Anatomy & Physiology: Current Research
ISSN: 2161-0940

Njoku et al., Anat Physiol 2017, 7:4
DOI: 10.4172/2161-0940.1000277

Research Article Open Access

Evaluation of Concurrent Administration of Leaf Extracts of *Vernonia Amygdalina* and *Gongrenema Latifolium* on some Liver Function Indices in Wistar Rats

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Received Date: June 06, 2016; **Accepted Date:** July 20, 2017; **Published Date:** July 31, 2017

Abstract

The effects of concurrent administration of ethanolic leaf extracts of *Vernonia amygdalina* and *Gongrenema latifolium* on some liver function indices in normal wistar rats. Seventy (70) male wistar rats were used in the study. The animals were divided into seven (7) groups of ten rats each. Group I was given 1 ml of distilled water and served as the control. Groups II and III received 100 mg/kg and 300 mg/kg bw of *Vernonia amygdalina*. Groups IV and V received 300 mg/kg and 600 mg/kg bw of Gongrenema latifolium, while groups VI and VII received 100 mg/kg *V. amygdalina* +300 mg/kg *G. latifolium*, and 300 mg/kg *V. amygdalina* + 600 mg/kg bw *Gongrenema latifolium* respectively orally once daily for a period of 28 days. Five (5) animals from each group were sacrificed after days 14 and 28 respectively, and blood samples collected through cardiac puncture for determination of liver function parameters viz: alanine transferase, aspartate transferase, alkaline transferase and total protein and albumin. The result obtained showed a significant ($p<0.05$) increase in serum alanine transferase (ALT) and aspartate transferase (AST) was observed for high dose of *V. amygdalina* on the day 14 while the serum level of alanine transferase (ALT) increased significantly ($p<0.05$) for the concurrent administered doses on day 28 relative to the control. A significant increase was recorded in the serum of alkaline phosphate for the combined doses. The serum levels of total protein and albumin remained with the reference range after extracts administration. A synergistic significant ($P<0.05$) increase was observed in serum ALP for the combined doses on day 28. This review suggests that the extracts possess possible hepatotoxic potentials particularly at the high dose of *V. amygdalina* and combined doses of the extracts administrated. However, its consumption for various purposes should be done cautiously.

Keywords: Liver function indices; *Vernonia amygdalina*; *Gongrenema latifolium*; Wistar rats

Introduction

Gongronema latifolium is an herbaceous plant that belongs to the family *Asclepiadaceae*. It is called utasi and arokeke in the South Southern and South Western parts of Nigeria respectively. It is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine. It has been traditionally used in the South Eastern part of Nigeria over the years in the management of diseases such as diabetes mellitus and high blood pressure [1]. The result of the phytochemical screening of the ethanolic extract of *G. latifolium* by showed that the root contains polyphenols in abundance while alkaloids, glycosides, and reducing sugars are also present [2,3]. Reported that *G. latifolium* has an antioxidant potential. 4 reported the anti-asthmatic potential of *G. latifolium*. The antiplasmodial activity of *G. latifolium* has been reported [4,5]. This supports the leaf extract of the plant for local treatment of malaria. In the review of it was also reported that *G. latifolium* is used in South Eastern Nigeria to treat various ailments such as cough, loss of appetite, stomach disorders and malaria [5]. According to them, the liquor obtained when the plant is sliced and boiled with lime juice or infused with water for over 3 days is usually taken as a purge for colic and stomach pains.

Reported that the *G. latifolium* plant contains flavonoids, tannins, terpenes, saponins, and alkaloids [6]. The anti-inflammatory property of the leaves of *Gongronema latifolium* has been confirmed [7].

V. *amygdalina* has a variety of names in various languages. *Vernonia amygdalina* is popularly known as bitter leaf because of its characteristic bitter taste. In English, it is referred to as bitter leaf [8,9] "Ewuro" in Yoruba, "Etidot" in Efik, Ijaw and Ibibio. The Igbos and the Etche people of Rivers State call it "Onugbo" or "Olubu". The bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides. Its nutritional, medicinal uses and scientific studies have respectively been articulated in two extensive reviews [10].

