

Comparative Effects of the Aqueous Extracts of *Gongronema Latifolium* and *Tetrapleura Tetraptera* on Serum Liver and Kidney Markers as well as Glucose Tolerance Test of Albino Rat

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ABSTRACT

The study investigated the effect of aqueous extract of *G. latifolium* and *T. tetraplura* on serum liver and kidney markers as well as glucose tolerance test of albino Rat. A total of twenty five male albino rats were randomly grouped into five groups. Group 1 served as control, Group 2 and 4 received 200 and 400mg/kg *G. latifolium* respectively. Animals in Group 3 and 5 received 200mg/kg and 400mg/kg *T. tetraplura* respectively for 28days. Result for AST was significantly ($P<0.05$) higher in group 5 (43.000 ± 3.033) when compared to the control group (30.400 ± 2.158). ALT concentration was also significantly ($P<0.05$) in group 5 (34.600 ± 1.077) when compared to the control (22.400 ± 1.326). Result for kidney function parameters showed a significant ($p<0.05$) increase in urea concentration of the group 5 (26.962 ± 0.6340) when compared to the control (24.242 ± 1.875) From the obtained results, it could be concluded that *T. tetraplura* could induce hepatotoxicity and nephrotoxicity. Also, from obtained results, the spices possess hypolipidemic and antioxidant activity. These ability of the spices to carry out these activities can be attributed to it rich phytochemical constituents.

INTRODUCTION

Medicinal plant are the most important source of life saving drug for the majority of the world population (Pripathi and Tripathi 2003). Plant have been an important source of medicine for thousands of years and even today, the world health organization estimate that up to 80% of people still rely on traditional remedies such as herbs for their medicine (Robert, 1988).

Gongronema Latifolium is widely used in West Africa for medicinal and nutritional purposes. An infusion of the aerial parts is taken to treat cough, intestinal worms, dysentery, dyspepsia and malaria. it is also taken as a tonic to treat loss of appetite. In Sierra Leone, an infusion or decoction of the stems with lime juice is taken as a purge to treat colic and stomach-ache. In Senegal and Ghana the leaves are rubbed on the joints of small children to help them walk. The boiled fruits in soup are eaten as a laxative. In Nigeria a leafy stem infusion is taken as a cleansing purge by Muslims during Ramadan. A decoction of leaves or leafy stems is commonly taken to treat diabetes and high blood pressure.

Tetrapleura Tetraptera, one of the medicinal plants in Nigeria, is known in the South Eastern Nigeria as *uhiokiriho*. The documented biological or pharmacological activities are found to be molluscicidal, cardiovascular, neuromuscular, hypotensive, anti-conversant, anti-ulcerative, anti-inflammatory and anti-microbial (Enwere, 1998). The pods notably have an appealing culinary use for mothers from the first day of delivery (post parturition) and as a lactation aid (Enwere.,1998). The pods notably have an appealing culinary use for mothers from the first day of delivery to post parturition and as a lactation aid (Enwere,1998).

The dry fruit has a characteristic pleasant aroma which makes it a popular seasoning spice in the southeastern Nigeria (Essien *et al.*, 1994; Okwu., 2004). At the same time, most of the folkloric chains agree in the traditional use of the fruit for management of convulsion, leprosy, inflammation and rheumatoid pains. *Tetrapleura tetraptera* is deciduous plant, it reaches 20-25 m in height, with a girth of 1.5-3 m. The bole is slender and older trees have very small, low, sharp buttresses. In the forest, the crown is fairly small, thin and rounded, becoming flat when old, but it tends to spread when in the open. Bark fairly smooth, grey-brown, and

very thin, slash reddish, strong smelling, fairly thick. Twigs and young foliage virtually glabrous or minutely hairy. Leaves are sessile, glabrous or minutely hairy with a common stalk 15-30 cm long, slightly channeled on the upper surface. Flowers are pinkish-cream turning to orange and are densely crowded in spikelike racemes 5-20 cm long, usually in pairs in the upper leaf axils. Fruit is very persistent, hanging at the ends of branches on stout stalks 25 cm long. It is shiny, glabrous, dark purple-brown, usually slightly curved; 15-25 cm long by about 5 cm across, with 4 longitudinal, winglike ridges nearly 3 cm broad. Two of the wings are woody, the other 2 filled with soft, sugary pulp, oily and aromatic. The seeds, which rattle in the pods, are small, black, hard, flat, about 8 mm long, embedded in the body of the pod, which does not split open. The kernel contains oil. The generic name comes from a Greek word meaning 'four ribs', referring to the ribbed fruit. The specific epithet means four winged. Orwa (2009).

