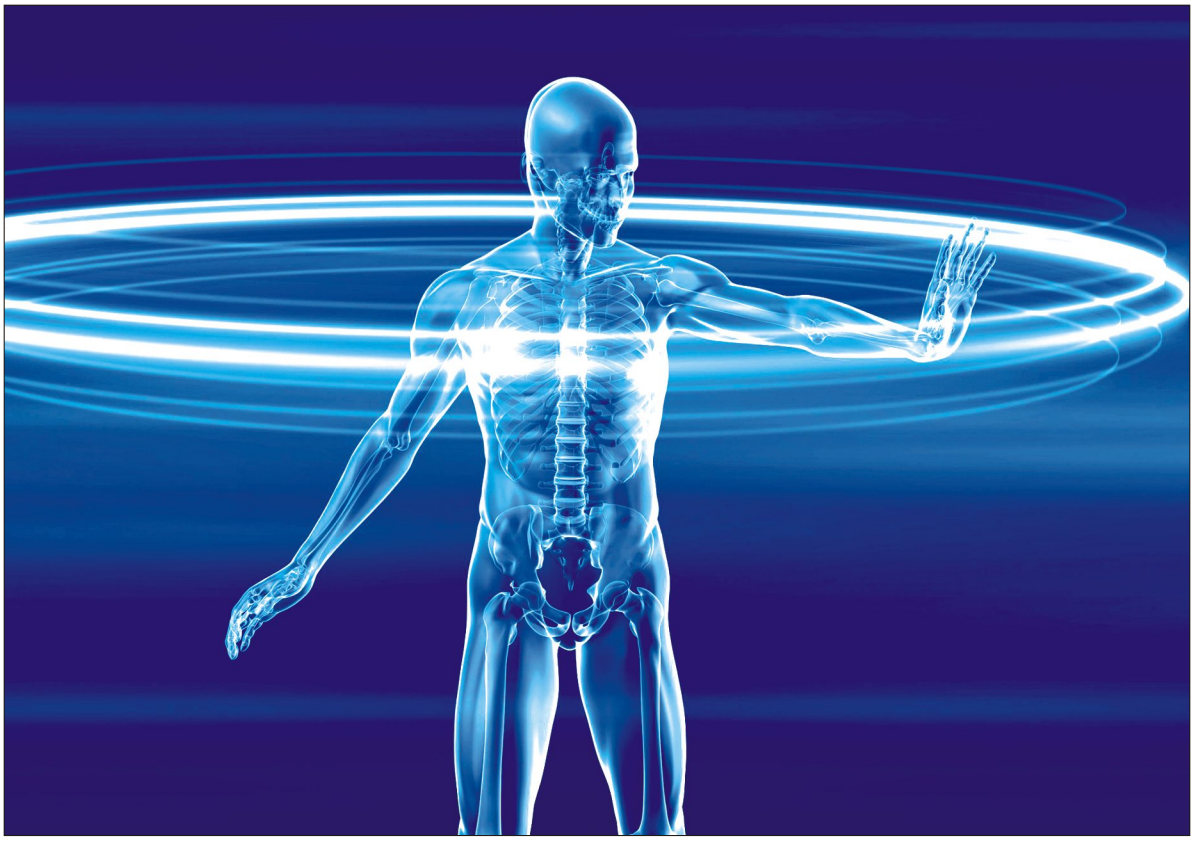

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Research Article

Comparative Studies on the Responses of Some Biochemical Markers in Immuno-Suppressed Rats Treated with *Gongronema latifolium* and *Vernonia amygdalina* Ethanol Leaf Extract

