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Effect of Vernonia amygdalina Ethanolic Root Extract on the Hepato- and Nephro-Protective Properties of Albino Rats (Rattus novergicus)

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Abstract
The hepato- and nephro-protective potentials of *Vernonia amygdalina* ethanolic root extract was evaluated for 14 days using standard bioassay in 45 normal male albino rats. The rats were divided into four treatment groups I – IV and a control group V. Groups I – IV were given 100mg.kg^{-1}, 200mg.kg$^{-1,}$ 400mg.kg^{-1} and 600mg.kg^{-1} body weight, respectively while the control (group V) was given equal volume of feed and water. The extracts were administered orally to the animals for 14 days. Blood samples were collected using the ocular puncture method before and weekly after administration to evaluate the extracts' effects on aspartate transaminase (AST), acid phosphatase (ACP), alanine transaminase (ALT), alkaline phosphatase (ALP), bilirubin, creatinine and blood urea nitrogen (BUN) levels. The mean serum levels of the liver marker enzymes AST, ACP, ALT, ALP, total bilirubin and direct bilirubin ranged from 10.00±0.53 to 11.44±0.44**,** 31.29±0.64 to 33.14±0.56, 27.22±0.94 to 29.67±0.37 and 37.83±0.59 to 40.57±1.02, 3.42±0.08 to 3.61±0.07 and 2.06±0.11 to 2.51±0.05 respectively. The mean levels of the nephrotic enzymes, creatinine and BUN also ranged from 39.87±1.79 to 43.04±1.57 and 6.62±0.21 to 15.98±0.17 accordingly. Although no significant difference ($p>0.05$) was observed in the serum levels of the liver marker enzymes and creatinine when compared with the control, a dose and duration dependent significant increase ($p<0.05$) occurred in the BUN level. This tends to suggest that the ethanolic root extract of *Vernonia amygdalina* on a short term basis has some hepato-protective property while its nephro-protective ability is still doubtful.
Keywords: *Vernonia amygdalina*, Ethanolic root extract, Liver maker enzymes, Nephrotic enzymes, Albino rats

1. Introduction

Despite the clamour for and availability of modern medicine in many countries, phyto-medicine or herbal medicine has become popular and indispensible all over the world as a result of easy accessibility, low cost and ancestral experience. It has been recognized as a potent tool in traditional medicines and in healthcare delivery. It is on record that as much as 80% of the population in the developing world depend on traditional medicines for primary healthcare (WHO, 1996). In view of the foregoing, ethno-botanical studies have been advocated as the most viable method of identifying new medicinal plants due to their high bioactive constituents. However, there is the growing interest to adopt systematic methodology to carefully evaluate scientific bases for the traditional herbal medicine so as to obviate the attendant severe undesirable side effects of synthetic drugs.

Vernonia amygdalina, a member of the *Asteraceae* family, is a small mercurial shrub that grows widely in tropical Africa, used variously for nutritional and medicinal purposes. The macerated leaves are used in preparing 'ofe olugbu' which is a delicacy among the Igbos of south east Nigeria. Also, in many parts of West Africa especially, Cameroon, Ghana and Nigeria, stem and roots divested of the bark are used as chew-sticks for oral hygiene and management of some dental problems (Burkill, 1985).

The plant has been reported to contain such important constituents as lipids (Ejoh *et al.,* 2007), proteins and essential amino acids (Igile *et al.,* 1994), carbohydrates (Eleyinmi *et al.,* 2008), anti-nutrient substances (Nwafor and Akah, 2001; Erasto *et al.,* 2007) and essential elements (Bonsi et *al.,* 1995). Pharmacologically, the leaf extracts have been reported to possess hypoglycaemic and hypolipidaemic properties (Aka and Okafor, 1992; Ekpo *et al.,* 2007), hepatoprotective effect (Ijeh and Obidoa, 2004; Iwalokun *et al.,* 2006; Arhoghro *et al.,* 2009) as well as anti-malarial properties (Abosi and Reaseroka, 2003). *Vernonia amygdalina* extracts are also reported to possess potent anti-schistosomal activity (Ogboli *et al.,* 2000) as well as anti-cancer effects (Izevbigie *et al.,* 2004; Sweeney *et al.,* 2005; Song *et al.,* 2005; Opata and Izevbigie, 2006; Yedjou *et al.,* 2008). Studies have also shown that *V. amygdalina* extracts may strengthen the immune system through the regulation of many cytokines (Sweeney *et al.,* 2005).