Ethnomedicine is an integral part of the culture of people of Odufor people in Etche Local Government of River State. A good number of the people rely on traditional medicine for health care delivery. Majority of the people still patronize herbal remedies despite the availability of orthodox medicine in their management of some diseases and ailments. Besides, polyherbal therapy (practice) is a common practice in the Etche traditional medicine as combination of roots, leaves and stem barks of various plants are often use in the treatment of a single disease. Moreover, polyherbal therapy is said to be a current pharmacological principle having the advantage of producing maximum efficacy with minimum side effects [11]. According to polyherbal therapies have the synergy, potentiative, agonistic and antagonistic pharmacological agents within themselves that work

together in a dynamic way to produce therapeutic efficacy with minimum side effects [12].

Ethnomedicinal information from some traditional medicine practitioners revealed that *V. amygdalina* and *G. gongrenema* constitutes bulk of the polyherbal combinations in the area for various purposes. The liver is concerned with the regulation of a wide variety of biochemical including the breakdown of complex molecules, many of which are central for vital functions.

Liver enzymes catalyze and regulate most biochemical reactions in the human body; from replication of DNA by DNA polymerases to metabolism of xenobiotics [13]. The functional integrity of the liver can be assessed by measuring the serum levels of some biochemical markers: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphate (ALP).

In this study, we investigated the effects of concurrent administration of leaf extracts of *Vernonia amygdalina* and *Gongrenema latifolium* on some liver function indices in normal wistar rats.

Materials and Methods

This study was conducted at the Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Choba, Nigeria; between May to July, 2014.

Collection and identification of plant materials

Fresh leaf samples of *Vernonia amgydalina* and *Gongrenema latifolium* were obtained from a local farm in Umuigwe, Odufor in Etche Local Government Area of Rivers State, Nigeria. It was identified and authenticated by Edwin Wosu, Chief Herbarium, Department of Plant Science and Biotechnology of the University of Port Harcourt.

Preparation of ethanolic leaf extracts of *Gongrenema latifolium*

Fresh leaves of *V. amygdalina* and *G. latifolium* were rinsed in clean water to remove dirt, and dried at room temperature (26°C) for a period of 3 weeks. The dried leaves were milled to fine powder using manuel engine grinder (model: Corene, A. 5 lander YCIA S.A.) and 500 g each of the plants were obtained. The weighed quantities were soaked in 400 ml of ethanol (80% v/v, BDH) for 48 hours. They were then filtered with Whatmann No. 1 filter paper to separate the filtrates from the residue. The extracts were concentrated under reduced pressure in a vacuum at 45°C using a rotary evaporator (Searl Instruments, Ltd., England). The yield of the crude ethanolic extracts of *V. amygdalina* and *G. gongrenema* leaves obtained weighed 63.1 g and 56 g respectively. The extracts were stored in a refrigerator at 4°C before use for the study.

Experimental animals

Seventy (70) male albino wistar weighing (160-270 g) used for this study were kept at the animal house, Department of Human Physiology, College of Health Sciences, University of Port Harcourt, Nigeria. The animals' were kept in a spacious and well ventilated cage at room temperature of about 28°C ± 1°C. They were allowed to acclimatize for 14 days and allowed free access to feed and water ad libitum. The ethical regulations in accordance with National and Institutional guidelines for the protection of animals' welfare were strictly adhered to during the experiments.

Experimental design

The seventy (70) rats were randomly distributed into seven (7) groups (I-VII) of 10 animals each. Group I served as control and received water and normal feed. The test groups (II-VII) received various doses of ethanolic leaf extracts as follows:

Group II: Received 100 mg/kg of *V. amygdalina*.

Group III: Received 300 mg/kg of *V. amygdalina*.

Group IV: 300 mg/kg of *G. gongrenema*.

Group V: 600 mg/kg of *G. gongrenema*.