ANTIOXIDANT

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C. Additionally, selenium, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category. The body cannot manufacture these micronutrients so they must be supplied in the diet.

The antioxidants zinc, vitamin C, and vitamin E play an important role in protecting cells and neutralizing free radicals during exercise. Antioxidants are found in many plant based foods—including green leafy vegetables, citrus fruits, legumes, nuts, grains, seeds, and oils—and are added to commercial foods as well as sports supplements and gels to extend their shelf life (Mcginle *et al.*, 2009)

It is well accepted that high-intensity exercise can result in damage to active muscle fibers, which is exhibited by soreness, stiffness, and a reduction in the force-producing capability of the muscle (muscle fatigue). Exercise produces reactive oxygen species, or free radicals, and it has been hypothesized that antioxidants enhance performance by scavenging these radicals (Stear *et al.*, 2009).

Aspartate Amino Transferase (Ast)

Aspartate Amino Transferase (AST) also called serum glutamate oxaloacetate transaminase is a cytoplasmic and mitochondrial enzyme widely distributed in the body tissues such as skeletal muscle, kidney and brain and also in the liver and heart where it can be found in higher concentrations. Hepatocellular damage possibly due to viral attack or any damage causes an increase in circulating AST. Generally high serum levels indicate extensive damage. Thus in acute myocardial infarction shows moderate increase starting after 6-8 hours of onset reaching approximately 5-10 times the normal value at about 48 hours and returns to normal between 4-6 days.

Alanine Aminotransferase (Alt)

Alanine Aminotransferase (ALT) which is also known as serum glutamate pyruvate transaminase, is found principally in the liver with only small amounts being present in other organs. It is used as a clinical marker for liver disease i.e. hepatocellular damage and other disease associated with hepatic necrosis. Alanine amino transferase is a better marker for liver disease than Aspartate Aminotransferase as it persists longer and is rarely observed in conditions other than Parenchymal liver disease characterized by hepatocellular damage. In infectious hepatitis and other inflammatory conditions that affect the liver, Alanine Aminotransferase is characteristically high.

Collection and Identification of Sample

Gongronema latioflum and *T.tetraptera* were bought from Eke market in Izhia, Ohankwu Local Government Area, Ebonyi state, Nigeria. It was identified by a plant taxonomist in the Department of plant and Biotechnology. Science in Micheal okpara university, umudike Nigeria.

Preparation of Extract

Gongronema Latioflium T.Teaptera fruits were weighed. Wet weight (10000kg and sun dried for seven days to get the corresponding dry weight 6000).the dried fruit were milled with a local grinder and the flour which directly macerated with cold water for 2 to 3 days with intermittent shaking and turning to facilitate extraction at a ratio of 340g each of the sample into 500ml of water.

The mixture was first filtered with a muslin cloth and then with a filter paper. The resultant filtrate was dried in a hot water bath at 50 stock solution of the extract was stored in a refrigerator (thermocool Nigeria). the volume of stock was administered based on body weight was calculated using the formular.(Nwafor *et al.*, 2009).

$$\text{Volume} = D \times P / C$$

Where D= Dose to be administered.

P= body weight of animal weight.

C= concentration of the stock.

Animal grouping

A total of 25 weaned male rats of weighing between 140-175g, of four to six weeks were purchased from Veterinary Research Institute, Umudike, Nigeria.

The animals were stored in iron cages of five animals per group and allowed to acclimatize for two weeks under humid tropical condition in the animal house in the department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia state, Nigeria. The rats were exposed to 12 hrs light/dark cycle and they were given clean tap water and normal rat feed throughout the acclimatization period.

