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RESEARCH ARTICLE

HEPATOTOXICITY OF THE AQUEOUS EXTRACT OF THE ROOTS OF *VERNONIA AMYGDALINA* DELILE (ASTERACEAE) IN RATS.

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Abstract

Conflicting scientific reports have trailed the hepatotoxicity of the roots of *Vernonia amygdalina* in animal studies. The study investigated the toxic effect of the aqueous extract of the roots of *V. amygdalina* on the integrity of the liver cells of adult female albino rats. LD₅₀ was determined using the Lorke's method (>3200 mg/kg, p.o. in 24 h). Biochemical assay of liver enzymes viz. aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and bilirubin (BIL) using graded doses (100, 500, 1000, 1500 mg/kg, p.o.) of the extract demonstrated a significant ($P < 0.05$) and dose dependent increase in these biochemical markers. The weight of the animals progressively decreased over the 14-day period of the experiment. The study, therefore, concluded that *V. amygdalina* possesses a strong hepatotoxic effect, and thus may explain why the roots of the plant are not very common ethnomedically.

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Introduction:-

Vernonia amygdalina Del. (Asteraceae) is a small shrub which is native to the tropical Africa, and typically grows to a height of 2-5 m (Luke *et al.*, 2013). The leaves are elliptical and up to 20 cm long and about 5 mm in diameter. Its bark is rough (Ijeh and Ejike, 2011). It is commonly called bitter leaf due to the bitter taste of the leaves. The leaves of *V. amygdalina* when cooked make a staple vegetable in soups and stews of various cultures (Onabanjo and Oguntona, 2003). The local names include onugbu (Igbo), ewuro (Yoruba), etidot (Ibibio), ityuna (Tiv), oriwo (Edo), shiwaka or chusar-doki (Hausa) and ndole (Cameroons). The use of *V. amygdalina* dates back to the time when veterinary pharmacologists observed that ill chimpanzees with empty stomach sucked pith and juice from the unsavoury *vernonia* plant stalk for self de-parasitization, enhanced body fitness, increased strength or appetite, and reduced constipation, especially during the rainy season (Clay and Wolf, 1993). Experimental studies on *V. amygdalina* have reported that the plant possesses antibacterial activity (Newbold *et al.*, 1997). Aqueous extract of the leaves of *V. amygdalina* has antifungal activity (Ogbebor *et al.*, 2007) while antiparasitic activity was reported by Hakizamungu *et al.* (1992). The leaf and root extracts of *V. amygdalina* possesses activity against the rodent parasite, *P. Berghei* *in vivo* (Abosi and Raseroka, 2003). Ethanol extract of the fruit has antiviral activity (Vlietinck *et al.*, 1995) while methanol extract of the leaves and roots has pesticidal or insecticidal property (Ohigashi *et al.*, 1991b). Other biological activities that have been reported include, anticancer and cytotoxic potential (Izevbigie,

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2003), antifertility effect (Desta, 1994), anticoagulant and antithrombotic activities (Awe *et al.*, 1998), analgesic and antipyretic effect (Iroanya *et al.*, 2010), anti-inflammatory effect (Koko *et al.*, 2008), antioxidant effect (Ayoola *et al.*, 2008), antidiabetic property (Gbolade, 2009) and antidyslipidaemic effect (Adaramoye *et al.*, 2008).

Materials And Methods:-

Research Design:-

The study investigated the acute toxic potential of the aqueous extract of the roots of *Vernonia amygdalina* on the integrity of the liver of adult female albino rats. It also, evaluated the effect of the extract on the weight of the experimental animals over a 14-day period, using different concentrations of the extract of *V. amygdalina*. Mature adult female albino rats (wistar strain) were used for the study, which were randomized into test and control groups, with each group consisting of three (3) rats. The tail of the animals was delineated with permanent markers for easy identification. The control group received distilled water 1 ml/100 g rat while the test groups received graded doses of the extract orally.

Experimental Procedures:-

Plant material:-

The roots of *V. amygdalina* Del. (Asteraceae) were harvested from a homestead farm in the month of January, 2013 at Agbani (a sub-urban town), Nkanu West Local Government Area, Enugu State, Southeastern Nigeria. The plant was identified and authenticated by Mr. C.N. Okoli, a taxonomist with the Department of Biological Sciences, Renaissance University, Ugbo-Awka, Enugu State, Nigeria. It was air-dried at laboratory temperature (33.7 °C) for 6-weeks before it was powdered using a milling machine.

Acclimatization of experimental animals:-

A total of fifteen (15) healthy mature adult female albino rats weighing between 165-170 g were used for the study. The animals were purchased from the animal facility of the Department of Physiology, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria. The animals were housed in galvanised cages under good environmental conditions of 12/12 h light/dark cycle, cross ventilated rooms, with the home cages regularly cleaned. The animals were fed with pelletized commercial diet (Top growers' mash of the Premier Feeds Company Ltd., Enugu, Nigeria) purchased from Ogbette market, Enugu, Southeastern Nigeria, and water was administered *ad libitum*. The guidelines for the care and use of animals for research was strictly adhered to (NIH, 1991; NRC, 1996).