Studies using root extracts of *V. Amygdalina* in different solvents have been very limited. Desta (1994) and Steen-Kamp (2003) have reported of the anti-fertility potential of aqueous root extract of *V. Amygdalina*. Similarly, a decoction of the roots has been utilized by traditional healers in Nigeria in the treatment of convulsion and as an expectorant (Okokon and Onah, 2004). According to Igbakin and Oloyede (2009) ethanolic

root extract of *V. Amygdalina* contains hypoglycaemic, hypocholesterolaemic, hypolipidaemic and hypoproteinaemic bioactive components. More recently, Eyo *et al.* (2013) reported of a duration dependent effect of the aqueous root extract on the haematological profile of experimental animals. To the best of our knowledge there is very scanty information in literature on the hepatic and renal protective effects of *V. amygdalina* root extract. This study therefore attempts to investigate the possible hepato- and nephro-protective potentials that may be associated with the oral consumption of the ethanolic root extract of *Vernonia amygdalina*.

2. Materials and Methods

2.1 *Collection, identification and preparation of plant material*

Fresh roots of *Vernonia amygdalina* were collected from a vegetable garden in Ozubulu in Ekwusigo Local Government Area of Anambra State, Nigeria. The plant materials were transported to the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka where they were authenticated by a Plant taxonomist. The fresh roots were then taken to the Physiology laboratory of the Department of Zoology and Environmental Biology, washed in clean tap water and cut into smaller sizes. The cut roots were weighed and air-dried for three weeks under room temperature. The dried roots were weighed again to ascertain the appropriate moisture content and then ground into a coarse powder using a laboratory milling machine (Model 4; Thomas Willey, USA).

100g of the powdered plant material was macerated in 300 ml of analytical absolute ethanol in a conical flask. The content was mixed thoroughly and left to stand for about 72 hours with an occasional shaking to increase the extraction capacity. Thereafter, the soaked substance was filtered with a muslin clothe (number 60 mesh size) and the resulting ethanolic extract was concentrated till a slurry form was obtained under 65^0C in a water bath. The solid residue referred to as the crude extract was stored in an air-tight experimental bottle in a refrigerator until ready for use.

2.2 *Procurement and management of experimental animals*

Forty-five adult male albino rats weighing between 124.3g – 134.5g were procured from the Genetics and Animal breeding Unit of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. Approval was obtained from the Ethical Committee of the University of Nigeria, Nsukka to use the rats in accordance with the stipulated protocol for laboratory animal experimentation. The rats had no history of drug consumption and were kept in stainless wire rat cages equipped with drinkers and faecal collecting trays, in a clean and fly proof experimental animal house. They were fed commercial growers chick mash (18 % crude protein) product of Vital Feeds, Nigeria Limited and clean drinking water *ad libitum* during the period of acclimatization and experimentation. All the animals were maintained under standard laboratory conditions for temperature, humidity and light throughout the experiment and were allowed unhindered access to food and water. The faecal droppings in the tray were removed daily.

2.3 *Experimental Design*

The Forty-five (45) adult albino rats were randomly divided into five groups I - V of 9 rats each and housed in separate stainless wire rat cages. Each group was further replicated three times consisting of 3 rats each. Groups I – IV were orally administered daily with a 2ml syringe an aqueous solution of the *V. amygdalina* ethanolic root extract corresponding to 100mg/kg, 200mg/kg, 400mg/kg and 600mg/kg body weight dose respectively for 14 days (Adebayo *et al.,* 2003). Contrarily, the Control group V, was fed daily also with commercial grower's chick mash and water only.

2.4 *Collection of Blood Sample*

About 5 ml of the blood sample was collected from each anaesthetized rat using the ocular puncture method (Hoff, 2000) before treatment (**Day 0**) and subsequently on the **7^{th}** and **14^{th}** days respectively. The blood samples were collected with heparinized haematocrit tubes and kept in ice-cold heparinized bottles. They were allowed to stand for 30 minutes, centrifuged at 5000rpm for 5 minutes while the plasma samples were collected and stored at 20^oC until required.

2.5 *Biochemical Assays*

The serum levels of the basic liver and kidney function enzymes were assayed using commercially available diagnostic assay kits (Randox Laboratories LTD, United Kingdom).

