

## Comparative Study on the Effects of Ethanol Extracts of *Piper guineense* and *Gongronema latifolium* Plants on Hematological Parameters in Albino Rats Exposed to Ethanol

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**Abstract:** Medicinal plants have been recorded for their haematological effects either at low or high concentration but very little is known about *G. latifolium* and *Piper guineense*. This study was undertaken to investigate effects of ethanol extracts of *Piper guineense* and *Gongronema latifolium* plants on haematology indices of albino rats exposed to ethanol. Methods: The animals were divided into three groups; A - C. Group A were fed with distilled water and rat feed only, served as the normal positive control, Group C was subdivided into four C1-C4. Group B and C were exposed with 70% v/v ethanol for seven days to evoke haem-toxicity lesions. Group B was not treated and acted as the negative control whereas Group C were further treated with 200, 400, 600 and 800 mg/kg respectively for 21 days and thereafter, full haematological parameters were evaluated on whole blood collected from rats twenty four hours after the administration of the last dose. All data were subjected to analysis of variance, with conclusions drawn at 5% probability level. Ethanol significantly ( $P < 0.05$ ) reduced haemoglobin content, white blood cell, packed cell volume, mean corpuscular haemoglobin concentration (MCHC) and increased mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) of the animals. Administration of ethanol extract of *Piper guineense* and *Gongronema latifolium* caused a concentration dependent and statistically significant ( $P < 0.05$ ), amelioration of deleterious effects of ethanol on hematological parameters in rats. The reversal of haematology indices to normal after exposure to ethanol observed in rats administered *G. latifolium* and *Piper guineense* in a dose dependent fashion, suggests that the extracts contains agents that could enhance the production of leucocytes and could serve as immune booster. It also indicated that the extracts were able to overcome the ethanol intoxication. The results of this work further strengthened the earlier works on the medicinal plants and virtue of *G. latifolium* and *P. guineense* as a good pharmacological source of haematopoiesis.

**Key words:** *G. latifolium* • *Piper guineense* • Haematology • Liver and Herbal

### INTRODUCTION

Herbal medicines have been used since earliest times to treat illnesses and restore good health and today, herbalism 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

report [3], it is estimated that 80% of people worldwide rely on herbal medicines for some aspects of their primary health care [4]. Plants which are medicinal and used by animals as foods have contributed immensely to health care [5]. These medicinal plants include *Piper guineense*, *Gnetumgnemon*, *Congrema latifolium*, *Azadirachta indica*, *Moringa orifera* and so on have been used in biological researches for aims of analysis or otherwise. *Piper guineense* of the plant family *Piperaceae* and contains over 700 species all over the globe. It is a medicinal plant known to provide medicinal, insecticidal,

culinary and dietary benefits to human beings. *Piper guineense* is a local spice that comprises of dillapiol, 5-8% piperine, elemicine, 10% of myristicine and saffrole. The effects of these local spice is associated with the presence of phytochemicals such as flavonoids, alkaloids, saponins, tannins, glycosides, essential oils, peptides and phenols[6].

*Gongronema latifolium* is an herbaceous nonwoody plant from the family of Asclepiadaceae [7]. 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 [8]. 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 [9]. Earlier reports on extract from this plant have focused mainly on their medicinal properties [10], with little attempts at investigating their food preservative potentials. Report has shown the use of bittering agents in brewing to produce the characteristic flavour, foam stability and preservative properties in beer [11]. Nigerian bitter vegetables of *Gongronema latifolium*, *Vernonia amygdalina* and *Garcinia kola* has been used as substitutes for commercial hops in lager beer production [12]. 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 [13].

Haematology refers to the study of the constituents of blood and its morphology. It can also be referred to as the study of the changes in some blood indices due the effect of some environmental or chemical substances [14]. Hence, haematological parameters are the features, indices, or characteristics relating to blood. Otherwise they are called indices of blood characterization, which include haemoglobin, red blood cells, packed cell volume, mean cell volume, mean cell haemoglobin, mean cell haemoglobin count, white blood cells and platelets. The study of these parameters is of ecological and physiological interest [15].

