Calcium, iron, potassium, phosphorous, manganese, copper and cobalt have also been found in significant quantities in *Vernonia amygdalina* (Bonsi *et al*., 1995; Ejoh *et al*., 2007; Eleyinmi *et al*., 2008).

Phytochemical importance

Phytochemicals was found abundantly in the leaves of *Vernonia amygdalina*. This include some of the identified Sesquiterpene lactonesare vernolide, vernodalol (Erasto *et al*., 2006), vernolepin, vernodalin and hydroxyvernolide (Jisaka *et al*., 1993). Igile *et al*., (1994) reported the presence of the flavonoids luteolin, luteolin 7-*O*-β-glucoroniside and luteolin 7-*O*-β-glucoside, in the leaves of *Vernonia amygdalina*. Other researchers have confirmed the presence of flavonoids in the plant (Udensi *et al*., 2002). These bio-active principles may act singly, or synergistically to produce the results for which the medicinal values of *Vernonia amygdalina* have been vigorously studied.

Liver diseases are serious medical problems, especially because of the central role of the liver in metabolic homeostasis and xenobiotic transformations. The search for alternative drugs for the treatment of liver diseases has produced some 'botanicals chief' among which is *Vernonia amygdalina.* Compounds of the sesquiterpene family have been shown to have anti hepatotoxic activity in tetrachloromethane-induced hepatic damage in rats (Babalola *et al*., 2001). A study by Ijeh and Obidoa (2004) found that a diet incorporated with *Vernonia amygdalina* protected weanling albino rats against afflatoxin B1-induced hepatotoxicity. Thus in this study, we investigated the effect of methanolic leaf extract against acetaminophen-induced hepatoxicity.

Materials and methods

Experimental animals

Twenty-five 16-weeks old wistar albino rats with an average weight of 185g were purchased from animal house of the Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Kwara State, Nigeria. They were kept in a well-ventilated animal house of the department of Biochemistry, Ladoke Akintola University of Technology, Ogbomosho, Nigeria with conducive atmospheric pressure and temperature.

The animals were randomly divided into 5groups each having five rats. The rats were housed in plastic cages and had access to feed and pipe-borne water. Experiments on animals and protocols conform to the guidelines of the National Institutional Health (NIH), (NIH publication 85-23, 1985) for laboratory animal care and use.

Experimental chemicals

Commercial Paracetamol injections manufactured by May and Makers Chemical Company Limited, Dagenham England and was purchased from Alkol Pharmacy, Ogbomosho. An intra-peritoneal method was adopted.

Vernonia amygdalina extraction

Fresh leaves of *Vernonia amygdalina* were harvested from neighboring bushes within Ogbomosho and were identified by a botanist at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The leaves were air-dried at room temperature and then pulverized to powder using electric blender. The powdered sample (200g) were weighed and soaked in 575ml of methanol and vortex for 72hrs. The leaf extract was removed by decanting the liquid content of the mixture and filtrate was dried using rotary evaporator at 45^{o}C to obtain the dried methanolic extract. The extract was dissolved in normal saline at a concentration base on the average of animals present in each group, and aliquots of different concentrations were given to the animals orally.

Experimental design

Twenty-five wistar rats were divided into five groups of five animals each. Group I (Control) was administered with normal saline, Group ii (Hepatotoxic control) were administered with 75.35mg/kg of Acetaminophen, Group iii were administered with 200mg /kg of methanolic leaf extract of *Vernonia amygdalina* only, Group iv were administered 75.35mg /kg of acetaminophen and 200mg/kg of methanolic leaf extract of *Vernonia amygdalina*. Group v were administered 75.35mg/kg of acetaminophen and 400mg/kg of methanolic leaf extract of *Vernonia amygdalina*.

All administrations were given once daily and Group iv &v were given the extract 5minutes after the acetaminophen for 7 days. An oral administration was adopted when administering the extract while intra-peritoneal administration was adopted for acetaminophen induced liver damage rats.

Collection of blood sample

The rats were sacrificed through cervical dislocation and blood samples obtained through cardiac puncture. The blood was collected into appropriately labelled heparinized sample bottles and spinned at 4000 rev/sec for 5 minutes to harvest the serum. The serum was analyzed for various biochemical parameters.

Preparation of liver homogenate

The livers were excised and homogenized in 0.25M sucrose solution using mortar and pestle.