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Abstract

Background and Objective: *Gongronema latifolium* and *Vernonia amygdalina* are medicinal plants that have been used in the treatment of nausea, hypertension, diabetes, stomach ache, high cholesterol level etc. However this research was set out to access comparatively the immuno-stimulatory effect of ethanol leaf extract of *Gongronema latifolium* and *Vernonia amygdalina* in cyclophosphamide immuno-suppressed rats. **Materials and Methods:** Thirty Wistar albino rats of 5 rats per group were used for this study and were divided into 6 groups. Group 1 rats were normal control; group 2 (positive control) rats were administered cyclophosphamide only, at the dose of 50 mg kg^{-1} b.wt. i.p. Group 3 rats were administered low dose (400 mg kg^{-1} b.wt.) of the *Vernonia amygdalina* leaf extract after cyclophosphamide induction while group 4 rats were administered high dose (600 mg kg^{-1} b.wt.) of the extract after cyclophosphamide induction. Group 5 rats were administered low dose (400 mg kg^{-1} b.wt.) of *Gongronema latifolium* leaf extract after cyclophosphamide induction while group 6 rats were administered high dose (600 mg kg^{-1} b.wt.) of the extract after cyclophosphamide induction. The results were analyzed using one way analysis of variance (ANOVA) using SPSS version 20. **Results:** Treatment with *Vernonia amygdalina* and *Gongronema latifolium* leaf extract (group 3, 4 and group 5, 6 respectively) showed a significant increase ($p<0.05$) in SOD activity, glutathione and vitamin C concentrations when compared with group 2 rats treated with cyclophosphamide only. More so treatment with *Vernonia amygdalina* and *Gongronema latifolium* leaf extract (group 3, 4 and group 5, 6 respectively) showed a significant decrease ($p<0.05$) in the liver marker enzymes (ALT, AST and ALP) when compared to group 2 rats treated with cyclophosphamide only, however group 2 rats showed a significant decrease ($p<0.05$) in total protein concentration and a significant increase ($p<0.05$) in the total cholesterol concentration and triacylglycerol concentration when compared to the treatment groups. **Conclusion:** Comparatively, these findings indicate that *Vernonia amygdalina* may have better antioxidant properties and also exhibited more potency in inhibiting liver damage by lowering the liver marker enzymes compared to *Gongronema latifolia*.

Key words: *Gongronema latifolium*, *Vernonia amygdalina*, cyclophosphamide, immuno-suppressed, enzymes, antioxidants, cholesterol

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and have remains relevant in both developing and the developed nations of the world for various chemotherapeutic purposes[1]. It has been medically proven that the rate of consumption of vegetable is concurrent with the state of health of an individual. This has been clearly observed among people in Asia and some part of Africa. In South-Eastern Nigeria, the inhabitants are known for high consumption of vegetables. These vegetables are not only eaten as food but also during ill health and times of convalescence[2]. Thus they are referred to as herbal medicine.

The increasing use of herbs in the treatment of diseases in developing countries has been proven to be due to their historical and cultural significance[3]. However, this also suggests that aside from culture, other factors such as poor governance, drug resistance, poverty, emergence and re-emergence of infectious diseases, have exacerbated the interest in ethno-pharmacological research. Also these plants contain secondary metabolites that influence biological processes and reverse disease states and unlike some conventional drugs, they seem to have lesser side effects and are also inexpensive[2]. For this reason and because they are also available they are taken by the majority of the population[4]. Among the numerous herbal plants used in traditional medicine *Gongronema latifolium* and *Vernonia amygdalina* have been well investigated. These plants have been reported to contain alkaloids, carbohydrate, tannins, saponins, flavonoids and non-cyanogenic glycosides[5]. Their extracts have been identified with certain properties such as anti-bacterial, anti-fungal, anti-microbial and antioxidant.

Cyclophosphamide is a potent immuno-suppressive agent, capable of inhibiting both humoral and cell mediated immune responses[6]. The main use of cyclophosphamide is together with other chemotherapy agents in the treatment of lymphomas, some forms of brain cancer, leukemia and some solid tumors[7]. It is a chemotherapy drug that works by slowing or stopping cell growth. In animal models, this cytotoxic agent can suppress graft-vs-host disease, prevent development of certain auto-allergic conditions and promote tolerance to certain antigens. Due to this activity, cyclophosphamide is being evaluated in treatment of certain human disorders believed to result from aberrant immunity and as an agent useful in preparing recipients for marrow transplant[6]. A previous study showed that the biological actions of cyclophosphamide are dose-dependent. At higher doses, cyclophosphamide undergoes biotransformatio by hepatic microsomal cytochrome P_{450} mixed function oxidase system to metabolites that enter the circulatory system[8]. Phosphoramide mustard and acrolein are the two active metabolites. The cytotoxic effects such as necrosis, cell death and oncosis are associated with acrolein, while the antineoplastic activity of cyclophosphamide is linked with phosphoramide[9].

Both immuno-suppressants and stimulants have serious side effects ranging from inflammatory disorders, hypersensitivities, immunodeficiency disorders etc; thus complementary or traditional remedies are other options to overcome this problem[10]. However the search for immune-stimulants from natural source with little or no side effect continues. Thus this project is a comparative study on the antioxidant properties and Immuno-stimulatory effect of *Gongronema latifolium* and *Vernonia amydalina* ethanol extract on cyclophosphamide immuno-suppressed rats. The results of this research will give a clue on immunological abilities of the different plants and give an insight on their mode of actions.

MATERIALS AND METHODS

Plant materials: Fresh leaves of *Gongronema latifolium* and *Vernonia amydalina* were purchased from Ogige market in Nsukka, Enugu State of Nigeria on the 10th day of June 2015 and were identified by Mr. Alfred Ozioko of the Herbarium Botany Department, University of Nigeria, Nsukka.

