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Impacts of Single and Combined Administration of Ethanol Leave Extracts of *Mangifera indica* and *Gongronema latifoluim* on Liver Enzymes and Electrolyte Profile of Male Albino Wistar Rats

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ABSTRACT

Investigation of the impacts of single and combined administration of ethanol leaf extracts of *Mangifera indica* and *Gongronema latifolium* on the Liver enzymes and electrolyte profile of male albino Wistar rats weighing 185-221 g was carried out for a period of one month. Sixteen animals were randomly grouped into four groups of four rats each. Groups 1 and 3 were treated orally with 200 mg/kg of *Mangifera indica* and *Gongronema latifolium* respectively. Group 2 was treated with the combined extracts at 50:50 dosage ratio. Group 4 was not treated and served as control. All animals were allowed free access to commercial rat's mash and water throughout the treatment period. Results of the serum liver enzymes revealed a significant increase ($p<0.05$) in AST in group 3 when compared to groups 1 and 2. All significant changes mentioned have a p- value <0.05 Serum ALT and ALP recorded significant increase and decrease respectively in all treatment groups when compared to the control group. A significant increase in the serum ALT and ALP were recorded in group 1 when compared to groups 2 and 3. Serum electrolyte levels revealed a significant increase for potassium (K^+) ion in all treatment groups when compared to the control. Group 3 showed significant increase in serum K^+ when compared to groups 1 and 2. Group 1 was significantly increased compared to group 2. Serum sodium (Na^+) ion recorded significant decrease in group 2 when compared to groups 1, 3 and control. Chloride (Cl^-) ion level recorded significant increase in groups 1 and 3 when compared to groups 2 and control. Biocarbonate (HCO_3^-) levels showed significant increase in groups 1 and 2 when compared to groups 3 and control. These results revealed that leaf extracts of the two plants could alter serum liver enzymes and electrolyte profile in single and combined treatment, however, traditional herbalists should use the plants with strict moderation for the treatment of some ailments.

Keywords: Liver enzymes, Electrolyte profile, *M. indica*, *G. Latifolium*, Albino Wistar rats.

1 Introduction

In many developing countries, it is estimated that about two-thirds of the population relies heavily on traditional practitioners and medicinal plants to meet primary healthcare needs (Farnsworth, 2009). As a result of the numerous problems associated with orthodox drugs, many plants species are now being revealed by researchers based on variation in plant species and their therapeutic chemical principles. Therefore, the need for thorough literature search on some species with a view to update the current state of knowledge is imperative. Two of such plant species are *Mangifera indica* and *Gongronema latifolium. Mangifera indica* (mango) has been an important herb in the *Agurvedic* and indigenous medical systems for over 4,000 years (Svensson *et al.*, 2004).

Mangoes belong to a genus *Mangifera* which consist of about 30species of tropical fruiting trees in the flowering plant family. *Anacardiaceae*, and its valued medicinal properties are attributed to different parts of the tree (Martinez *et al.*, 2012). The plant contains different bioactive consitutuents especially polyphenolics flavonoids, triterpenoids and mangiferin, a xanthone glycoside, isomangiferin, tannins and gallic acid derivatives (Andreas *et al.*, 2000). The bark is known to contain protocatechin acid, catechin, mangiferin,

alanine, glycine etc. the leaf and flower yield an essential oil containing humulene, element, ocimene, linalool, nerol and many others (Nunez-selles *et al.*, 2002; Kuhn *et al.*, 2017). Various parts of this plant are used medicinally as antiseptic, astringent, diaphoretic, stomachic vermifuge, tonic, laxatie and diuretic for the treatment of several ailments such as tootache, leucorrhoea, haemorrhage, piles, asthma, bronchitis, cough, hypertension, insomnia and rheumatism (Scartezzini and Speroni, 2000; Subha *et al.*, 2007; Fzunga *et al.*, 2010).