Reagents and Chemicals:-

Assay kits *viz.* alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and bilirubin (BIL), acetic acid, hydrochloric acid, sulphuric acid, methanol, ferric chloride, diethyl ether and distilled water were employed in the study.

Equipment:-

Digital photocolormeter (EI Products, India Model No. 312), centrifuge (Medfield Equipment and Scientific Ltd., China, Model No. 80-2B), test tubes, electrothermal drying oven (Medfield Equipment and Scientific Ltd., China, Model No. DHG-9023 A), water bath (Jiangsu Zhengji Instruments Company Ltd., China, Model No. DK-8A), electric hot plate (Sonik Japan, Model No. SHP 102-D7), gulf electric balance (Gulf Scale, Sharjah, United Arab Emirate, Model No. AF-600) and Ph meter (Medfield Equipment and Scientific Ltd., China, Model No. PHS-25).

Plant extraction:-

300 g of the powdered plant material was subjected to cold extraction in a percolator using 2.5 litres of water for 72 h, with occasional stirring. The extractive (filtrate) was concentrated *in vacuo* in a rotary evaporator at a temperature of 40 °C to prevent the decomposition of constituents of the extractive. The extraction process yielded 38.0 g of dark brown, non-sticky crude extract (12.6 %).

Animals:-

Experimental animals were distributed into five (5) groups (1,2,3,4 and 5) with three (3) animals per cage. They were acclimatized for seven (7) days in the laboratory before the study. Group one (1), the control received only distilled water (1 ml/100 g rat), while groups 2,3,4 and 5 were administered with graded doses of the extract (100, 500, 1000, 1500 mg/kg, p.o.) respectively to healthy mature adult female albino rats once daily for fourteen (14) days. After the 14th day of the experiment, the liver enzymes (ALT, AST, ALP and BIL) assay were performed to determine if the aqueous extract of the roots of *V. amygdalina* is toxic to the liver.

Acute toxicity testing:-

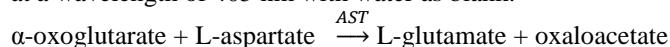
The median lethal dose (LD₅₀) was determined using the Lorke's method (Lorke, 1983).

Phytochemical screening:-

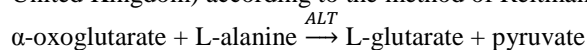
Phytochemical analysis of the roots of *Vernonia amygdalina* will be used to determine the constituents of the extractive using the protocol previously described by Kupcham *et al.* (1969).

Biochemical markers Assay:-**Aspartate transaminase (AST):-**

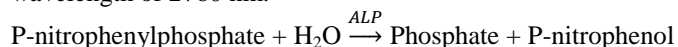
Protocol: The estimation of serum AST activity was performed using the AST test kit (Randox Laboratories Ltd., United Kingdom) purchased from Ogbette market, Enugu, Southeastern Nigeria, using the method previously described by Reitman and Frankel (1957), in which the absorbance of the samples were read on a spectrophotometer at a wavelength of 405 nm with water as blank.

**Alanine transaminase (ALT):-**

Protocol: The determination of the serum ALT activity was done using the ALT test kit (Randox Laboratories Ltd., United Kingdom) according to the method of Reitman and Frankel (1957).

**Alkaline phosphatase (ALP):-**

Protocol: The serum ALP activity was estimated by employing a commercial ALP test kit (Randox Laboratories Ltd., United Kingdom) using the method of Baker and Silvertown (1985), with the absorbance of samples taken at a wavelength of 2760 nm.

**Total bilirubin (BIL):-**

Protocol: The serum BIL was evaluated by the method of Malloy and Evelyn (1937) with minor modification by Tietz (1976), and absorbance of samples read at 578 nm. BIL was estimated using BIL test kit (Randox Laboratories Ltd., United Kingdom) in the presence of caffeine benzoate, which liberates caffeine-bound bilirubin. The BIL reacts with diazotised sulphanilic acid to form a pink coloured azo-bilirubin, which is subsequently converted to a greenish colour in the presence of alkaline tartrate.

Collection of blood Samples:-

The blood samples of the animals were collected through a retro-orbital bleeding while observing caution to avoid haemolysis. The samples were then put into a non-coagulant tubes and spun at 1000 rpm for 10 min in order to separate the red cells from the serum.

Protocol for retro-orbital Bleeding:-

1. Application of digital (right thumb) pressure to the external jugular vein (ejv) caudal to the mandible while gently elevating the upper eyelid with the index finger of the same hand.
2. Insertion of the edge of a broken haematocrit tube into the conjunctiva of the mid-dorsal globe of the eye.
3. Direction of the broken haematocrit tube into the caudo-medial (infero-medial) aspect of the mid-dorsal globe of the eye until blood begins to ooze.
4. As soon as the required volume of blood is obtained, digital pressure on the ejv is released and the broken haematocrit tube removed.