Group VI: Received 100 mg/kg of *V. amygdalina* plus 300 mg/kg of *G. gongrenema*.

Group VII: Received 300 mg/kg of *V. amygdalina* and 600 mg/kg of *G. gongrenema.*

The extracts were administered to the animals by oral gavage, once daily (9am-10am) for 14 and 28 days respectively. The various doses of the two extracts were based on previous studies showing that *Vernonia amygdalina* leaf extract has LD50 of 500 mg/kg [9], while *Gongrenema latifolium* has LD50 of 1050 mg/kg [14]. After the 14^{th} and 28^{th} days of extract administration, five animals were sacrificed from each group on days 15 and 29 of the study. Body weights of the animals were measured before commencement of extract administration, and before sacrificing the animals.

Blood sample was collected by cardiac puncture into lithium heparin bottles for evaluation of some liver function parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline transferase (ALP); total protein and albumin using commercial kits from Randox Laboratories, UK; and a Mindray auto-analyzer (Model: BS 800M) in the Department of Chemical Pathology, University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Nigeria.

Statistical Analysis

Data were expressed as Mean ± SEM and data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0; one way analysis of variance (ANOVA). Students't-test was used to assess significant difference between the animals' body weight. Differences were considered significant at p value less than 0.05.

Results

Effects of concurrent administration of *V. amygdalina* and *G. latifolium* on body weight

The percentage body weight changes of the rats in each group before and after extract administration was determined. From the result in Figure 1, there was a non-significant percentage change in the body weight of animals on day 0.

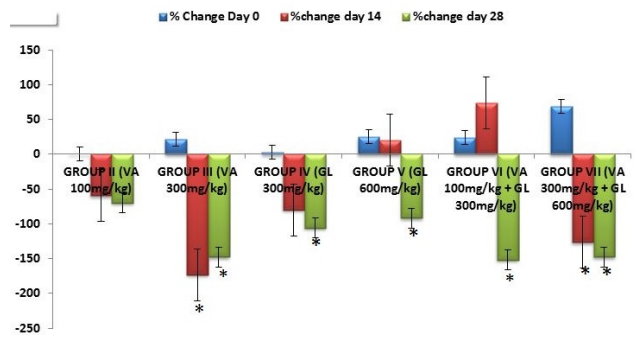

Figure 1: Percentage (%) change in animals' body weight after the administration of extracts. N/B: *Significant at P<0.05.

A significant (p<0.05) percentage reduction was observed in the body weight of animals administered with high dose of *V. amygdalina* and combined doses of the extracts on day 14 relative to control.

Following 28 days of extracts administration, a significant (p<0.05) percentage reduction in body weight of animals was recorded when compared.

Effects of concurrent administration of *V. amygdalina* and *G. latifolium* on liver enzymes

Following administration of ethanolic leaf extracts of V. amygdalina and G. latifolium. There was a significant (p<0.05) increase in serum alanine transferase (ALT) for high dose of *V. amygdalina* on day 14 relative to the control (Figure 2). However, a non - significant increase was recorded for other administered groups on day 14.

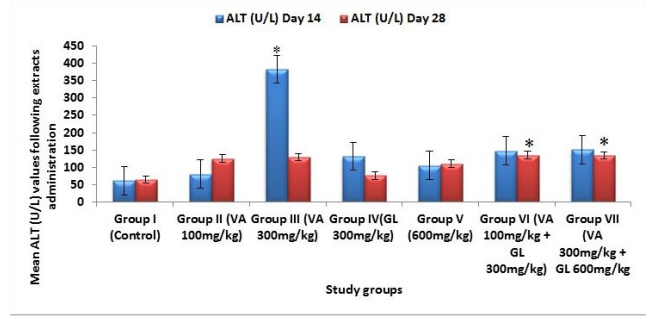

Figure 2: Mean ALT (U/L) values following administration of ethanolic leaf extracts of *V. amygdalina* and *G. latifolium*. Values are expressed as mean ± SEM, n=5, *Significant at p<0.05.