The serum levels of AST and ALT were ascertained with the methodology of Reitman and Frankel (1957) while the ACP level was determined using the α-Naphthylphosphate kinetic method (Sood, 2006) and the ALP level was verified with the pNPP kinetic method (Sood, 2006). On the other hand, the total and direct bilirubin levels were determined using the modified Jendrassik and Grof method (Sood, 2006).

The blood urea nitrogen (BUN) content was ascertained according to the modified Berthelot and Searcy method (Sood, 2006) using the formular:

Serum BUN concentration (mg urea nitrogen/100 ml) =

$$\frac{A_{sample}}{A_{standard}} x\ 50$$

Similarly, the serum creatinine level was determined by employing the modified Jaffa's kinetic method (Sood, 2006) using the formular:

Serum creatinine concentration (mg/dl) =

$$\frac{A_{sample}}{A_{standard}} x\ standard\ concentration$$

2.6 *Statistical Analysis*

The data obtained was analyzed using SPSS version 20.0 (IBM Statistics, UK). One-way analysis of variance (ANOVA) was used to test for variations of the various parameters. All results were expressed as Mean ± Standard error, while the level of significance was placed at $p < 0.05$.

3. RESULTS

3.1 The Effects of Ethanolic Root Extracts of V. amygdalina on Hepatic Enzymes and Bilirubin levels of normal Albino rats.

Table 1 shows the result of the effects of the *V. amygdalina* ethanolic root extracts on the hepatic enzymes and Bilirubin serum levels. There was no significant difference ($p>0.05$) observed in the serum levels of the hepatic enzymes assayed despite the somewhat minimal wavelike but insignificant duration dependent increases observed in AST **(400mg/kg)**, ACP **(100mg/kg, 400mg/kg and 600mg/kg)**, ALT **(200mg/kg – 600mg/kg)**, ALP **(100mg/kg and 600mg/kg)**, Total bilirubin **(400mg/kg and 600mg/kg)** and Direct bilirubin **(100mg/kg – 600mg/kg)**.

3.2 Effects of Ethanolic Root extracts of V. amygdalina on Nephrotic enzymes of normal Albino rats.

Table 2 shows the result of the *V. amygdalina* root extract effect on the nephrotic enzymes of the albino rats. There was no overall dose and duration dependent significant change ($p>0.05$) observed in the serum creatinine levels of the treated rats when compared with the control group. However, a minimal wavelike but insignificant dose and duration dependent increase in the serum creatinine levels was observed on the 14th day of treatment in virtually all the treatment groups.

Contrarily, there was an overall dose and duration dependent significant increase ($p<0.05$) observed in the BUN serum levels of the treated rats in comparison with the control. It is equally noteworthy that the least increase in the serum BUN level was observed in the 7th day of 100mg/kg dose **(9.88±0.14)** while the highest value was obtained on the 14th day of the rats administered the 600mg/kg dose **(15.98±0.17)**.

4. Discussion

Data accumulated in this study showed that the ethanolic root extract of *V. amygdalina* recorded no significant difference in the serum levels of the liver marker enzymes assayed namely, AST, ACP, ALT, ALP and Bilirubin. Usually the serum levels of the above enzymes have been used as a good indicator of not only the functionality and cellular integrity of the liver (Lavanaya *et al.,* 2011) but as well, to assess the functional health status and the internal environment of the organism (Rehman *et al.,* 2006). Normally an elevation in their serum levels may indicate an inflammation or damage to the hepatocytes (Sood, 2006) especially whenever the liver undergoes such pathological conditions as cirrhosis or subjected to abnormal onslaught that accompany the presence of toxins or usage of some drugs (Nyblom *et al.,* 2004; Crook, 2006). Inflamed or injured liver cells release higher than normal amounts of certain chemicals, including liver enzymes into the blood stream thereby resulting in elevated concentration of the liver enzymes after blood tests. The fact that the extract had no significant effect on the serum levels of these liver marker enzymes is an indication that it had no negative interaction on the hepatocytes and as such did not increase the activities of the lysosomes. In addition, the extract interaction with the animal appeared to have not caused any damage to the mitochondria nor did it affect the membrane permeability of the liver cells thus, not inducing any form of liver damage nor dysfunction (Crook, 2006). Furthermore the dose levels of the ethanolic root extract used did not adversely interfere with the functions of the liver involved in such processes as calcification, metabolism, production and excretion of bilirubin (Edem and Usoh, 2009). Our present observation largely corroborates past findings on the hepato-protective potentials of various extracts of *V. amygdalina* in experimental animals (Ijeh and Obidoa, 2004; Iwalokun *et al.,* 2006; Arhoghro *et al.,* 2009). It also supports an earlier observation that the aqueous root extracts increases the formation rather than destruction of red blood cells in experimental animals (Eyo *et al.,* 2013).