The use of blood examination as a way of assessing the health status of animals has been documented [16]. Report has apart from genotype, age, sex, differences in haematological indices or parameters may be caused by nutritional, environmental and hormonal factors [17] Haematological assays seldom provide an ethiological diagnosis but they remain nevertheless indispensable

diagnostic tools to evaluate health and disease conditions in human beings and animals for monitoring the response and progress of patients to therapeutic regimes and to offer a prognosis. The routine collection and processing of blood samples allows the evaluation of haematological responses to diseases [18].

The use of Wister or albino rats as case study for biological researches is an increasing practice by scientists. This is solely because of the much resemblance in the body systems of the rats and that of human being [19]. In other words, the albino rats and human being belong to the same class of organisms-the mammals in the animal kingdom. The Wister rats survive the same environmental conditions just as human beings.

## MATERIALS AND METHODS

**Collection and Preparation of Plant Materials:** The plant materials were sourced from Aakpa Market in Ebonyi State and authenticated by Prof. S.E Okafor Department of biology Ebonyi State University Abakaliki Nigeria and voucher specimen deposited in the herbarium unit. They were dried at room temperature for two weeks and ground into powder using mechanical grinder (model 241c). One hundred gram of each sample were dissolved in 300ml of 98% ethanol and system allowed to stand for 48 hours. It was further filtered with a muslin cloth, the filtrate was allowed the ethanol to evaporate. The resulting extract paste was then stored in the refrigerator until needed.

**Experimental Design and Animal Treatment Regimen:** Thirty male albino rats of two and half months age, weight 150-220g were obtained from Animal House of Department of Veterinary Medicine University of Nigeria Nsuka. The animals were housed in standard cages, with light 12h day, water and feed *ad libitum*. The animals were assigned into six groups of five rats each: A, B, C1, C2, C3 and C4. Group A were fed with distilled water and rat feed only, served as the normal control, Group C was subdivided into four C1-C4. Group B and C were exposed with 70% v/v ethanol for seven days to evoke haemotoxicity lesions. Group B was not treated and acted as the negative control whereas Group C were further treated with 200, 400, 600 and 800 mg/kg respectively via oral gavage for 21 days. Acclimatization and quarantining was allowed for one week before commencement of treatment and they were handled in accordance with the standard guide for use of laboratory animals. The animals were treated daily with the stated doses of plant extract via oral gavage for 21 days.

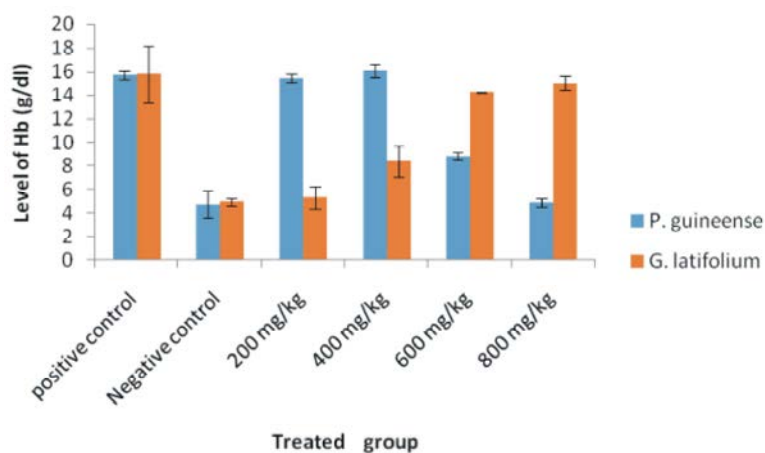


Fig. 1: Effect of *P. Guineense* and *G. latifolium* extract on the level of Hb in ethanol-exposed albino rats