A significant (p<0.05) increase in serum alanine transferase (ALT) was observed for the concurrent compared administered doses on day 28 when with the control (Figure 2). The serum levels of ALT increased in the singly administered doses of the extracts, but were not significantly impacted when compared with the control.

This study observed a significant (p<0.05) increase in serum aspartate aminotransferase (AST) for high dose of *V. amygdalina* on the day 14 when compared (Figure 2). The serum levels of aspartate aminotransferase (AST) at low dose of V. *amygdalina* and the concurrently administrated doses of the extracts decreased non-significantly on the day 14. However, a dose dependent non-significant increase was recorded for the low and high doses of *G. latifolium* on the day 14 when compared (Figure 2). Following 28 days of extracts administration, a non-significant increase was observed in the serum aspartate aminotransferase (AST) for the low dose of *V. amygdalina* (Figure 2). The high dose of *V. amygdalina*, low and high doses of *G. latifolium* and the concurrent administered doses of the extracts showed a non-significant decrease in serum aspartate aminotransferase (AST) when compared with the control (Figure 3).

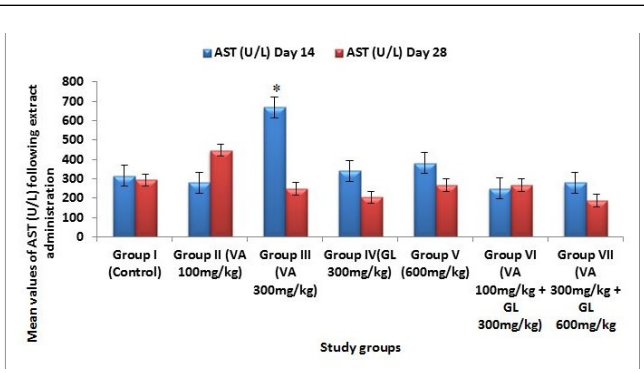

Figure 3: Mean AST (U/L) values following administration of ethanolic leaf extracts of *V. amygdalina* and *G. latifolium*. Values are expressed as mean ± SEM, n=5, *Significant at p<0.05.

There was a non-significant reduction in serum alkaline phosphatase (ALP) for the high dose of *G. latifolium* on day 14; however, this differed from the non-significant increase observed in the serum levels of alkaline phosphatase (ALP) for the low dose of *G. latifolium*, low and high doses of *V. amygdalina* and the concurrent administered doses when compared (Figure 4).

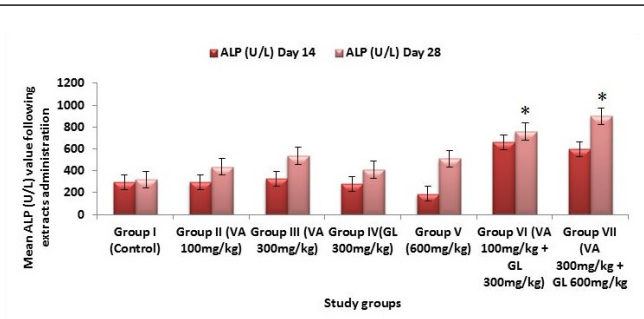

Figure 4: Mean ALP (U/L) values following administration of ethanolic leaf extracts of *V. amygdalina* and *G. latifolium*. Values are expressed as mean ± SEM, n=5, *Significant at p<0.05.

A significant (p<0.05) increase in serum alkaline phosphatase (ALP) for the concurrent administered doses of the extracts on day 28 was recorded when compared with the control (Figure 4). A non-significant increase in serum alkaline phosphatase (ALP) was observed for the single administered doses of the extracts (Figure 4).