An elevation in the serum levels of BUN and creatinine in clinical analyses presupposes renal dysfunction (Sood, 2006; Mehrdad *et al.,* 2011). This informed our decision to ascertain the serum values of BUN and creatinine in the treated animals. The absence of a significant difference in the serum creatinine levels

of treated and control albino rats obtained in the present report simply indicates that the extract had no negative effect on the kidneys (Atangwho *et al.,* 2007). This presupposes that the root extract did not impair the functioning renal tubular mass as well as its regulatory functions. Secondly, it shows that there was no form of abnormality in the metabolism of protein molecules in the muscles following the introduction of the root extract. This is more so, as creatinine is a major catabolic product of protein metabolism in the muscle tissue usually excreted by the kidneys (Mehrdad *et al.,* 2011). Our opinion is strongly supported by the view of Nwangwu *et al.* (2011) who reported that ethanolic leaf extract of *V. Amygdalina* caused a non-significant reduction in the total protein content of experimental animals. Nevertheless, the observed oscillating minimal non-significant increase in the serum creatinine levels (Table 2) is very disturbing. We are prone to assume among other things, that such may be indicative of the root extract's propensity to negatively impair renal function with increasing dosage and duration of usage. Or at best may be some sort of a compensatory mechanism of the kidney to ameliorate the effect of the ethanolic extract (Medubi *et al.,* 2010).

Similarly, the observed dose and duration dependent significant increase in the BUN level of the treated rats when compared with the control is instructional. Urea equally is the end product of protein metabolism which may be as a result of renal disease, urinary obstructions, shock, congestive heart failure or burns (Sood, 2006). Presently, it is extremely difficult to be categorical on the extract's negative effect on renal function of the treated animals. We can only suspect a gradual tendency of the extract to manifest significantly its harmful renal tendencies (Adebayo *et al.,* 2003). This is because, although the extract showed a dose and duration dependent significant increase, yet the least serum BUN level at the 7th day of the rats administered 100mg/kg (**9.88±0.14mg/dL)** and the highest level at the 14th day of the rats that received 600mg/kg **(15.98±0.17mg/dL)** still fall within the normal BUN serum level (**8 – 25mg**) in humans (Ficker *et al.,* 2003). Nevertheless, caution should be exercised in the prolonged usage of the ethanolic root extract of *V. Amygdalina.* This is more so as several past studies have linked the usage of high doses of plant extract with the possibility of toxic injury to the kidney which may result in acute renal failure (Ijeh and Ukweni, 2007).

5. Conclusion

The trend of the results obtained in the present study tends to suggest that although the ethanolic root extract of *V. amygdalina* may have some hepato-protective property, its nephro-protective ability is still doubtful. Therefore more studies must be carried out on the *V. Amygdalina* root extract using various solvents. This will go a long way to fully ascertain the most beneficial means of harnessing its abundant medical potentials to benefit mankind the more.

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Table 1: The effects of Ethanolic root extracts of *V. amygdalina* on Hepatic enzymes and Bilirubin of normal Albino Rats.