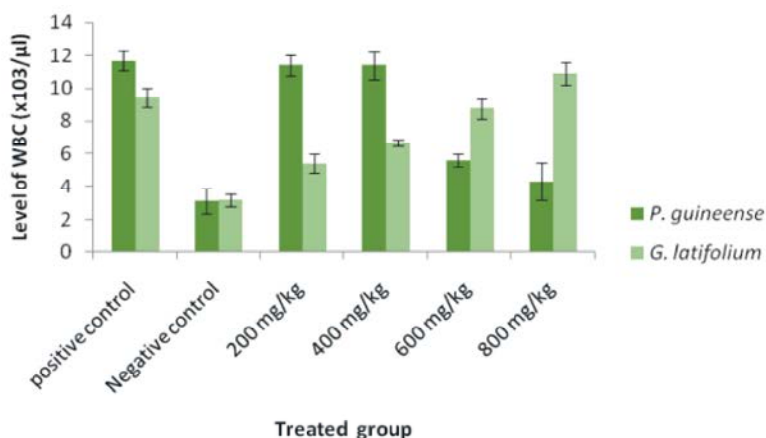


Fig. 2: Effect of *P. Guineense* and *G. latifolium* extract on the level of WBC in ethanol-exposed albino

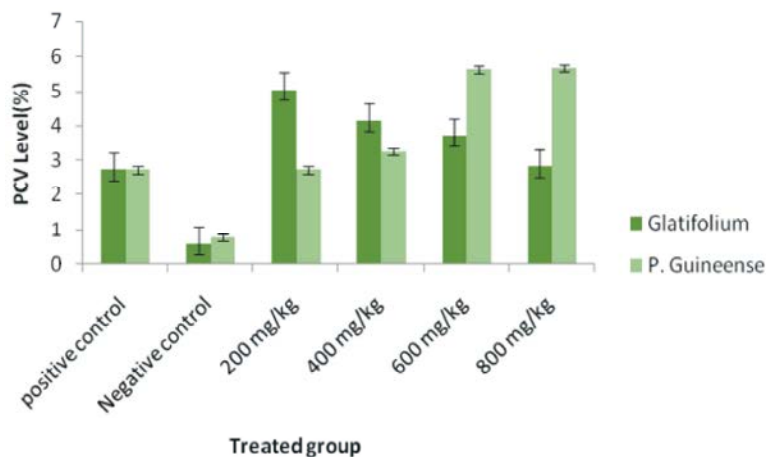


Fig. 3: Effects of *P. Guineense* and *G. latifolium* extract on level of PCV in ethanol exposed albino rats

**Determination of Haematological Parameters:** Blood were collected via ocular puncture into EDTA tubes. The haemoglobin content ( $\text{gdL}^{-1}$ ), red blood count ( $10^9\text{L}^{-1}$ ), mean corpuscular volume (MCV), mean corpuscular

haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), packed cell volume (%) and white blood cell were determined according to the method of Dacie and Lewis [20].

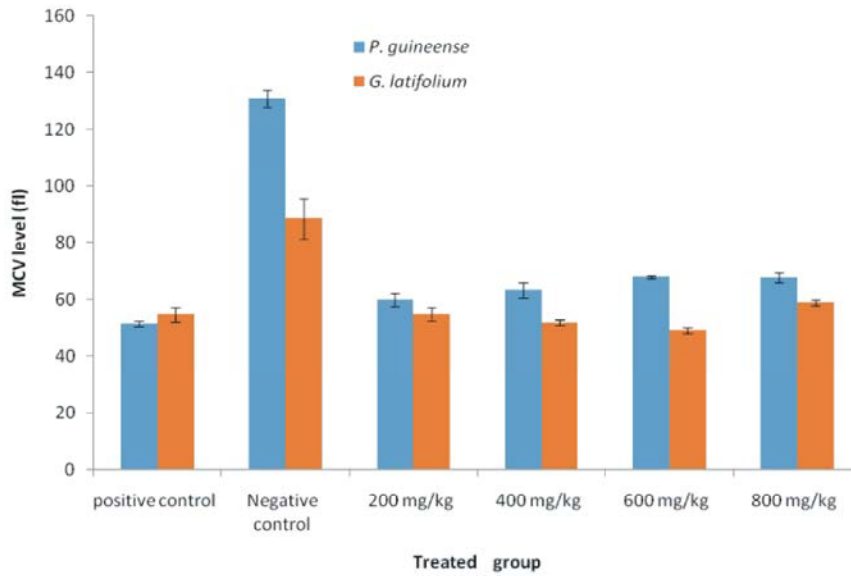


Fig. 4: Effect of *P. guineense* and *G. latifolium* extract on the level of MCV in ethanol-exposed albino rats