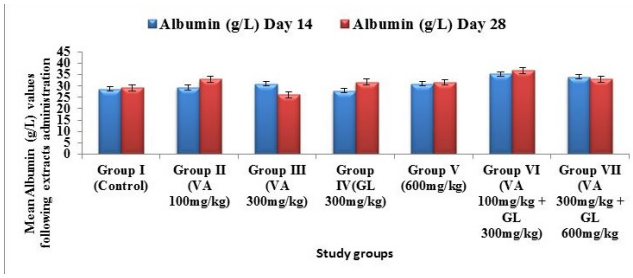

Figure 5: Mean Albumin (g/L) values following administration of ethanolic leaf extracts of *V. amygdalina* and *G. latifolium*. Values are expressed as mean ± SEM, n=5.

Following the administration of ethanolic leaf extracts of *V. amygdalina* and *G. latifolium*, there were no-significant changes in the serum levels of albumin and total protein for the administered doses on day 14 and 28 (Figures 5 and 6) respectively.

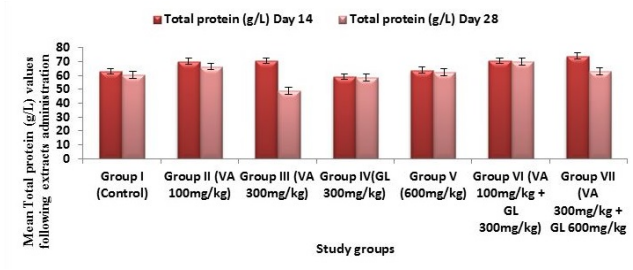

Figure 6: Mean Total protein (g/L) values following administration of ethanolic leaf extracts of *V. amygdalina* and *G. latifolium*. Values are expressed as mean ± SEM, n=5.

Effects of concurrent administration of *V. amygdalina* and *G. latifolium* on liver histology

The Photomicrographs of liver of animals administered with *V. amygdalina* and *G. latifolium* (GL) for 14 and 28 days are shown in Figure 7. The histology of the doses administered for *V. amygdalina* and concurrently administered doses showed cytoplasmic degeneration and hepatocytes distortion especially at high dose of *V. amygdalina* when compared to the control which showed normal hepatic histological features. This however, differed from the effect of the *G. latifolium* administered doses, which showed normal hepatic integrity with fatty change consistent with the control.

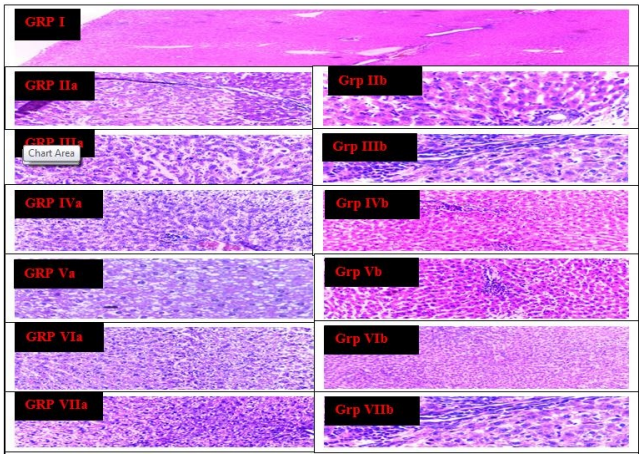

Figure 7: Photomicrographs of liver of rats administered with *V. amygdalina* and *G. latifolium* (GL) for 14 and 28 days (H&E X400). Group I (control groups): Shows normal liver histology. Group IIa and Ib (VA 100 mg/kg): Administered group for days 14 and 28 respectively: Shows slight cytoplasmic degeneration and hepatocytes distortion. Group IIIa and IIIb (VA 300 mg/kg): Administered group for days 14 and 28 respectively: Shows slight hepatic intraparenchymal inflammation. Group IVa and IVb (GL 300 mg/kg): Administered group for days 14 and 28 respectively: Shows normal hepatic intergrity with fatty change. Group Va and Vb (GL 600 mg/kg): Administered group for days 14 and 28 respectively: Shows hepatic intraparenchymal inflammation. Group VIa and VIb (VA 100 mg/kg + GL 300 mg/kg): Administered group for days 14 and 28 respectively: Shows severe hepatic intraparenchymal inflammation. Group VIIa and VIIb (VA 300 mg/kg + GL 600 mg/kg): Administered group for days 14 and 28 respectively: Shows severe hepatic intraparenchymal inflammation.