PARAMETERS	Dosage (mg/kg)	Duration (Days)		
		0	7	14
AST (U/L)	Control	9.67±0.78^{a1}*	10.56±0.58^{a1}	10.33±0.37^{a1}
	100	10.11±0.74^{a1}	10.78±0.72^{a1}	10.44±0.44^{a1}
	200	10.11±0.66^{a1}	10.56±0.60^{a1}	10.44±0.38^{a1}
	400	10.56±0.63^{a1}	11.22±0.60^{a1}	11.44±0.44^{a1}
	600	10.00±0.53^{a1}	10.11±0.26^{a1}	10.11±0.39^{a1}
ACP (U/L)	Control	31.89±0.45^{a1}	31.28±0.64^{a1}	31.90±0.62^{a1}
	100	32.49±0.92^{a1}	32.73±0.71^{a1}	33.04±0.70^{a1}
	200	31.31±0.49^{a1}	31.29±0.64^{a1}	32.73±0.50^{a1}
	400	32.20±0.51^{a1}	33.14±0.56^{a1}	33.08±0.65^{a1}
	600	31.89±0.45^{a1}	32.73±0.66^{a1}	32.84±0.38^{a1}
ALT (U/L)	Control	26.00±1.258^{a1}	26.67±0.97^{a1}	26.78±0.85^{a1}
	100	27.67±0.87^{a1}	27.33±0.97^{a1}	29.56±0.84^{a1}
	200	27.22±0.94^{a1}	27.67±1.12^{a1}	28.78±1.06^{a1}
	400	27.33±0.67^{a1}	28.11±0.75^{a1}	29.00±0.58^{a1}
	600	27.67±0.67^{a1}	28.78±0.55^{a1}	29.67±0.37^{a1}
ALP (U/L)	Control	41.38±2.22^{a1}	41.46±2.16^{a1}	41.51±1.91^{a1}
	100	39.84±1.11^{a1}	39.81±1.08^{a1}	40.57±1.02^{a1}
	200	37.83±0.59^{a1}	39.37±0.87^{a1}	38.24±0.80^{a1}
	400	39.03±0.96^{a1}	38.32±0.59^{a1}	39.22±0.52^{a1}
	600	38.82±0.52^{a1}	38.83±0.51^{a1}	39.33±0.83^{a1}
Total Bilirubin (g/dL)	Control	3.34±0.13^{a1}	3.41±0.12^{a1}	3.39±0.10^{a1}
	100	3.45±0.13^{a1}	3.57±0.12 ^{a1}	3.47±0.06^{a1}
	200	3.46±0.12^{a1}	3.46±0.12^{a1}	3.42±0.08^{a1}
	400	3.53±0.07^{a1}	3.61±0.07^{a1}	3.58±0.06^{a1}
	600	3.43±0.09^{a1}	3.50±0.08^{a1}	3.57±0.09^{a1}
Direct Bilirubin (g/dL)	Control	2.37±0.12^{a1}	2.40±0.11^{a1}	2.37±0.09^{a1}
	100	2.17±0.11^{a1}	2.28±0.14^{a1}	2.38±0.11^{a1}
	200	2.06±0.11^{a1}	2.28±0.10^{a1}	2.38±0.06^{a1}
	400	2.14±0.08^{a1}	2.18±0.07^{a1}	2.27±0.07^{a1}
	600	2.14±0.07^{a1}	2.51±0.05^{a1}	2.50±0.05^{a1}

*Values with different alphabetic (lower case) superscripts differ significantly (P<0.05) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly (P<0.05) between different exposure periods within the same concentration. Results are expressed as Mean

Table 2: The effects of Ethanolic root extracts of *V. amygdalina* on Nephrotic enzymes of normal Albino Rats.

PARAMETERS	Concentrations (mg/kg)	Duration (Days) 0	7	14
Creatinine (mg/dL)	Control	39.87±1.89^{a1}*	40.16±1.89^{a1}	38.42±1.28^{a1}
	100	39.87±1.79^{a1}	41.31±1.86^{a1}	40.73±1.56^{a1}
	200	40.16±1.89^{a1}	43.04±1.57^{a2}	42.76±1.44^{a2}
	400	40.16±1.44^{a1}	40.44±1.57^{a1}	41.89±1.59^{a1}
	600	40.42±1.89^{a1}	41.89±1.76^{a1}	42.18±1.55^{a1}
Blood Urea (mg/dL)	Control	7.07±0.16^{a1}	7.24±0.18^{a1}	7.12±0.17^{a1}
	100	6.62±0.21^{a1}	9.88±0.14^{b2}	13.48±0.17^{b3}
	200	6.91±0.19^{a1}	11.08±0.10^{b2}	14.36±0.15^{b3}
	400	7.14±0.17^{a1}	11.32±0.14^{b2}	15.04±0.17^{b3}
	600	6.93±0.17^{a1}	12.47±0.15^{b2}	15.98±0.17^{b3}

*Values with different alphabetic (lower case) superscripts differ significantly ($P<0.05$) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly ($P<0.05$) between different exposure periods within the same concentration. Results are expressed as Mean ± SEM.

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