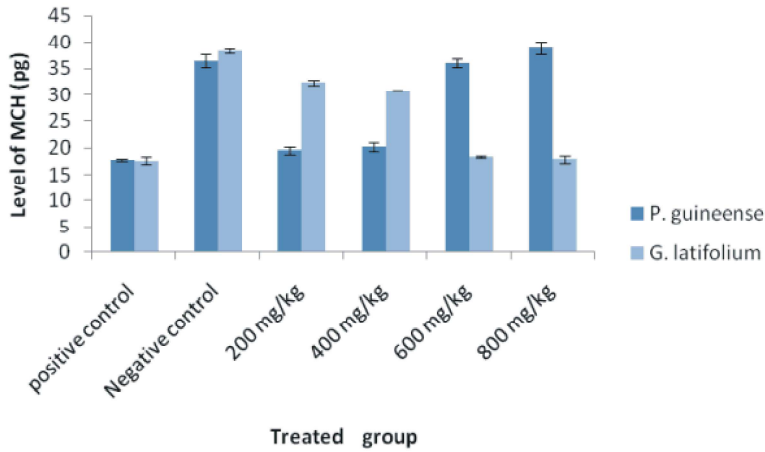


Fig. 5: Effect of *P. Guineense* and *G. latifolium* extract on the level of MCH in ethanol-exposed albino rats

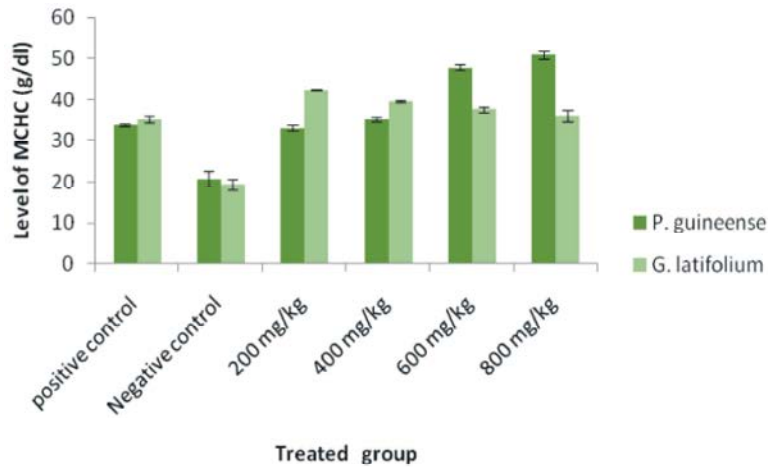


Fig. 6: Effect of *P. Guineense* and *G. latifolium* extract on the level of MCHC in ethanol-exposed albino rats

**Statistical Analysis:** All data generated were subjected to analysis of variance (ANOVA) and significant differences were obtained at  $P \leq 0.05$ . The results were expressed as mean  $\pm$ SEM.

## RESULTS AND DISCUSSION

The liver is the key organ involved in numerous metabolic functions and detoxification of hazardous substances and is frequently targeted by a number of toxicants [21]. Reactive oxygen species play an important role in pathological changes in the liver, particularly in the cases of alcoholic and toxic liver diseases [22]. It is now generally accepted that the hepatotoxicity of by-products of ethanol metabolism readily interacts with molecular oxygen to form adduct. These are capable of binding to proteins or abstracting a hydrogen atom from an unsaturated lipid, causing lipid peroxidation and liver damage. These metabolites by doing so play a significant role in pathogenesis of diseases [23].

The results of the haematological parameters of ethanol exposed animals and the control subjects are as shown in Figure 1-6. The groups exposed with ethanol without treatment records a significantly lower values ( $p < 0.05$ ) in haemoglobin, white blood cell, packed cell volume, mean corpuscular haemoglobin concentration, high mean corpuscular volume and mean corpuscular haemoglobin. All the haematological parameters in ethanol exposed rats were adversely altered by ethanol, it suggest that ethanol may have negative implications and may said to possess toxicity potential [24]. The decrease in hemoglobin in ethanol exposed rats has been attributed to impairment on the rate of RBC formation as a result and may have negative effect on the oxygen carrying capacity of the animals [25].