Extraction of the leaves of *Gongronema latifolium* and *Vernoniaamydalina*: The leaves of *Gongronema latifolium* and *Vernonia amydalina* were air-dried separately at room temperature, then grinded into powdery form using electrical grinding machine. The ground samples were extracted with 95% ethanol solution, using cold maceration techniques. The samples were filtered using Whatman filter paper No. 1. The filtrates concentrated to solid matter using rotary evaporator at the temperature of 45°C. The extracts were stored in the refrigerator until further use.

Animals: Adult male Wistar albino rats of 10-16 weeks old and average weight of 160±15 g were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were acclimatised for duration of 7 days under standard environmental conditions with a 12 h light/dark cycle maintained on a regular feed (vital feed) and water *ad libitum*. Clearance and approval for the humane use and handling of laboratory animals were given by the ethical committee of Department of Biochemistry, University of Nigeria, Nsukka which is in accordance to CIOMS[11].

Drug (cyclophosphamide): Cyclophosphamide (potent immuno-suppressant) was purchased from Elophez Pharmacy, Nsukka in Enugu State of Nigeria.

Administration of cyclophosphamide: The rats were administered intraperitoneally with a dose of 50 mg kg^{-1} b.wt., of cyclophosphamide (potent immuno-suppressant).

Chemicals/reagents/samples: All chemicals used in this study were of the analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstadt, Germany. Reagents used for all the assays were commercial kits and products of Randox, USA; QCA, Spain; Teco (TC), USA; Biosystem Reagents and Instruments, Spain.

Experimental design: Twenty male albino Wistar rats were acclimatized at the same conditions of temperature and pressure and the same animal feeds were used for all the rats. The rats were divided into 6 groups of 5 rats each as shown below:

Group 1: Normal/negative control that were treated with normal saline only

Group 2: Positive control received cyclophosphamide intra-peritoneally at the dose of 50 mg kg^{-1} b.wt.

Group 3: Test group received cyclophosphamide intra-peritoneally first then a low dose (400 mg kg^{-1} b.wt.) of *Vernonia amydalina* ethanol extract

Group 4: Test group received cyclophosphamide intra-peritoneally first then a high dose (600 mg kg^{-1} b.wt.) of *Vernonia amydalina* ethanol extract

Group 5: Test group recieved cyclophosphamide intra-peritoneally first then a low dose (400 mg kg^{-1} b.wt.) of *Gongronema latifolium* ethanol extract

Group 6: Test group received cyclophosphamide intra-peritoneally first then a high dose (600 mg kg^{-1} b.wt.) of *Gongronema latifolium* ethanol extract

Biochemical investigation: The animals were treated for 7 days and on the 8 day the animals were sacrificed and blood was collected and used for biochemical analysis.

Assay of aspartate aminotransferase activity: A randox commercial enzyme kit according to the method of Reitman and Frankel[12] was used in the study.

Assay of alanine aminotransferase activity: A randox commercial enzyme kit based on the method of Reitman and Frankel[12] was used.

Assay of alkaline phosphatase activity: This was done using the QCA commercial enzyme kit which is based on the phenolphthalein monophosphate method of Klein *et al.*[13].

Direct and total bilirubin: Direct and total bilirubin were measured using the method of Jendrasskik and Grof[14].

Assay of superoxide dismutase activity: Superoxide dismutase (SOD) activity was assayed using the method as described by Fridovich[15] as contained in Randox commercial kit.

Assay of catalase activity: Catalase activity was assayed using the method of Aebi[16].

Determination of glutathione concentration: The concentration of glutathione was determined according to the method of Habig *et al.*[17].

Determination of vitamin C: The concentration vitamin C (ascorbic acid) was determined according to the method of Baker *et al.*[18].

Determination of total cholesterol concentration: The concentration of total cholesterol was determined according to the method of Allain *et al.*[19] as outlined in the QCA test kit.

Determination of triacylglycerol concentration (randox enzyme kit): The concentration of triacylglycerol was determined according to the method of Trinder,[20] as outlined in the QCA test kit.

Determination of total protein concentration: The determination of protein concentration was carried out according to the method of Slater[21].

Statistical analysis: Data obtain was expressed as Mean ± SD, analysis for significance of disparity using a one-way analysis of variance (ANOVA). The Statistical Product and Service Solution (SPSS), version 20 was used for the analysis where $p < 0.05$ was regarded as significant[22].

