

Evaluation Of Synergistic Effects Of *Gongronema Latifolium* And *Piper Guineense* Ethanol Extracts On Selected Biochemical Indices In Ethanol Exposed Wistar Rats

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Abstract: Combination therapy has been identified as a promising strategy to improve disease management. This work was designed to estimate the synergistic effects of *Gongronema latifolium* and *Piper guineense* in selected biochemical indices in ethanol exposed wistar rats.

A total of thirty five wistar rats were used for this study. It was grouped into A, B and C, with C subdivided into five; C1, C2, C3, C4 and C5. Groups B and C were exposed to 70 % ethanol for seven days, and further treated group C1-C3 with the combine extract 100-300 mg/kg respectively while group C4 and C5 were treated with 400 mg/kg and 500 mg/kg of separate doses of the two plants extract for 21 days ,thereafter,the blood samples were collected and assay for biochemical indices, the results obtained were analysed using ANOVA. The results revealed that liver marker enzymes increased after exposure to ethanol whereas treatment with the extracts significantly ($p < 0.05$) decreased AST, ALP, ALT, total bilirubin and total cholesterol in groups C1-C3. While the total protein increased significantly compared to control groups and individual separate doses. Selected biochemical parameters due to ethanol toxicity in rats fed chronically with high dose of ethanol were altered. This study indicates that treatment with combined extract could reverse damages due to ethanol toxicity than separate doses.

Keywords: Synergistic effect, *Gongronema latifolium*, *Piper guineense* and Wistar rats.

I. Introduction

The practice of herbal medicine is an ancient tradition. Herbal medicines have been used since earliest times to treat illnesses and restore good health, and today, herbalism still remains the most widely practised form of medicine worldwide [1]. Globally, medicinal plants are very useful for the treatment and management of diseases or infections. They are mostly particularly useful in countries, where, due to their low income status, they can hardly afford imported and expensive conventional medicine [2]. According to the World Health Organization (WHO) report, it is estimated that 80 % of people worldwide rely on herbal medicines for some aspects of their primary health care [3].

Drug combinations have been identified by many to be a promising approach to treat complex diseases such as tuberculosis, cancer, inflammation and gonorrhoea [4]. Thus, when used in combination, drugs interact in different unexpected ways and show many outcomes [5].

Drug synergy, the combined boost of drug efficacy, is a highly pursued goal of combinational drug development [6]. Synergistic drug combinations have been reported to be highly efficacious, therapeutically more specific and economically affordable [7].

Piper guineense, popularly known as African black pepper or hot leave is widely consumed in some part of West Africa especially Nigeria and Ghana on account of its nutritional and medicinal properties [8]. It belongs to the family Piperaceae or Sapotaceae [9]. In traditional herbal medicine, the seeds are put into a variety of uses, for instance, in some parts of Nigeria, the seeds are consumed by women after child birth, to enhance uterine contraction for the expulsion of placenta and other remains from the womb [10], as an adjuvant in the treatment of rheumatic pains and as antiasthmatics [11] and also for the control of weight [12]. The seed and leaf extracts are capable of exhibiting a depolarizing neuromuscular activity in a concentration related manners [13]. The antiparasitic, antimicrobial and antifungal activities of the leaf and seeds of *P. guineense* have also been reported [14]. According to [15], leaves of *P. guineense* have been used by traditional medical practitioners for the treatment of respiratory diseases and correction of female infertility problems, and the seeds as an aphrodisiac [16]

Gongronema latifolium is an herbaceous nonwoody plant from the family of Asclepiadaceae [17]. It is widespread in the tropical, and subtropical regions, especially, in Africa and South America, with a moderate

representation in Northern and South Eastern Asia [18]. In South Eastern and South Western Nigeria, *Gongronema latifolium* is commonly called “utazi” and “arokeke”, respectively, and is primarily used as spice and vegetables in traditional folk medicine [19]. Apart from this, there is a dearth of information on the preservative potential of extracts from this plant with particular reference to its effect on some food-quality-related enzymes. It has been reported that the extracts of *Gongronema latifolium* contain phytochemical compounds including alkaloids, saponins, tannins (flavonoids), and glycosides [20]. Studies have shown that these phytochemicals found in *Gongronema latifolium* may influence cellular proteins with enzymic activities [21]. Phytochemicals like tannins have been shown to be strong inhibitors of oxidative enzymes present in foodstuffs [22]. The research was carried out to evaluate the synergistic effect of *Gongronema latifolium* and *Piper guineense* ethanol extract on selected biochemical indices in ethanol exposed Wistar rats.