Animal Feeding

The animals were randomly divided according to body weight into five groups represented in the table below.

Group	Feed composition
Group A	Normal Rat Feed
Group B	received 200mg/kg of aqueous extract of <i>Gongronema latifolium</i> with body weight.
Group C	received 200mg/kg of aqueous extract of <i>T.tetraptera</i> with body weight.
Group D	received 400mg/kg of aqueous extract of <i>Gongronema latifolium</i> with there body weight.
Group E	received 400mg/kg of aqueous of <i>T.tetraptera</i> with there body weight.

Liver Enzyme

Determiration of Alanine –S-Amino Transferase (Randox Kit)

500ml of Ransod solution (R₁) was pipetted into a test tube and 100ml of sample was subsequently added. The mixture was incubated for 30minutes at 37°C. subsequently 500ml of Ransodsolution (R₂)was added. The mixture was incubated for 20 minutes at 25°C. Finally 5.0ml of NaOH solution was added to the mixture before the absorbance of the sample against a reagent blank at 546nm was read.

Aspartate S- Amino Transferase Determiration (Randox Kit)

100ml of the sample with 500ml of the reagent 1, the mixture was mixed, incubated for 30minutes at 37°C.Subsequently,500ml of the reagent R was added to the mixture which was mixed and allowed to stand for 20minutes at 25 °C. The absorbance was read of sample against reagent blank after 5minutes at 546nm.

Statistical Analysis of the Research Data

Results are expressed as mean \pm standard deviation and all data were subjected to analysis of variance (ANOVA) as described by Steel and Torrie, (1960). Significant differences between the group means were detected at 5% confidence level using Duncan’s multiple range test (Duncan, 1955).

RESULT AND DISCUSSION

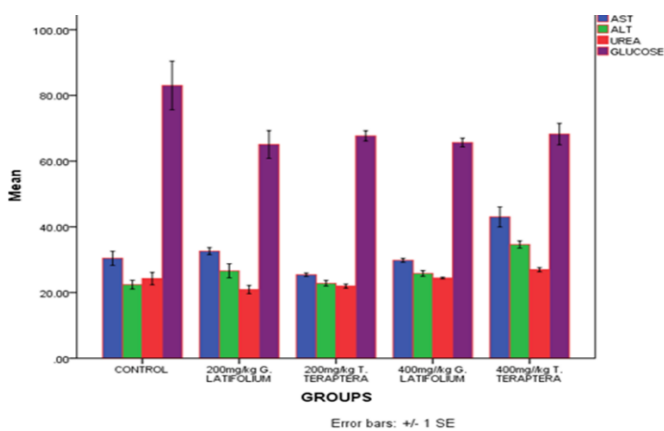
Result

Table 1: Effect of *Gongronema Latifolium* and *T. Tetraplura* on serum liver function marker albino rat.

Group	AST(U/l)	ALT(U/l)	Total Protein(mg/dl)	Bilirubin(mg/dl)
Group I Control	30.400 \pm 2.158	22.400 \pm 1.326	5.314 \pm 0.1767	1.092 \pm 0.1180
Group II 200mg/kg/bwt of <i>G. latifolium</i>	32.600 \pm 1.077	26.600 \pm 2.135	5.529 \pm 0.1894	0.6964 \pm 0.0103
Group III 200mg/kg/bwt of <i>T. tetraplura</i>	25.400 \pm 0.5099	22.800 \pm 0.860	5.230 \pm 0.2134	0.9240 \pm 0.1245
Group IV 400mg/kg/bwt of <i>G. latifolium</i>	29.800 \pm 0.5831	25.800 \pm 0.8602	5.656 \pm 0.1634	0.7220 \pm 0.3550
Group V 400mg/kg/bwt of <i>T. tetraplura</i>	43.000 \pm 3.033	34.600 \pm 1.077	5.816 \pm 0.2298	0.7260 \pm 0.2830
Total	32.240 \pm 1.398	26.440 \pm 1.0505	5.522 \pm 0.0916	0.8321 \pm 0.4511

Values are means \pm standard deviation of duplication determination.