RESULTS AND DISCUSSION

In the present study Table 1 and 2 show that administration of cyclophosphamide cause a significant

Table 1: Effect of *Vernonia amygdalina* and *Gongronema latifolium* ethanolic leaf extracts on aspartate aminotransferarse and alanine aminotransferarse activity in immunosuppressed Wistar albino rats

Treatment groups	AST (IU L^{-1})	ALT (IU L^{-1})
Group 1 = Normal/negative control	51.00±4.45*	35.00±2.72*
Group 2 = Positive control	95.00±3.76	65.00±4.65
Group 3 = 400 mg kg^{-1} b.wt., of V.A. extract	59.00±4.74*	40.00±3.65*
Group 4 = 600 mg kg^{-1} b.wt., of V.A. extract	53.00±2.67*	37.00±3.89*
Group 5 = 400 mg kg^{-1} b.wt., of G.L. extract	65.00±3.96*	49.00±4.49*
Group 6 = 600 mg kg^{-1} b.wt., of G.L. extract	62.00±3.50*	45.00±3.76*

Values are expressed as Mean±SEM; *p<0.05 is considered significant when compared with positive control group using one-way analysis of variance, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Table 2: Effect of *Vernonia amygdalina* and *Gongronema latifolium* ethanolic leaf extracts on alkaline phosphatase activity in immunosuppressed Wistar albino rats

Treatment groups	ALP (IU L^{-1})
Groups 1 = Normal/negative control	75.00±3.50*
Groups 2 = Positive control	130.00±2.67
Groups 3 = 400 mg kg^{-1} b.wt., of V.A. extract	82.00±3.43*
Groups 4 = 600 mg kg^{-1} b.wt., of V.A. extract	78.00±4.60*
Groups 5 = 400 mg kg^{-1} b.wt., of G.L. extract	90.00±3.98*
Groups 6 = 600 mg kg^{-1} b.wt., of G.L. extract	84.00±3.45*

Values are expressed as Mean±SEM, *p<0.05 is considered significant when compared with positive control group using one-way analysis of variance, ALP: Alkaline phosphatase

increase (p<0.05) in the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) as shown in group 2 when compared to the normal control and this suggests possible case of necrosis or hepatocellular injury in the rats[23]. However treatment with treatment with *Vernonia amygdalina* and *Gongronema latifolia* leaf extracts (group 3, 4 and group 5, 6 respectively) showed a significant decrease (p<0.05) in the liver marker enzymes (ALT, AST and ALP) when compared to group 2 rats treated with cyclophosphamide only. The significant decrease (p<0.05) in the aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities of the rats treated with different doses (400 and 600 mg kg^{-1} b.wt.) of *Vernonia amygdalina* and *Gongronema latifolium* ethanol leaf extracts when compared to the group 2 animals indicates no form of myocardial infarction or viral hepatitis in the rats[24]. This is consistent with the works of Iweala *et al*.[25] who studied the biochemical effects of leaf extracts of *Gongronema latifolium* in alloxan induced diabetic rats. However, *Vernonia amygdalina* leaf extract showed a higher potency when compared to *Gongronema latifolium* leaf extract.

From Table 3 it was observed that intra-peritoneal administration of cyclophosphamide caused a significant increase (p<0.05) in the concentration of direct and total bilirubin when compared to the normal control rats which is an indication of hyper-bilirubinemia might be as a result of an increase in breakdown of haemoglobin by the free radicals generated by acrolein or damage of the biliary duct. Treatment with different doses (400 and 600 mg kg^{-1} b.wt.) of *Vernonia amygdalina* and *Gongronema latifolium* leaf extracts showed a significant decrease (p<0.05) in both total and direct bilirubin concentrations. This suggests an effective control of bilirubin concentration which points towards an improved secretory mechanism of the hepatic cells by the extract. This study is in tandem with the works of Parker *et al*.,[26] who demonstrated effective control of bilirubin by an alkaloid-rich fraction of an extract.

Administration of cyclophosphamide alone gave a significant decrease (p<0.05) in SOD, catalase, glutathione and vitamin C concentration of group 2 rats when compared with the normal control group 1 and other test groups as shown in Table 4 and 5. This might be because the activity of the metabolite of cyclophosphamide, acrolein, leads to the generation of ROS. These cause oxidative stress that may lead to the reduction or inactivation of these antioxidants. The oxidative stress also leads to the disruption of pro-oxidant and antioxidant balance as a result of an increase in Reactive Oxygen Species (ROS) generation, impairment of anti-oxidant defence systems or an insufficient capacity to repair oxidative damage[27]. This decrease observed in these antioxidants level is consistent with the results of Ismail *et al*.[28]. A study by Kedzierska *et al*.[29] affirmed that immune-suppressants lead to generation of Reactive Oxygen Species (ROS) which cause oxidative stress. More so if due to decrease in SOD activity, hydrogen peroxide is produced in large amounts, catalase also takes part in its degradation. Also vitamin C an antioxidant nutrient plays an important role in removing ROS. Furthermore, a study by Rajasekaram and Kalaivani[30], confirmed that oxidant stress can be marked by LPO, SOD, GSH and catalase levels. While LPO increases in oxidative stress, GSH is reduced and SOD and catalase are inactivated.