II. Materials And Methods

Plant collection and identification

The plant was sourced from Abakpa main market in Abakaliki Local Government Area of Ebonyi State and identified by a Taxonomist- Prof.S.E Okafor of herbarium section of department of Applied Biology Ebonyi State University Abakaliki. A voucher specimen number:1874 was assigned to it for future reference.

Preparation and extraction of plant material

The plant material was air dried in the laboratory. The size was reduced using grinding machine and stored in an air tight container. This was further sieved with 2 mm size sieve. A 100 g weight of the plant powdered material was macerated with 400 ml of 95 % methanol. The extract obtained was evaporated to dryness at room temperature.

Experimental animals

Thirty five male wistar rats weighing between 100 -200 g were obtained from the breed of the animal house of the Clinical Pharmacy Department, University of Nigeria, Nsukka Nigeria. They were kept in cages under standard environmental conditions and fed *ad libitum* with water and pellets from Obum Farm Number 3 Zik Avenue Abakaliki.

Ethical clearance

The experiment was performed with the permission of the University’s Animal Ethical Committee, and in accordance with approved institutional and national guidelines for the care and use of laboratory animals. All experiments on animals were in line with the guidelines of both the committee and the International Guidelines for handling of research animals [23]. There was no conflicting interest in this work declared by the authors.

Research design

The animals were grouped into three, A, B, and C, with C subdivided into C1-C5. Group A served as the normal control, group B and C were exposed to 70 % ethanol for seven days. Group B were not treated and served as negative control, group C1- C3 were treated with 100, 200, and 300 mg/kg of the combined extract whereas group C4 and C5 were treated with separate doses 400 and 500 mg/kg of *G. latifolium* and *P. guineense* respectively for twenty one days.

Sample collection

Twenty four hours before the assay, the animals were fasted and sacrificed under anaesthesia using cardiac puncture. The blood samples were centrifuged for 10 minutes at 3000 rpm and the serum was used to assay for selected biochemical indices: ALT, AST, ALP, Total protein, Total bilirubin and cholesterol.

Statistical analysis

Values were expressed as mean \pm S.E.M. Statistical significance were determined by the student’s t-test compared with control. Values with $p < 0.05$ were considered significant.

III. Results

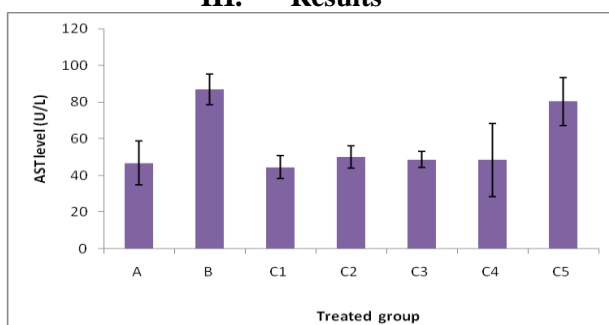


Fig. 1: Synergistic effects of *P. guineense* and *G. latifolium* extracts on AST level in ethanol-exposed albino rats. A = Normal control, B = Negative control, C1 = 200 mg/kg, 300mg/kg, C3=400mg/kg, C4=400mg/kg *P. guineense* only, C5=400mg/kg of *G. latifolium*, only.

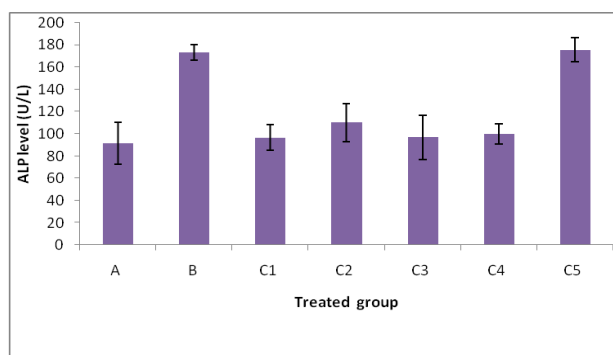


Fig. 2: Synergistic effects of *P. guineense* and *G. latifolium* extracts on ALP level in ethanol exposed albino rats. A = Normal control, B= Negative control, C1 = 200 mg/kg, 300mg/kg, C3=400mg/kg, C4=400mg/kg *P. guineense* only, C5=400mg/kg of *G. latifolium*, only.