Gongronema latifoluim belongs to the class of medicinal plants that are beneficial in preventing and a treating certain diseases and ailments that are detrimental to human health. The fundamental ingredients used for medicinal purposes in the plant are stored in various parts of the plant, such as essential oils, alkaloids saponins, glycosides, tannins and various minerals, vitamins and some essentials amino acids (Apori *et al.*, 2023). These bioactive compounds in *Gongronema latifoluim* exhibit the following herbal actions, analgesic, anti-tumor, broad spectrum and antimicrobial (antibacterial, antifungal, anti-parasitic, anti-inflammatory, antiulcer, anti-sickling, anti-asthmatic, mild expectorants, hypoglycemic, hypolipidemic, hepatoprotective, digestive and laxative properties (Hanley *et al.*, 2005; Apori *et al.*, 2012; Enyi- Idoh *et al.*, 2012).

The liver is a large, complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It is a largest organ of human body saddled with the responsibility of detoxifying chemicals and other xenobiotics and inactivating and metabolizing them (Izunga *et al.*, 2010). Thus, it is a direct victim for toxicity that could result in liver disease. Most liver diseases cause only mild symptoms initially, but, these diseases must be detected early. Liver enzymes are used to detect the presence of this diseases. These enzymes include alanine transaminase (ALT), aspartate transaminase (AST) alkaline phosphatase (ALP). Elevation of these enzymes above their normal ranges in the blood signify disease condition of the liver. The kidneys play a significant role in ensuring that electrolyte levels remain invariant despite any changes the body may undergo. However, having an excess or an insufficiency of electrolytes in the body can be dangerous and sometimes fatal (Syzdek, 2009). Therefore, that was why the effects of administration of leaf extracts of *M. indica* and *G. Latifolium* on the liver enzymes and electrolyte profile of male albino Wister rats was studied.

2 Materials and Methods

2.1 Collection and Preparation of Plant Samples

Fresh leaves of *Mangifera indica* and *Gongronema latifolium* were collected at different locations in the botanical garden of Akwa Ibom State Polytechnic, Ikot Osurua. The two leaves were authenticated by taxonomist in the Department of Botany and Ecological Studies, Faculty of Sciences, University of Uyo, Akwa Ibom State, Nigeria. The leaves were plucked from their stems, washed with distilled water to remove dirt, sliced separately with knife into tiny pieces and dried separately at room temperature for three days (Essien *et al.*, 2023). The dried leaves were later ground separately using a clean, dry mortar and pestle and 400g each of sample were soaked in 100ml of 70% ethanol for 72 hours, with occasional shaking, at room temperature" for clarity and to improve extraction efficiency Essien *et al.*, 2022). The macerated leaves were separately filtered using Whatman No. 1 filter paper by means of a funnel. The filtrates were separately concentrated for three consecutive days in a water bath at a temperature of 400 – 500C after which slurry form of the extracts was obtained and preserved in a refrigerator at 40C for further use (Essien *et al.*, 2022).

2.2 Experimental Design, Grouping and Treatment of the Animals

A total of sixteen (16) healthy adult male albino Wistar rats weighing (185-221 g) were obtained from the disease-free stock of the animal house, Biochemistry Unit, Department of Chemical Sciences, Akwa Ibom State Polytechnic Ikot Osurua, Ikot Ekpene, Nigeria. The animals were housed in a cage with four sizable

compartments with wooden bottom and were mesh tops, randomly assigned four animals to each of four groups. The rats were maintained under standard conditions of temperature and natural light – dark circles for 7 days acclimatization in the animal house, Akwa Ibom State Polytechnic, Ikot Osurua. Groups 1 and 3 animals were treated with 200mg/kg of *M. idica* and *G. latifoluim* respectively. Group 2 animals were treated with the combined extracts at 50:50 dosage ratio and Group 4 was not treated and served as control. Treatments were administered daily via oral route for one month.

2.3 Collection of Blood sample and preservation of serum

At the end of one-month experimental period, the rats were fasted for 12hours and were anaesthetized under chloroform vapour and were sacrificed by dissecting medioventrically and blood was obtained via cardiac puncture using a syringe and needle into sterile EDTA sample bottles and then centrifuged at 3,000 rpm for 15 minutes to separate serum from plasma ((Essien *et al.*, 2023). The serum was used to determine the levels of liver enzymes (AST, ALT and ALP) and electrolyte profile (K^+, Na^+, Cl^- and HCO_3^-).