The results also showed that treatments with the ethanol leaf extract of both *Vernonia amygdalina* and *Gongronema latifolium* gave significant increase (p<0.05) in SOD and

Table 3: Effect of vernonia amygdalina and *Gongronema latifolium* ethanolic leaf extracts on direct and total bilirubin concentrations in immunosuppressed wistar albino rats

Treatment groups	D. BIL (mg dL^{-1})	T. BIL (mg dL^{-1})
Groups 1 = Normal/negative control	0.56±0.04*	0.75±0.06*
Groups 2 = Positive control	1.72±0.09	2.78±0.09
Groups 3 = 400 mg kg^{-1} b.wt., of V.A. extract	0.42±0.02*	1.40±0.08*
Groups 4 = 600 mg kg^{-1} b.wt., of V.A. extract	0.42±0.08*	1.30±0.09*
Groups 5 = 400 mg kg^{-1} b.wt., of G.L. extract	1.12±0.07*	2.14±0.07*
Groups 6 = 600 mg kg^{-1} b.wt., of G.L. extract	1.23±0.03*	2.21±0.04*

Values are expressed as Mean±SEM, *p<0.05 is considered significant when compared with positive control group using one-way analysis of variance

Table 4: Effect of *Vernonia amygdalina* and *Gongronema latifolium* ethanolic leaf extracts on superoxide dismutase and catalase activity in immunosuppressed Wistar albino rats

Treatment groups	Catalase (IU L^{-1})	SOD (IU L^{-1})
Groups 1 = Normal/negative control	8.63±0.98*	52.86±3.28*
Groups 2 = Positive control	3.79±0.50	20.28±5.50
Groups 3 = 400 mg kg^{-1} b.wt., of V.A. extract	6.70±0.33*	49.22±4.02*
Groups 4 = 600 mg kg^{-1} b.wt., of V.A. extract	7.75±0.40*	50.49±3.58*
Groups 5 = 400 mg kg^{-1} b.wt., of G.L. extract	7.20±0.50*	39.96±4.34*
Groups 6 = 600 mg kg^{-1} b.wt., of G.L. extract	7.50±0.70*	43.59±3.40*

Values are expressed as Mean±SEM, *p<0.05 is considered significant when compared with positive control group using one-way analysis of variance, SOD: Superoxide dismutase

Table 5: Effect of *Vernonia amygdalina* and *Gongronema latifolium* ethanolic leaf extracts on glutathione and vitamin C concentrations in immunosuppressed Wistar albino rats

Treatment groups	GSI (mg dL^{-1})	VIT C (mg dL^{-1})
Groups 1 = Normal/negative control	8.46±0.29*	4.03±0.33*
Groups 2 = Positive control	2.33±0.33	1.50±0.05
Groups 3 = 400 mg kg^{-1} b.wt., of V.A. extract	5.00±0.80*	5.33±0.17*
Groups 4 = 600 mg kg^{-1} b.wt., of V.A. extract	5.33±0.57*	6.00±0.33*
Groups 5 = 400 mg kg^{-1} b.wt., of G.L. extract	4.00±0.33*	2.96±0.14*
Groups 6 = 600 mg kg^{-1} b.wt., of G.L. extract	4.33±0.57*	3.03±0.12*

Values are expressed as Mean±SEM; *p<0.05 is considered significant when compared with positive control group using one-way analysis of variance

catalase activities and glutathione and vitamin C concentrations. This may be due to the antioxidants possessed by the plants extract. These antioxidants such as vitamin C, vitamin E, flavonoids, tannins, polyphenols, terpenes, phenolic acids and saponins mop up reactive oxygen species in the body. Studies by Nwanjo *et al.*[31] and Ayoola *et al.*[32] confirmed the presence of these antioxidant phytochemicals in these plants extract. A study by Buher and Miranda[33] confirmed that flavonoids may help provide protection against diseases by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defence system of the human body. In addition, it was observed that *Vernonia amygdalina* treatments (group 3 and 4) show higher recovery in SOD activity, GSH and vitamin C concentrations compared to *Gongronema latifolium* treatments (group 5 and 6) while both plants show similar catalase activity recovery. This shows that *Vernonia amygdalina* might have better antioxidant property. This may be because *Vernonia amygdalina* has higher antioxidant content than *Gongronema latifolium.* A study by Farombi and Owoeye[34] confirmed that in phytochemical screening, *Vernonia amygdalina* tested positive for most phytochemicals. They stressed that the presence of flavonoids and tannins in the plant is likely to be responsible for the free radical scavenging effects observed.