Results

Table 1. Effects of methanolic leaf extract of vernonia amygdalina on serum lipids profile in acetaminophen – induced liver damage in rats

Group	Total Cholesterol	(HDL)	Triglycerides	LDL)
Control (n=5)	62.37 ± 6.61^{a}	40.77 ± 2.27^{a}	44.43 ± 0.74^{a}	13.15 ± 7.8^{ab}
Acetaminophen (n=5)	98.10 ± 8.25^{b}	42.78 ± 2.41^{a}	84.46 ± 13.36^{b}	37.7 ± 4.22^{c}
Extract Only (n=5)	65.80 ± 5.15^{a}	46.06 ± 1.76^{a}	57.38 ± 10.05^{ab}	9.4 ± 5.09^{a}
Normal dose (n=5) (200mg/Kg)	80.00 ± 5.98^{ab}	40.80 ± 1.00^{a}	42.60 ± 5.93^{a}	30.68 ± 5.59^{bc}
Over dose (n=5) (400mg/Kg)	77.40 ± 15.71^{ab}	40.78 ± 2.43^{a}	52.0 ± 1.17^{b}	29.86 ± 7.30^{bc}

Values are expressed as Mean±SE. same superscripts indicate values are not significantly different while different superscripts indicates values are significantly ($p \leq 0.05$) different.

There is increased in cholesterol level in acetaminophen induced liver damage rats when compared to control and group administered methanolic leaf extract of *Vernonia amygdalina* only. However administration of methanolic leaf extract of *Vernonia amygdalina* (VA), 200 mg/Kg body weight of rats along with 75.35 mg/kg of acetaminophen showed reduction in cholesterol level when compared to the rat administered acetaminophen and 400 mg/Kg of *Vernonia amygdalina*.

There is increased in Triglycerides level in acetaminophen induced liver damage rats when compared to control and group administered methanolic leaf extract of *Vernonia amygdalina* (200 mg/Kg) only. However, administration of methanolic leaf extract of *Vernonia amygdalina* (VA) 200 mg/Kg and 400 mg/Kg body weight of rats along with 79.35 mg/kg of acetaminophen showed reduction in triglycerides level.

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Table 2. Effects of methanolic leaf extract of vernonia amygdalina on serum enzymes in acetaminophen – induced liver damage in rats

GROUP	Aspartate aminotransferase	Alanine aminotransferase	Gamma-Glutamyl aminotransferase
Control (n=5)	3.17± 5.77a	24.57± 3.48a	7.70± 0.00a
Acetaminophen Only (n=5)	61.88± 8.26b	78.58 ±23.92b	7.78± 1.21a
Extract Only (n=5)	36.80 ±6.98ab	29.50 ±0.00a	6.82 ±1.31a
Normal dose (n=5) 200mg/Kg)	58.90 ±13.97b	29.42 ±8.06a	7.78 ±1.21a
Over dose(n=5) (400mg/Kg)	39.38 ±10.86ab	44.22 ±10.16ab	7.40 ±1.83b

Values are expressed as Mean±SE. same superscripts indicate values are not significantly different while different superscripts indicate values are significantly different. Administration of different doses acetaminophen caused significant ($p \leq 0.05$) increase in the activity of serum enzyme compared with corresponding activity in control group and group administered 200 mg/Kg of methanolic leaf extract of *Vernonia amygdalina* only.

Administration of 200 mg/kg and 400 mg/kg body of methanolic leaf extract of *Vernonia amygdalina* moderate effects of acetaminophen by decreasing the activity of serum enzymes.

Table 3. Effects of methanolic leaf extract of vernonia amygdalina on some serum proteins in acetaminophen-induced liver damage in rats.

Group	**Total Protein**	**Albumin**
Control (n=5)	7.02 ± 0.56^{b}	3.27 ± 0.14^{b}
Acetaminophen Only (n=5)	5.32 ± 0.20^{a}	2.10 ± 0.15^{a}
Extract Only (n=5)	6.36 ± 0.29^{ab}	3.06 ± 0.32^{b}
Normal dose (n=5) (200mg/Kg)	5.70 ± 0.94^{a}	2.26 ± 0.11^{a}
Over dose (n=5) (400mg/Kg)	5.66 ± 0.18^{a}	2.28 ± 0.12^{a}

Values are expressed as Mean±SE. same superscripts indicate values are not significantly different while different superscripts indicate values are significantly different. Serum concentration of total protein and albumin were significantly ($p \leq 0.05$) reduced in groups given different doses of acetaminophen when compared with corresponding concentrations in control group and group administered 200 mg/Kg of methanolic leaf extract of *Vernonia amygdalina* only. Administration of 200 mg/kg and 400mg/kg body of methanolic leaf extract of *Vernonia amygdalina* five minutes after administration of 79.35 mg/kg body weight of acetaminophen causes elevations in serum concentrations of albumin and total protein when compared with groups given acetaminophen only.