Statistical Analysis:-

All data were expressed as mean \pm standard error of the mean (mean \pm S.E.M.). Analysis of data was performed using the student T-test. The level of statistical significance was accepted at 5 % ($P < 0.05$).

Results:-

Acute toxicity Testing:-

LD₅₀ of the aqueous extract of the roots of *Vernonia amygdalina* was found to be greater than 3200 mg/kg, p.o. in 24 h.

Phytochemical Screening:-

The preliminary phytochemical analysis of the aqueous extract of the roots of *Vernonia amygdalina* revealed the presence of carbohydrates, tannins, glycosides, flavonoids, phlobatannins, steroids, terpenoids and saponins (Kupcham *et al.*, 1969).

Bioassay result of the biomarker liver enzymes (100-1500 mg/kg, p.o.) on the Hepatotoxic effect of *V. amygdalina* in adult female albino rats.

Treatment	AST (μL)	ALT (μL)	ALP (μL)	BIL (μL)
Control	16.00 ± 1.73	8.30 ± 0.81	331.20 ± 47.81	9.25 ± 1.06
100 mg/kg	53.66 ± 2.73*	32.30 ± 12.30*	3121.50 ± 11.89*	18.30 ± 6.58*
500 mg/kg	30.00 ± 2.08*	37.30 ± 6.98*	1230.00 ± 29.26*	19.53 ± 6.98*
1000 mg/kg	56.30 ± 13.24*	45.00 ± 15.35*	1476.60 ± 47.23*	17.10 ± 2.83*
1500 mg/kg	66.53 ± 15.20*	44.00 ± 4.96*	3488.60 ± 159.60*	21.30 ± 2.14*

Discussion:-

The median lethal dose (LD₅₀) (the index of acute toxicity) of the aqueous extract of the roots of *Vernonia amygdalina* was found to be greater than 3200 mg/kg, p.o. in 24 h. The animals progressively decreased in weight over the period of the study, which is an indication of a toxic potential of the plant. Biochemical markers, e.g., alanine transaminase (ALT) formerly called serum glutamate pyruvate transaminase (SGPT), aspartate transaminase (AST) formerly called serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP) and bilirubin (BIL) are often used to indicate liver damage in a patient or animal with some degree of intact hepatic function (McClatchey, 2002; Mengel, 2005 and Ozer *et al.*, 2008).

Earlier study on the stem and root ethanol extracts of *Vernonia amygdalina* has demonstrated that the plant possesses a hepato-protective activity (Luke *et al.*, 2013). Ejere *et al.* (2015) also, reported that the ethanolic root extract of *V. amygdalina* has hepato-protective property. This study, however, has evidently demonstrated that the aqueous root extract of *V. amygdalina* possesses a hepatotoxic activity, and thus disagrees with the previous studies on this common but important nutritional plant.

The different concentrations of the extract demonstrated a marked elevation in the biochemical markers of hepatic injury viz. AST, ALT, ALP and BIL. These enzymes were significantly increased at all the doses of the extract tested (100, 500, 1000, 1500 mg/kg, p.o.), and the pattern of increment in the concentration of these liver enzymes were somewhat similar.

Drug-induced liver injury, including natural products (e.g. medicinal plants) is a cause of acute, sub-acute, chronic and sub-chronic liver disease. The liver plays a cardinal role in the biotransformation and elimination of drugs and herbs, and is liable to toxicity from these substances. Chemicals have been demonstrated to cause clinical (tender hepatomegally, jaundice, oedema and ascites) and pathological (sub-clinical) injury to the liver, which manifests as abnormal (elevated) liver enzymes (Mumoli *et al.*, 2006). Natural products, including medicinal plants can be toxic (Pak *et al.*, 2004; Kim, 2013; Kumar *et al.*, 2013). A multiplicity of cellular and molecular mechanisms are responsible for either inducing or aggravating the damage cascade (Keeffe *et al.*, 2004). First, a host of chemical substances can damage the mitochondria, an intracellular organelle that produces energy. The dysfunction of the mitochondria releases excess amount of oxidants, which in turn, injure the liver cells. Second, the activation of the cytochrome P₄₅₀ family of enzymes, for instance, CYP_{2E1} leads to oxidative stress (Jaeschke *et al.*, 2002). When there is injury to the liver cells, and the cells of the bile duct, an aggravation of hepatic damage ensues (Patel *et al.*, 1998).

Conclusion:-

The study, therefore, concluded that the roots of *V. amygdalina* possesses a strong hepatotoxic effect, and thus may explain why the roots of the plant are not very common ethnomedically. Further toxicological screening is recommended to enrich our knowledge of this common but important edible plant.

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Conflicts of Interests:-

The authors declare that there is no conflicts of interest.

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