2.4 Methods

2.4.1 Determination of Aspartate Amino transferase (AST)

Serum AST was determined using the method by Reitman and Frankel (1975). Exactly 0.1 ml of distilled water and serum sample were measured into two sample tubes, labelled blank and substrate respectively. Next 0.5ml of the substrate prepared (R1) was also added to each of the two tubes, mixed and incubated for exactly 30 minutes at 37^0C. Thereafter, 0.5ml of 2, 4-dinitrophenyl-hydrazine solution (R2) was added to the tubes after incubation and allowed to stand for 20 minutes at room temperature, finally, 5ml of sodium hydroxide (0.4 mol) was added to each tube and mixed. Then absorbance of the sample was then read immediately against reagent blank at 546nm.

2.4.2 Determination of Alanine Transaminase (ALT)

The method of Reitman and Frankel (1957) was adopted to determine serum ALT. To two sample tubes labelled blank and sample, 0.1ml of distilled water and serum sample were measured into each respectively, and 0.5ml of the substrate prepared (R1) was also added, mixed and incubated at 37^0C for 30 minutes. Thereafter, 0.5ml of 2, 4-dinitrophenylhydrazine solution (R2) was added to all the tubes after incubation and allowed to stand at room temperature for 20 minutes. Finally, 5 ml of sodium hydroxide (0.4mol) was added to each tube and the absorbance of the sample was read against reagent blank with a spectrophotometer at 546nm wave length.

2.4.3 Determination of Alkaline Phosphatase (ALP)

Serum ALP was determined with the method of Englahondt (1970). To two sample tubes, 0.1ml of the sample was pipetted into each and 0.5ml of the reagent was added to the sample, mixed and the initial absorbance was read immediately while starting a timer simultaneously. Absorbance was read again at 1, 2- and 3-minute intervals all at 405nm, at room temperature.

2.4.4 Determination of Potassium (K^+)

The test tubes were labelled accordingly as sample, control, standard and blank, and 10µl potassium reagent was added to all tubes and allowed to mixed at room temperature for 3 minutes, after which the absorbance was read with spectrophotometer at 500nm (Nurminen *et al.*, 1998)

2.4.5 Determination of Sodium (Na^+)

The test tubes were labelled as blank standard and sample and 50µl of the sample was pipetted into the sample tubes and distilled water to the blank, the tubes were shaken vigorously and allowed to mix for 3 minutes the contents of the tubes were then centrifuged at 1500g for 10 minutes and the resulting supernatant filtered carefully to avoid disturbing the protein precipitate. Later, 50µl supernatant was pipetted into the various tubes and 1.0ml of acid reagent was added to each tube, after which 50µl of colour reagent was added to each tube. The absorbance of each of each sample was read at 550nm with a spectrophotometer (Tietz, 2006).

2.4.6 Determination of Chloride (Cl^-)

The test tube was labelled as sample, control, standard and blank. Later, 10µl sample was pipetted into the sample tubes and 10ml chloride reagent added to each and incubated at room temperature at 5 minutes. The absorbance of each tube was taken at 480nm with a spectrophotometer (Tietz, 2006).

2.4.7 Determination of Bicarbonate (HCO^{-3})

The carbon dioxide (CO_2) reagent was prepared by Teco diagnostics, 10µl of sample was pipetted into the sample tubes and 10ml of carbon (iv) oxide reagent into each tube and were incubated for 5 minutes at 37^0C after proper mixing. The absorbance was determined at 340nm with a spectrophotometer against the reagent blank (Tietz, 2006).

Calculation

The formula below was used to calculate the concentration of each of the electrolytes determined.

$$Concentration\ of\ Sample\ (MEq/L) = \frac{Absorbance\ of\ sample\ \times Concentarion\ of\ standard}{Absorbance\ of\ standard}$$

2.5 Mathematical Expressions and Symbols

Data obtained from the analysis were subjected to one-way analysis of variance (ANOVA). Statistically significant difference was obtained at ($P<0.05$) by the Bonferroni's multiple range test. The results were express as meant ± standard error of mean (SEM) estimated using statistical package of social science (SPSS) version 23.