Also a study by Farombi and Owoeye[34] showed that unlike *Gongronema latifolium, Vernonia amygdalina* contains phenolic acids. These phenolic acids, example ferrulic acid, have similar structures to flavonoids and tannins and thus contribute to their antioxidant property.

From Table 6, the results showed a significant increase ($p<0.05$) in the total cholesterol levels and triacylglycerol levels of group 2 rats that received only cyclophosphamide when compared with the normal group (group 1). The liver is central to the regulation of cholesterol levels in the body, not only does it synthesize cholesterol for export to other cells but it also removes cholesterol from the body by converting it to bile salts and excreting it into the bile. Levels of triglyceride in the serum or liver could increase due to several processes, including increased availability of free fatty acid, glycerol-phosphate, decreased VLDL in the serum and decreased removal of triglyceride and cholesterol from serum due to diminished lipoprotein activity[35]. However, treatment

Table 6: Effect of *Vernonia amygdalina* and *Gongronema latifolium* ethanolic leaf extracts on total cholesterol and of triacylglycerol concentrations in immunosuppressed Wistar albino rats

Treatment groups	T. CHOL (mg dL^{-1})	TAG (mg dL^{-1})
Groups 1 = Normal/negative control	2.90±0.3*	1.45±0.15*
Groups 2 = Positive control	6.80±0.2	3.50±0.13
Groups 3 = 400 mg kg^{-1} b.wt., of V.A. extract	3.35±0.18*	1.59±0.20*
Groups 4 = 600 mg kg^{-1} b.wt., of V.A. extract	3.30±0.27*	1.45±0.17*
Groups 5 = 400 mg kg^{-1} b.wt., of G.L. extract	3.37±0.20*	1.59±0.20*
Groups 6 = 600 mg kg^{-1} b.wt., of G.L. extract	3.28±0.25*	1.50±0.16*

Values are expressed as Mean±SEM; *p<0.05 is considered significant when compared with positive control group using one-way analysis of variance

Table 7: Effect of *Vernonia amygdalina* and *Gongronema latifolium* ethanolic leaf extracts on total protein concentration in immunosuppressed Wistar albino rats

Treatment groups	T. PROT (mg dL^{-1})
Groups 1 = Normal/negative control	7.25±0.30*
Groups 2 = Positive control	3.49±0.25
Groups 3 = 400 mg kg^{-1} b.wt., of V.A. extract	5.32±0.35*
Groups 4 = 600 mg kg^{-1} b.wt., of V.A. extract	6.15±0.20*
Groups 5 = 400 mg kg^{-1} b.wt., of G.L. extract	5.90±0.16*
Groups 6 = 600 mg kg^{-1} b.wt., of G.L. extract	6.50±0.23*

Values are expressed as Mean±SEM, *p<0.05 is considered significant when compared with positive control group using one-way analysis of variance

with the extract showed a dose dependent reversal of such increased which could be as a result of the extract containing some phytochemical contents that could revive the functionality of the liver[36].

Intra-peritoneal administration of cyclophosphamide cause a decrease in the total protein concentration of the group 2 animals when compared to normal control group as can be seen in Table 7. These low levels of total protein could be as a result of specific oxidative damage of some of the susceptible amino acids of the intracellular proteins of the body. However treatment with *Vernonia amygdalina* and *Gongronema latifolium* leaf extract was able to cause a significant increase ($p<0.05$) in the total protein concentration of the rats in the treatment group (group 3, 4, 5 and 6) when compared to the untreated control group 2. This points to the effectiveness of the extracts in inhibiting the breakdown of structural proteins of the body which could be by membrane stabilization effect or other unknown mechanism. More so stimulation of protein synthesis can be contributory to its effect which could accelerates the regeneration process and the production of liver cells. This improvement in the total protein level of the rats treated with the extracts is in tandem with the works of Kanchana and Sadiq[37], where the hepato-protective effect of *Plumbago zeylanica* on positive induced liver toxicity in rats was determined.

CONCLUSION

The study showed that ethanol leaf extracts of *Gongronema latifolium* and *Vernonia amygdalina* possess an immune-stimulatory effect by its ability to revitalise hepatic functions and improve antioxidant properties of the immuno-suppressed rats. However, comparatively *Vernonia amygdalina* showed a better potency when compared to *Gongronema latifolium*.