Discussion

The liver is a major target organ for toxicity of xenobiotics and drugs, because most orally ingested xenobiotics and drugs pass through the liver and some chemicals are metabolized into toxic intermediates in the liver (Jaeschke *et al*., 2002). Paracetamol, when used at high doses, could cause acute liver injury most probably via formation of N-acetyl-p-benzoquinone imine, a toxic metabolite, by cytochrome P4502E1 (CYP2E1). N-acetyl-p-benzoquinone imine is usually inactivated by hepatic glutathione, but when produced excessively, covalently binds to centrilobular hepatic proteins, contributing to hepatic toxicity (Gardner *et al*., 1998; 2002).

Vernonia amygdalina leaves are commonly used in Nigeria traditional medicine for the treatment of various diseases such as cough, cold, influenza, chest infections, diabetes mellitus (Alabi *et al*., 2005). The leaves are used as green leafy vegetable and may be consumed either as a vegetable (leaves are macerated in soups) or aqueous extracts used as tonics for the treatment of various illnesses in which the participation in reduction of liver damage has been implicated (Bonsi *et al*., 2005). This may be as a result of the hepatoprotective effect ability of the plant.

In the assessment of liver damage by acetaminophen (paracetamol), the determination of enzyme activities such as ALT and AST is largely used. In the present study, the increase in serum activities of ALT, AST and GGT in acetaminophen (paracetamol) treated rats had been attributed to the damaged structural integrity of the liver, because these are normally located in the cytoplasm, mitochondria or microsomes and are released into the circulation after cellular damage (Sallie *et al*., 1991) or due to alterations in the permeability of cell membrane and increased synthesis or decreased catabolism of aminotransferases (Nuduka *et al.*, 1999). These results were in accordance with those of Kuvandik *et al*., (2008) who found that the serum levels of both ALT and AST were elevated almost four-fold in acetaminophen (paracetamol) treated group in comparison with the control group. Also, Kanchana and Sadiq (2011) mentioned that oral administration of 400 mg/kg paracetamol in rats increased serum activities of ALT, AST, LDH, ALP and GGT.

Moreover, chronic administration of paracetamol statistically decreased serum albumin and total proteins fractions which were evident for chronic hepatic necrosis. Albumin is decreased in chronic liver disease and is generally accompanied by an increase in the β and γ globulins as a result of production of IgG and IgM (Kaplan and Pesce, 1996). The present results were in harmony with Lotkova *et al*., (2009) who revealed that paracetamol induced toxic injury of rat hepatocytes as assessed by significant decrease in albumin level and total proteins. Also, Abdel-Azeem *et al*., (2013) mentioned that acute paracetamol toxicity induced remarkable increase in plasma ALT, AST, ALP activities and significant decrease in plasma level of total protein and albumin of rats.

In the present study, intra-peritoneal administration of acetaminophen was accompanied by elevation of markers of lipid peroxidation i.e malondialdehyde when compared with concentrations in control group, which may result in depletion in GSH stores and reduced GPx activity in the liver. Though it was not determined in this study but it has been generally accepted that P450-dependent bioactivation of paracetamol is the main cause for potentially fulminant hepatic necrosis upon administration or intake of lethal doses of paracetamol (Bailey *et al.*, 2003; Lee *et al.*, 2004). NAPQI is initially detoxified by conjugation with reduced GSH to form mercapturic acid (Moore *et al*., 1985). Under conditions of NAPQI formation following toxic paracetamol doses, GSH concentrations become very low in the centrilobular cells (Oz *et al*., 2004; Volmar and Menger, 2009) which could account for the observed depletion in liver GSH stores.

Administration of methanolic extract of *Vernonia amygdalina* reduced hepatic lipid peroxidation, maintained antioxidant enzymes within normal levels and increased level of reduced glutathione. It could be due to antioxidative properties of *Vernonia amygdalina* extracts.

Administration of paracetamol decreased serum, HDL concentration and VLDL concentration, with increased serum total cholesterol level, triglyceride with increased serum LDL level. Paracetamol seems to cause impairment in lipoprotein metabolism and also alterations in cholesterol metabolism.

Conclusion

Paracetamol most notably caused hepatic toxicity as indicated by increased serum liver enzymatic activities, decreased albumin and increased globulin fractions, induction of oxidative stress and depletion of antioxidant.

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In addition, methanolic leaf extract of *Vernonia amygdalina* reduce lipid peroxidation, indicating the antioxidant properties and anti –lipid peroxidative effects along with the hepatoprotective effect (by lowering the hepatic marker enzymes and restored the level of proteins) by the extract.

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