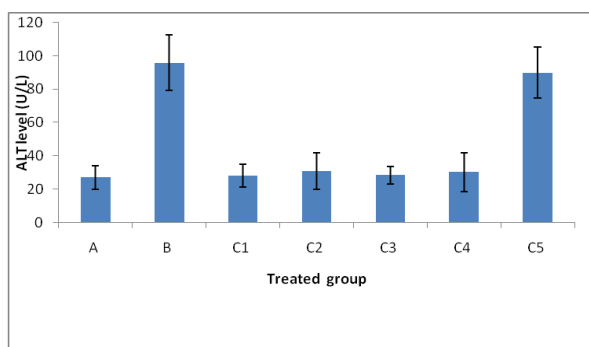


Fig. 3: Synergistic effects of *P. guineense* and *G. latifolium* extracts on ALT level in ethanol-exposed albino rats. A = Normal control, B = Negative control, C1 = 200 mg/kg, 300mg/kg, C3=400mg/kg, C4=400mg/kg *P. guineense* only, C5=400mg/kg of *G. latifolium*, only.

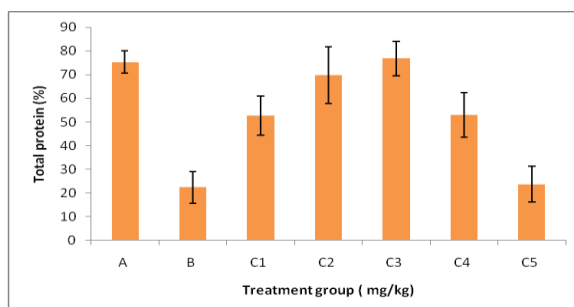


Fig. 4: Synergistic effects of *P. guineense* and *G. latifolium* extracts on Total protein level in ethanol-exposed albino rats. A = Normal control, B = Negative control, C1 = 200 mg/kg, 300mg/kg, C3=400mg/kg, C4=400mg/kg *P. guineense* only, C5=400mg/kg of *G. latifolium*, only.

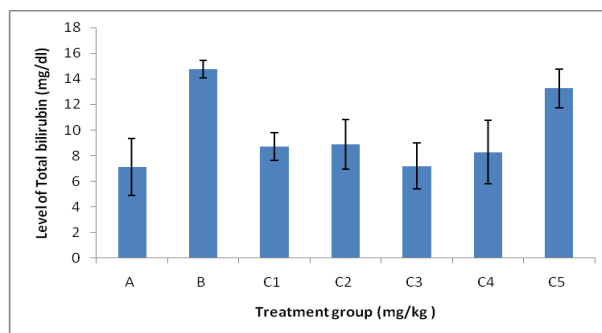


Fig. 5: Synergistic effects of *P. guineense* and *G. latifolium* extracts on Total bilirubin level in ethanol-exposed albino rats. A = Normal control, B = Negative control, C1 = 200 mg/kg *P. guineense* + *G. latifolium*, C2 = 300 mg/kg *P. Guineense*

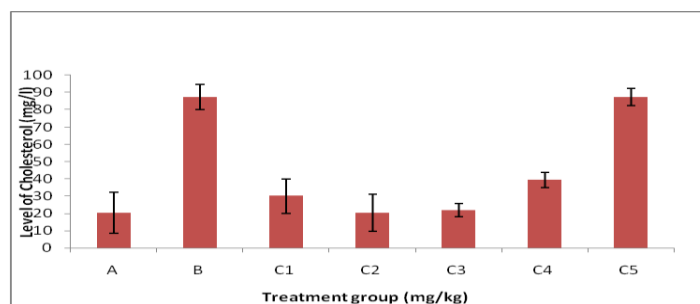


Fig. 6: Synergistic effects of *P. guineense* and *G. latifolium* extracts on Cholesterol level in ethanol-exposed albino rats. A = Normal control, B = Negative control, C1 = 200 mg/kg *P. guineense* + *G. latifolium*, C2 = 300 mg/kg *P. guineense*

IV. Discussion

The increasing acceptance of combination therapy for the treatment of ailments/diseases such as cancer, AIDS and other deadly diseases, scientists have developed interest to study the pharmacological effects of multiple-component herbal preparations. This is further supported by the observations that many herbal extracts show superior effect when compared to single chemical constituents at the equivalent dose [24]. Reports of such effects in which examples of herbal interactions using *in vitro* and animal models as well as clinical observations were highlighted [25]. Such interactions were referred to as “synergy” [25].