3 Results

Table 1: Mean Serum Liver Enzymes in Albino Wistar Rats Treated with Ethanol leave Extract *Mangifera indica* and *Gongronema latifolium*

GROUP	AST (IU/L)	ALT (IU/L)	ALP(IU/L)
1	0.75±0.25	75.25±1.25	212.24±0.63
2	2.25±0.25	41.25±0.95	177.25±16.84
3	94.24±1.03	34.25±0.48	152.74±1.11
Control	0.25±0.25	6.75±1.12	262.50±1.32

Result presented as mean ±SEM (N=4)

Table 2: Mean Electrolyte Profile in Albino Wistar Rats Treated with Ethanol leave Extract *Mangifera indica* and *Gongronema latifolium*

GROUP	POTASSIUM (K+) (MEq/L)	SODIUM (Na^+) (MEq/L)	CHLORIDE (Cl^-) (MEq/L)	BICARBONATE (HCO_3^-) (MEq/L)
1	5.80±0.07	147.00±0.91	104.25±0.48	22.25±0.25
2	5.45±0.06	143.25±0.48	101.50±0.50	21.50±0.65
3	7.25±0.05	147.00±0.41	104.25±0.48	18.75±0.24
Control	3.15±0.06	145.50±0.65	101.25±0.75	19.75±0.25

Result presented as mean ±SEM (N=4)

4 Discussion

Liver enzymes viz; alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate amino transferase (AST) are enzymes produced and stored in great amounts in the liver and they offer an important approach to the diagnosis of disorders of the liver. On the other hand, electrolyte profile (panel) is a blood test that measures levels of body's main electrolytes namely; sodium (Na^+), Potassium (K^+), Chloride (Cl) and Bicarbonate (HCO_3^-) which are essential for basic life functions such as maintaining electrical neutrality in cells and generating and conducting action potentials in nerves and muscles. Most diseases are generated from the exposure of the overall metabolic or biochemical functions of the body's cells, tissues or organs to certain substances, such as drugs.

The results of this study revealed a significant increase in serum AST in group 2 animals treated with only *G. Latifolium* when compared to the control group. Significant increase was seen in serum AST levels in group 3 compared to group 1 treated with only *M. indica*. These revealed that treatment of the animals with *G. Latifolium* alone had a greater effect on the liver causing elevated AST more than the combine extracts treatment which seems to have exhibited an antagonizing effect against each other. Normal serum AST for human is 5 – 40 IU/L. Meanwhile, this result was not in agreement with the report by Izunya et al., (2010) who reported significantly decreased serum AST activity following treatment of mice with ethanol leave extract of *M. indica*. This may be due to the species of animals used for the study. ALT levels showed a significant increase in all treatment group when compared to the control group. But, a significant decrease was observed in groups 2 and 3 when compared to group 1. Normal serum ALT level for human is 7 – 55 IU/L. However, the results of this work were within this range which buttress the fact that the liver was able to handle *G. latifulium* and the combine extracts at that administered dosage, except M. indica which showed elevated concentration of ALT above the normal range, indicating liver injury. ALT and AST are

found in most tissues, though in relative amount in the liver, but AST in the heart (Emeka and Obiora, 2019). Hence, increased or elevated ALT marks for liver abnormality (injury).

The results of serum level of ALP showed a significant decreased in groups 1, 2 and 3 when compared to the control. This is in accordance with the report of Izunya et al. (2010) which showed a significant reduction in the activity of serum ALP in rats treated with ethanol leave extract of *M. indica*, that was an indication that the plant had the ability to protect rat's hepatocytes from damage. Normal serum level of ALP for human ranges from 20 – 140 IU/L. This result as proven that single administration of either of the extracts on the animals may affect the liver causing leakage of the hepatic contents. More so, the significant reduction in the co-administered group might have resulted from plant extract antagonizing themselves, thus, preventing to elicit a synergistic effect or may be due to the present of some bioactive compounds that might have performed antioxidant activity by counterbalancing the effect of the free radicals generated during normal cellular metabolism in the animals.