Discussion

This study was conducted to determine the effects of ethanolic leaf extract of *Vernonia amygdalina* and *Gongrenema latifolium* on some liver function indices in normal wistar rats. Changes in animals' body weight after extract administration have been used as a valuable index of organ damage [15] and thus, used in this study to justify the effects of the plant on the body weights of studied animals. Following extracts administration, a significant reduction in animals' weight was observed, especially at high dose of *V. amygdalina* and combined doses of the extracts. The effect of the extracts on body weight may be due to the interference with metabolic processes and absorption or inflammatory effects on hepatocytes, which may have led to the reduction in weight observed especially at higher concentration.

The liver maintains and regulates homeostasis in living systems. It is involved in some biochemical pathways which are necessary for growth and fight against diseases. It also supplies nutrients and energy. Therefore, maintenance of a healthy liver is essential for the overall wellbeing of an individual [16,17]. The changes in enzyme activities observed in these experimental animals are possible steps of determining the toxicity of these leaf extracts. Changes observed in biochemical processes of experimental animals are as a result of increase or decrease in some enzyme activities such as alanine

aminotransferase (ALT), aspartate aminotransferase (AST) are present in the hepatic and biliary cells [18]. Alanine aminotransferase (ALT) is a specific marker for hepatic damage while aspartate aminotransferase (AST) is not a good indicator for liver dysfunction and is also secreted in by cardiac, skeletal, hepatic and renal tissues [19]. These enzymes are usually released from the hepatocytes and leak into circulation causing in their serum levels under hepatocellular injury.

Observation from this study indicates that the extracts seem to alter significantly the levels of serum ALT and AST activity especially at high dose of *V. amygdalina* (Figures 2 and 3). This indicates a possible hepatotoxic tendency of the extracts. This may be due to the effect of their bioactive (saponin) components on the membrane of hepatocytes [20]. Saponins seem to possess membrane permeating activity due to their interaction with the hydrophobic moieties combined with cholesterol component of the hepatic membrane [21,22]. A non-significant change was observed in serum level of liver enzymes for the low dose of *G. latifolium*. This might be attributed to anti-inflammatory action of flavonoids observed in these extracts. This is consistent with the report of [17].

The results obtained from this study indicate that the concurrent or combined administration of *Vernonia amygdalina* and *Gongrenema latifolium* showed a great significant increase in serum level of ALP on day 28.

This significant effect is possibly due to synergism usually associated with bioactive compounds from medicinal plants and other agents over a prolonged period of time [22]. The great significant increase in serum alkaline aminotransferase (ALT) obtained from the combined doses of the extracts was expected, since the single administration of the extracts showed non-significant increases of the enzyme. Although, we do not understand for now, how this synergism was achieved; it is plausible that both extracts may have produced a long lasting permeability of hepatic cell membranes and inhibition of antioxidants components (flavonoids) of the extracts. This is however, a subject for further research.

Conclusion

From the foregoing, it can be concluded that leaf extracts of *V. amygdalina* and *G. latifolium* at the studied doses may possess hepatotoxic potentials particularly at the high dose of *Vernonia amygdalina* and combined doses of the extracts administered. However, its consumption for various purposes should be done cautiously.

Acknowledgement

The authors appreciate the technical assistance of Ojeka, Sunday Ogbu of the Department of Human Physiology and Pharmacology respectively, College of Health Sciences, University of Port Harcourt, Nigeria for their immense technical assistance.

Competing Interest

The authors have declared that no completing interests exist.

Author's Contribution

Author CCPR conceived the study, designed the protocol and coordinated the experiment. The animal feeding, laboratory procedures, performed statistical analysis and manuscript writing were carried out by author NB while authors OMO and AWC carried out data interpretation, contributed in the manuscript writing. All authors read through and approved the final manuscript.

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