The development of hepatotoxicity induced by exposing of the test animals to 70 % ethanol corresponds to the events in early age of lipid peroxidation which is an important features of oxidative stress [26]. This is in agreement with [27]. The liver is an organ involved in many metabolic functions and is prone to xenobiotic injury because of its central role in xenobiotic metabolism [28]. Administration of alcohol increased the mean value of liver marker enzymes significantly ($p < 0.05$). Exposure of hepatocytes to ethanol alters the membrane structure and functions by increasing the leakage of enzymes into the circulation and causes oxidative stress [29]. Hence, statistically significant ($p < 0.05$) differences were found between the liver marker enzymes of the test animals.

The results of the present study show that many biochemical test values are altered in the serum of Wistar rats at the end of seven days exposure to 70 % ethanol. However, as shown in Figure 1-3, mean serum ALT, AST and ALP level was raised by 60 % in the alcohol –treated wistar rats compared to the groups treated with the plant extracts. Report on the previous studies showed that excess acetaldehyde, lipid peroxides, oxidative stress and other toxic effect of alcohol are possible key reasons in the pathogenesis of alcohol associated injury and biochemistry in tissues [30]. Once ethanol has been absorbed after consumption, it is distributed to all tissues and fluids of the body in direct proportion to the water content [31]. The antihepatotoxic activity displayed by the combined extract compared to their individual separate doses (Figure 1- 4) by decreasing significantly ($p < 0.05$) AST, ALP, ALT, total bilirubin and total cholesterol in groups C1-C3 were indication of hepatoprotective of the combined extract. The ability of *Piper guineense* and *G. latifolium* to effectively inhibited lipid peroxidation in the treated animals, reducing the levels of marker enzymes cholesterol, bilirubin and had increased level of total protein at 100 -300 mg /kg in this study is in consonance with [32]. Thus, this trend towards improved normal in comparison to controls and those receiving single separate doses may be attributed to the phytochemicals present in both extracts. This reduces the risk associated toxicity and justifies their folkloric uses in treating various liver damages in the African traditional medicine. Furthermore, the total protein increased significantly compared to control groups and individual separate doses,

this could be attributed to inhibition of lipid peroxidation or its products released by exposure of the test animals with 70 % ethanol (Figure 5). Inhibition of lipid peroxidation and associated products has been proposed as a mechanism of anti – hepatotoxic action in an ethanol – induced liver damage model [32].

Hence, our finding is consonance with the reports [33] for appropriate plants. The results of our evaluation on extracts synergy of *G. latifolium* and *P.guineense* showed significant ($p<0.05$) anti –hepatotoxic activities compared with their individual doses. It may be inferred that the plant extracts exhibit equipotent at the doses investigated. Earlier reports expressed that in alcohol metabolism NADH is produced which may be used directly in the ETC to synthesize ATP as a source of energy. This may have a direct effect of inhibiting the normal oxidation of fats and citric acid cycle [34], hence large chronic high dose alcohol consumption can lead to excess lactate production, acetyl COA or triglyceride accumulation. In this study, total cholesterol level was significantly changed in wistar rats in the negative control group, whereas combined extract treated groups were decreased significantly ($p< 0.05$). Reports by many authors noted that the serum concentrations of total lipids and cholesterol significantly increased in chronic alcohol-treated albino rats for 30 days [35]. These findings in alcoholism pathogenesis may be important for coronary artery diseases. It is often implicated that chronic heavy intake of alcohol is associated with increases in both overall mortality and cardiovascular mortality [35]. The resulting increase of serum total cholesterol may be explained by enhanced synthesis of cholesterol or impairing of cholesterol metabolism in the body owing to alcohol-induced toxicity in liver, as observed (Figure 6). However, it may be inferred that the combined extract have proved better curative effect in a dose dependent manner by reversing the status of total cholesterol compared to individual separate dose, thus confirming the synergy in both extract.

V. Conclusion

The investigation of synergy of *G. latifolium* and *P.guineense* which exhibited comparatively better than their individual doses. The combined doses depending on the concentration being more potent, could serve as possible alternatives for treatment of liver damages due to ethanol toxicity.

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