Serum liver function markers tested were AST, ALT, TP, Bilirubin Group v shows a significant increase of (P>0.05) when compared to group 1 which is the control, and other groups at a comparable range.



Graph representation of AST, ALT, UREA and GLUCOSE

DISCUSSION

LD₅₀ of *G. latiflioum* and *Tetrapleura Tetraptera* do not produce any death during acute toxicity period even at 5000mg/kg body weight as all treated animal appears physically healthy and emotional stable following

treatment with varying dose level of the extracts. These suggest that both *G.latiflioum* and *Tetrapleura Tetraptera* are safe and do not posse potential or toxicity. The OECD guideline for acute toxicity studies had state that death is the only sign of acute toxicity and hence lack of death even at a dose that ordinary should produce death indicate the high level safety for the agent under study (OECD 2001).No wonder both *G.latiflioum* and *Tetrapleura Tetraptera* over the years had serves as both food and medicine in Nigeria .(Nwanjo *et al.*, 2006).although Ugochukwu and Babady,(2002).reported that dose of ethanol extract of *Tetrapleura Tetraptera* cause dead in laboratory animals these difference may attribute to the extraction solvent.

The most common parameters used to assay liver function are AST, ALT, and ALP (Tolman and Rej, 1999; Hilaly *et al.*, 2004) with the AST and ALT being used for diagnosis of underlying cellular injuries (Karthikeyan *et al.*, 2006). ALT which is localized primarily in the cytosol of the hepatocytes is a liver specific enzyme in dogs, rats and primate (Farah *et al.*, 2011). It is a more sensitive marker for liver damage when compared to AST and can provide a quantitative evaluation of extent of damage sustained by the liver (Al mammary *et al.*, 2002). The ALT activities of the groups that received 200mg *Gongronema latifolium* (group 2), 200mg *T. tetraplura* (group 3), and 400mg *Gongronema latifolium* (group 4) were all within comparable range compared to the control group (group 1) whereas the ALT activity of group that received 400mg *T. tetraplura* (group 5) showed a significant ($p<0.05$) increase compared to that of the control group (group 1) which could be indicative possible liver injury.

AST is an enzyme found in the cytoplasm and mitochondria of tissues such as heart, skeletal muscles, liver kidney and erythrocytes (Aniagu *et al.*, 2004). The AST activity of the group that received 200mg *Gongronema latifolium* (group 2) was significantly ($p<0.05$) higher compared to that of the control group suggesting possible liver damage. There was also a significant ($p<0.05$) increase in the AST activity of the group that received 400mg *Gongronema latifolium* (group 4) compared to the control group also suggesting possible liver injury. However, the AST activity of the groups that received 200mg *T. tetraplura* (group 3) and 400mg *T. tetraplura* (group 5) were not significantly different. AST and ALT are often diagnostic of underlying cellular injuries (Karthikeyan *et al.*, 2006).

Liver is the primary site of the synthesis of plasma proteins. A disturbance of protein synthesis therefore occurs as a consequence of impaired hepatic function which will lead to a decrease in their plasma concentration (Okediran *et al.*, 2014). Increase in serum total protein has been attributed to increased hepatic protein synthesis (Ozolua *et al.*, 2009) while its reduction has been linked to possible liver damage (Yakubu *et al.*, 2003).

CONCLUSION

Researches in herbal medicine have attained an incredible global level in the recent past. The application of some plants constituents in pharmaceutical industries has gone a long way in the elevation of the status of traditional herbal medicine in Africa and in Nigeria in particular. The use of herbal drugs by elites and non-elites for the treatment and prevention of disease in south eastern parts of Nigeria is very common particularly in rural areas. The results obtained from analysis of some biochemical profile (sodium, potassium, cholesterol, triglyceride, AST and ALT activity *T. tetraptera* treated rats have shown that these spices have hypolipidemic, and hypokalaemic effect. The spices possess hypolipidemic bioactive component(s). *Gongroneam Latifolium* and *T. tetraptera* possess no or low hepatotoxic activity. They were also observed to contain high antioxidant potentials and justifying their therapeutic uses which could be utilized in drug formulation.

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