SIGNIFICANCE STATEMENTS

The present research tends to discover the possible synergistic effect of *Vernonia amygdalina* and *Gongronema latifolia* ethanol extract in boasting cyclophosphamide immuno-suppressed rats. Although there are some inorganic immuo-stimulants but there is a continuous search for immuo-stimulants with little or no side effect. Thus the results of this research will give a clue on immunological abilities of the different plants and also give an insight on their mode of actions. Finally this research will take a lead in the discovery of new organic immunological agents with little or no side effects from both plants.

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REFERENCES

1. Akpaso, M., I.J. Atangwho, A. Akpantah, V.A. Fischer, A.O. Igiri and E.P. Ebong, 2011. Effect of Combined leaf extracts of *Vernonia amygdalina* (bitter leaf) and *Gongronema latifolium* (utazi) on the pancreatic β-cells of streptozotocin-induced diabetic rats. Br. J. Med. Med. Res., 1: 24-34.
2. Nwangwu, S., D.A. Ofusori, S. Josiah, O.F. Amegor, H. Njoya and A.O. Oyaka, 2011. The effects of aqueous and ethanolic leaf extracts of *Vernonia amygdalina* on some vital organs in adult wistar rats. J. Cell Mol. Biol., 9: 75-82.
3. Nwachukwu, M.I., R.N. Dike, I.O. Nwachukwu, C.N. Ohalete and V.E. Anyanwu, 2012. Preliminary phytochemical assessmentof extract from leaves and root of *Vernonia amygdalina* on *E. coli*. World J. Pharm. Pharm. Sci., 2: 44-51.