Alcohol exerts a direct pathologic effect to the bone marrow causing vacuolization of the bone marrow precursor cells, anaemia, thrombocytopenia and leukaemia. It also affects the roles of the leukocytes and platelets. Ethanol causes hyperactivity of bone marrow, which leads to the production of red blood cells with impaired integrity that are easily destroyed in the circulation, as well as marked leucopenia [26]. Haematological functions may also be affected indirectly from nutritional factors, chronic alcoholic liver disease and other metabolic derangement associated with toxicants [27]. Kaplan *et al.* [28] reported that alcohols have a wide spread direct and indirect effects on the

haematological indices which mimic and obscure other disorders. Other researchers have indicated that Leukocytes, erythrocytes and thrombocytes synthesis and functions are affected directly [29]. Furthermore, liver damage secondary to alcohol abuse also impact on red blood cells and haemostatic mechanism [30]. Alcohol has effect the consumption, storage and neutralization of vitamins [31]. Results obtained by other authors showed that ethanol consumption affects the absorption, distribution and excretion of each of the vitamins in rats fed a diet containing a low-vitamin mixture. Our results are in line with Kiran and Khan [32], which showed that chronic alcohol abuse individuals frequently indicate lowered plasma levels of pyridoxal 5'-phosphate, the coenzyme form of vitamin B6. This is because the liver is the primary source of this coenzyme in plasma coupled with the fact that it is the principal organ that oxidizes ethanol [33]. Ethanol is known to damage the stomach lining, causing stomach inflammation, a condition known in medical term as atrophic gastritis. In patients suffering from atrophic gastritis, the stomach cells cannot produce and secrete an important protein called intrinsic factor (IF), absolutely essential for the absorption of vitamin B12.

The effects of the extract on the haematological parameters investigated showed that there was a significant ( $p < 0.05$ ) change on the Hemoglobin, WBC count, Packed cell volume, mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration in extract treated groups compared to the controls (Figure 1-6). However, treatment with graded doses of ethanol extract of *P. guineense* and *G. Latifolium* and ethanol exposed animals ameliorated ethanol induced haematotoxicity toward normal in a dose dependent fashion. These results showed that the leaf extracts of *P. Guineense* and *G. latifolium* has a potency to induce haematological parameters recovery in the towards normal values.

The increase in the level of PCV in the treated groups may be attributed to concentration of iron in the extract, which increases the amount of iron available for erythropoiesis [34]. Increase in iron leads to an increase in the production of red blood cells (RBC) and haemoglobin. This could be the cause of the observed increase in packed cell volume, Hb concentration and restoration of other haematological parameters in the treated groups compared to ethanol exposed groups.

It could be possible that some of the chemical constituents of the extracts may have erythropoietin-like effect on the bone marrows. Leading to the increase in the rate of erythropoiesis and resultant increase in PCV and normalizing other indices [35]. The increase in total white blood cell count, Haemoglobin, PCV, MCHC and reversal of MCV and MCH to normal indicates that the extract was able to overcome ethanol intoxication. This finding is in consonance with our previous research [36], which reported that alcohol causes highly significant positive association with blood electrolytes like potassium, phosphorus and calcium and these plants extracts can ameliorates it effect.

However, it can be inferred that *G. latifolium* and *Piper guineense* can therefore be used in building up the blood level in physiological conditions like pregnancy and during menstruation when there is a drop in haemoglobin concentration and packed cell volume.

### CONCLUSION

The findings of this work is quite promising as the leaves extract of *P. guineense* and *G. latifolium* were found to possess haematotoxic protection against ethanol induced liver damage in experimental animals. The ameliorative effect observed could be attributed to the presence secondary metabolites in plants, which might be responsible for restoration of liver damage. This is reflected in the reversal and the correction of haematological parameters.

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