Furthermore, the impact of single and combined administration of ethanol leave extracts of the two plants on electrolyte panel (Na^+, K^+, Cl^- and HCO_3^-) revealed a significant increase in serum potassium (K^+) ion level in treatment groups 1, 2 and 3 when compared to the control group. Significant increase was also recorded in treatment group 3 when compared to groups 1 and 2. Moreso, significant increase in serum K^+ level was observed in group 1 when compared to group 2. These results implied that single treatment with *G. latifulium* extract resulted in an increase serum K^+ level compared to treatment with *M. indica* as well as the one treated with combined extracts. Meanwhile, serum K^+ levels in groups 1 and 2 were slightly above normal range of 3.5 - 5.3 MEq/L for human, whereas group 3 animals recorded elevated serum K^+ level above normal range. Here, the significant increase in serum K^+ level in group 3 might have been due to the shock *G. latifulium* exerted on the liver. It might have assaulted the liver acutely thereby leaking the contents into the blood circulation, resulting in elevated serum K^+ level above the normal range. The value obtained in this study ranges from 5.45 – 7.25 MEq/L. This condition is termed hyperkalemia which is potentially life – threatening; the most common cause of kidney failure. It may also result from severe burns or injury (excessive potassium released from injured cells), inadequate adrenal hormones (Addison's disease), the use of certain medications such as excessive use of potassium supplement (Krismamuri and Shyamala, 2001). This result is in line with findings by Ugochukwu et al. (2003), who reported toxic effect of *G. latifulium* on serum electrolytes which resulted in an increased serum potassium level at 175 and 350 mg/kg doses.

Serum sodium (Na^+) level in group 2 co-treated with the two leaf extracts recorded significant decrease when compared to groups 1, 3 and control. This result may be attributed to the ability of the kidneys to utilize and excrete adequate electrolytes from the body. Sodium is the major positive ion in the extracellular fluid, regulating the total amount of water in the body (Ezekwesili *et al.*, 2008). Normal serum sodium levels range from 135-145 MEq/L; however, the level obtained in this work was 143.25-147.00 MEq/L. The value of the control fell within the normal range. Meanwhile, serum sodium levels less than 129MEq/L are termed hyponatremia and is considered severe when it is below 120MEq/L (William, 2001). The difference is Na^+ and K^+ levels in this work is a clear known fact that sodium and potassium ions work and operate inversely as a result of the activity of Na^+/K^+ pump driven by the Na^+/K^+ ATPASE (Carbone *et al.*, 2005; Ali and Ibiam, 2011).

Serum chloride (Cl^-) ion level showed a significant increase in group 1 when compound to groups 2 and control, with a significant increase in group 3 compared to groups 2 and control with a significant increase in group 3 compared to group 2 and control. Meanwhile, the range of serum Cl^- in this study (101.50-104.20 MEq/L) was within the normal range for human (97-107 MEq/L). Chloride is involved in regulating blood pressure. High concentration in blood is termed hyperchloremia. This could be as a result of kidney failure or dialysis, and overproduction of parathyroid hormones (Kenney, 2004). Furthermore, the result of this work also revealed a significant increase in serum bicarbonate (HCO_3^-) level in group 1 when compared to

groups 3 and control, with a significant increase in group 2 compared to group 3. Bicarbonate is a chemical (buffer) that keeps the pH of blood in check (Schmidt, 2010). However, elevated or depressed bicarbonate level suggest problems in maintaining acid/base balance or disturbances in electrolyte level perhaps by ions or retention of fluid (Rebbolz *et al.* 2021; Ali and Ibiam, 2011). Bicarbonate levels obtained in this study were within the normal limit (22 – 30 MEq/L) (Schmidt, 2010). Meanwhile, serum bicarbonate levels in the work ranged from 18.75 – 22.25 MEq/L. this result agrees with the report by Abdulahi et al. (2015), suggesting that *M. indica* and *A. paniculata* leaves have therapeutic efficacy by maintaining the levels of bicarbonate, sodium, potassium and chloride toward normal at moderate dose use. However, the slight variation in this work may be due to the combination of *M. indica* with *G. latifulium* instead of *A. paniculata.*

5 Conclusion

There was no adverse impact of ethanol leaf extracts of *Mangifera indica* and *Gongronema latifulium* at single or combined treatment on serum liver enzymes and electrolyte profiles and male albino Wistar rats, though the extracts should be used with moderation in human treatment of diseases.

6 Ethical Approval

Animals ethic committee approval has been collected and preserved by the authors.

7 Competing Interests

Authors have declared that no competing interest exist.

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