4. Hassan, H.S., M.T. Odunola, M. Garba and M.A. Usman, 2007. Effect of *Vernonia amygdalina* leaves on the pharmacokinetics of chlorpropamide in man. Nig. J. Pharm. Sci., 63: 34-39.
5. Mohammed, A.A. and A.S. Zakariyau, 2012. Bitter leaf (*Vernonia amygdalina*) as a feed additive in broiler diets. Res. J. Anim. Sci., 6: 38-41.
6. Winkelstein, A., 1973. Mechanisms of immunosuppression: Effects of cyclophosphamide on cellular immunity. Blood, 41: 273-284.
7. Young, S.D., M. Whissell, J.C. Noble, P.O. Cano, P.G. Lopez and C.J. Germond, 2006. Phase II clinical trial results involving treatment with low-dose daily oral cyclophosphamide, weekly vinblastine and rofecoxib in patients with advanced solid tumors. Clin. Cancer Res., 12: 3092-3098.
8. ShalizarJalali, A., S. Hassanzadeh and H. Malekinejad, 2011. Cyclophosphamide immune-suppression. Vet. Res. Forum, 2: 266-273.
9. Nicolini, A., P.A. Mancini, P. Ferrari, L. Anselmi and G. Tartarelli *et al*., 2004. Oral low-dose cyclophosphamide in Metastatic Hormone Refractory Prostate Cancer (MHRPC). Biomed. Pharm., 58: 447-450.
10. Sethi, J. and J. Singh, 2015. Role of medicinal plants as immuno-stimulants in health and disease. Ann. Med. Chem. Res., Vol. 1.
11. CIOMS., 1985. International guiding principle for biomedical research, involving animals. Council for International Organization of Medical Sciences (CIOMS), C/O WHO 121, Switzerland, pp: 1-9.
12. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.
13. Klein, B., P.A. Read and L.A. Babson, 1960. *In vitro* determination of alkaline phosphate in serum or plasma. J. Clin. Chem., 6: 269-275.
14. Jendrassik, L. and P. Grof, 1938. Determination of total and direct bilirubin in serum or plasma on manual system. Biochem. Zeitschrift., 297: 82-89.
15. Fridivich, I., 1989. Superoxide dismutases: An adaptation to a paramagnetic gas. J. Biol. Chem., 264: 7761-7764.
16. Aebi, H.E., 1983. Catalase. In: Methods of Enzymatic Analysis, Bergmeyer, H.U., J. Bergmeyer and M. Grassl (Ed.). 3rd Edn., Verlag Chemie, Weinhem, pp: 273-286.
17. Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.
18. Baker, E.M., R.E. Hodges and J. Hood, 1971. Metabolism ^{14}C and ^{3}H-labelled L-ascorbic acid in human scurvy. Am. J. Clin. Nut., 24: 444-480.
19. Allain, C.C., L.S. Poon, C.S.G. Chan, W. Richmond and P.C. Fu, 1976. Enzymatic determination of serum total cholesterol. J. Clin. Chem., 20: 470-475.
20. Trinder, P., 1969. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. J. Clin. Pathol., 22: 158-161.
21. Slater, R.J., 1986. Experiments in Molecular Biology. Humana Press, Clifton, NJ., USA., ISBN: 978-1-60327-405-0, pp: 269.
22. Sokal, R.R. and F.J. Rohlf, 1995. Biometry: The Principle and Practice of Statistics in Biological Research. 3rd Edn., W.H. Freeman, New York, USA., Pages: 887.
23. Uboh, F.E., E.E. Edet, I.J. Atangwo and E. David-Oku, 2012. Effect of *Gongronema latifolium* leaf extract on some kidney enzymes and protein levels in diabetic and non-diabetic rats. J. Pharm. Biomed. Sci., Vol. 21.
24. Ekpo, A., O.A. Eseyin, A.O. Ikpeme and J.E. Edoho, 2007. Studies on some biochemical effects of *Vernonia amygdalina* in rats. Asian J. Biochem., 2: 193-197.
25. Iweala, E.E.J., F.O. Uhegbu and O.A. Adesanoye, 2013. Biochemical effects of leaf extracts of *Gongronema latifolium* and selenium supplementation in alloxan induced diabetic rats. J. Pharm. Phytotherapy, 5: 91-97.
26. Parker, J.E., O.C. Nwodo, C.Y. Ukegbu and O.O. Prince, 2016. Prophylactic effect of an alkaloid-rich fraction of *Abrus precatorius* seed methanol extracton paracetamol-induced liver damage in rats. Global Vet., 17: 126-134.
27. Pal, R., T. Ahmed, V. Kumar, S.G. Suke, A. Ray and B.D. Banerjee, 2009. Protective effects of different antioxidants against endosulfan-induced oxidative stress and immunotoxicity in albino rats. Indian J. Exp. Biol., 47: 723-729.
28. Ismail, O.O., I.B. Ridwan, O.G. Olaiya, A. Abdulbasit, J.S.O. Rukayat, F.O. Roehan and S.A. Abass, 2013. Effects of ascorbic acid on immunosuppressive drug-azathioprine-induced alteration in the liver and kidney of adult wistar rats [*Rattus norvegicus*]. Eur. J. Exp. Biol., 3: 371-378.
29. Kedzierska, K., K. Sporniak-Tutak, J. Bober, K. Safranow, M. Olszewska, K. Jakubowska and L. Domanski, 2011. Oxidative Stress Indices in Rats Under Immunosuppression. Elsevier, New York, pp: 3938-3939.
30. Rajasekaran, A. and M. Kalaivani, 2011. Antioxidant activity of aqueous extract of Monascus fermented Indian variety of rice in high cholesterol diet fed-streptozotocin diabetic rats, an *in vivo* study. Int. J. Cur. Sci. Res., 1: 35-38.
31. Nwanjo, H.U., M.C. Okafor and G.O. Oze, 2006. Anti lipid peroxidative activity of *Gongronema latifolium* in streptozotocin-induced diabetic rats. Nig. J. Physiol Sc., 21: 61-65.
32. Ayoola, G.A., A.D. Folawewo, S.A. Adesegun, O.O. Abioro, A.A. Adepoju-Bello and H.A.B. Coker, 2008. Phytochemical and antioxidant screening of some plants of Apocynaceae from South West Nigeria. Afr. J. Plant Sci., 2: 124-128.

33. Buher, D. and C. Miranda, 2000. Antioxidant activities of flavonoids. The Linus Pauling Institute.
34. Farombi, E.O. and O. Owoeye, 2011. Antioxidative and chemopreventive properties of *Vernonia amygdalina* and *Garcinia biflavonoid*. Int. J. Environ. Res. Public Health, 8: 2533-2555.
35. Sugimoto, T., S. Yamashita, M. Ishigami, N. Sakai and K. Hirano *et al*., 2002. Decreased microsomal triglyceride transfer protein activity contributes to initiation of alcoholic liver steatosis in rats. J. Hepatol., 36: 157-162.
36. Ukegbu, C.Y., O. Arome, E. Affiong, A. Ogechukwu and C. Ike, 2016. Anti-diabetic effect of the methanolic leaf extract of *Axonopus compressus* (*P. beauv*) in alloxan induced diabetic rats. Int. J. Biochem. Res. Rev., 12: 1-5.
37. Kanchana, N. and M.A. Sadiq, 2011. Hepatoprotective effect of *Plumbago zeylanica* on paracetamol induced liver toxicity in rats. Int. J. Pharm. Pharm. Sci., 3: 151-154.