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Nephroprotective and Anti-hyperlipidemic Effects of Methanol Leaf Extract of *Gongronema latifolium* (Utazi) against Thioacetamide-induced Renal Injury in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to evaluate the nephroprotective and anti-hyperlipidemic potential of methanol leaf extract of *Gongronema latifolium* (utazi) in albino rats. Freshly harvested leaves of *G. latifolium* were processed into fine powder with which extract was formed. Twenty adult male albino rats were divided into four groups of five rats per group. Group I was the normal control and was administered with 2 ml of distilled water. Group III and IV were pretreated with 200 and 400 mg/kg of extract respectively for 28 days prior to oral administration of 100 mg/kg of thioacetamide (TAA)

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on groups II, III and IV. Animals were denied food overnight and subsequently sacrificed by cervical dislocation. Collected blood samples were analyzed using standard procedures. The result obtained from the qualitative phytochemical analysis performed on the leaf of *G. latifolium* revealed that saponins and flavonoids are the most abundant of the phytochemicals reportedly present. Oral administration of methanol leaf extract of *G. latifolium* significantly ($p<0.05$) demonstrated the potential to normalize serum lipid profile, as well as indices of renal functions. In conclusion, *Gongronema latifolium* leaf is nephroprotective and anti-hyperlipidemic.

Keywords: Nephroprotective; anti-hyperlipidemic; gongronema latifolium; flavonoids.

1. INTRODUCTION

The liver, likewise the kidneys, is one of the crucial organs of the body saddled with the task of ridding the body of metabolic waste products [1]. Very often, human beings are intentionally or unintentionally exposed to a wide array of chemical agents that harm these delicate and sensitive organs which results in injury to the said organs and consequently impair their ability to optimally perform their metabolic functions. An estimated 10% of the global populations are victims of this problem [2]. On the other hand, the role of the liver in the synthesis of lipids cannot be overemphasized, and when injured, may not be able to handle lipid homeostasis.

Conventionally, dialysis and transplant are the most frequently employed procedures for individuals with these problems. Unfortunately, there are characterized by shortcomings such as immunological rejection of kidney grafts, immune suppression, and its attendant consequences [3].

The use of plant-based therapies in the treatment of human ailments dates back to prehistoric times. Its use is widespread, evident by the fact that an estimated 80% of the populations in developing countries depend solely on it to meet their health needs [4]. *Gongronema latifolium* is a climbing plant known for its broad, heart-shaped leaves with a characteristic sharp, bitter and slightly sweet taste, especially when eaten fresh. The stems have soft and hairy, yielding milky latex or exudates [5]. It belongs to the *Asclepiadaceae* family. *Gongronema latifolium,* locally known to the South-Easterners of Nigeria as Utazi and commonly known as amaranth globe leaf, is an edible rainforest plant [5]. In folk medicine, it is considered a medicinal spice and vegetable owing to the fact that it has been successfully used in the treatment of diseases such as diabetes [6,7]. In South-Eastern Nigeria, the leaf is employed in soup making for mothers who have recently given birth. it is believed to stimulate appetite, reduce post-partum contraction, and enhance the return of the menstrual cycle [6]. The crude extract of *G. latifolium* is used in the treatment of malaria, hypertension, and laxative [6]. Research efforts have shown that the leaf of this plant contains essential oil, fibre, and essential phytochemicals such as saponins, alkaloids, flavonoids, among others [8]. The plethora of existing data on the therapeutic values points to the fact that the plant could be further explored for more therapeutic benefits. Hence, the importance of this study is defined.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh leaves of *Gongronema latifolium* (Utazi) were harvested from a farm in Uturu in Abia State, Southeast Nigeria. The leaves were subsequently identified at the herbarium unit of the Department of Forestry, Michael Okpara University of Agriculture, Umudike Abia State Southeast Nigeria.

2.2 Processing and Extraction of Plant Material

Leaves of *G. latifolia* were thoroughly washed with tap water. The leaves were dried at room temperature and afterwards, dried and ground into fine powder. 500 g of powdered *G. latifolia* leaf sample was steeped in one litre of 50% methanol for a period of 72 h. The mixture was shaken twice daily. The solvent was filtered over a layer of gauge, and then the filtrate evaporated to dryness in vacuo at 55°C.

2.3 Phytochemical Analysis

Extract derived from leaf of *Gongronema latifolium* (Utazi) was assayed to identify the quantity of phytochemicals present in accordance with the method described by Trease et al. [9].

2.4 Animals

Adult male Wistar rats weighing 120-150 g were purchased from the Animal House of the Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Uwana, Afikpo. The rats were housed in aluminium cages under standard laboratory conditions. They were given food and water *ad-libthum.* Acclimatization lasted for 14 days.

2.5 Median Lethal Dose 50% (LD50)

Determination of median lethal dose 50% involved two phase of the experiment. At the initial phases, nine adult male Wistar rats were divided into three groups of three rats each of which was separately administered with 10, 100 and 1000 mg/kg of extract orally. Animals were observed for 24 h for signs of toxicity. Owing to the fact that mortality was not observed after the first phase, the second phase comprising another three groups of one rat each was separately administered with 1600, 2900 and 5000 mg/kg of extract, after which animals were observed for 48 h for signs of toxicity according to Lorke [10].

2.6 Experimental Design

Twenty adult albino rats were starved of food for 24 h prior to the commencement of experiment. The rats were divided into five groups of five rats per group.

Group 1: (Normal control) rats were administered 2 ml of distilled water

Group 2: Rats were administered TAA without treatment

Group 3: Rats were pretreated with 200 mg/kg of MEGLU

Group 4: Rats were pretreated with 400 mg/kg of MEGLU

Pretreatment with extract lasted for 28 days during which the body weight was determined on weekly basis, while TAA was administered on the 28th day by a single dose subcutaneous injection of 100 mg/kg of TAA. Animals were denied food overnight, and subsequently sacrificed by cervical dislocation. Collected blood samples collected in plain tubes for analysis.

2.7 Biochemical Analysis

To determine creatinine and urea, 2 mL of blood introduced into plain tube was subjected to centrifugation at 4,000 rpm for 15 min and the plasma obtained was stored for biochemical analysis. Kits were used to determine the levels of urea and creatinine.

2.8 Determination of Lipid Profile

Cholesterol, HDL and triacyglyceride levels were estimated from serum by CHOD-PAP. LDL and HDL were calculated. While the artherogenic index was calculated using the method described by Muruganandan et al. [11].

2.9 Statistical Analysis

Data obtained from the study were expressed as mean ± standard deviation using SPSS (Ver. 23). Data were analysed using one way analysis of variance (ANOVA). Variation in mean values was compared using Turkey Test. *P-values* less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

Liver and and kidney are the two major organs involved in the detoxification and elimination of xenobitics [1]. Owing to which they are adjudged the most susceptible organs to the toxic influence of foreign substances. Table 1 shows the qualitative phytochemical composition of methanol leaf extract of *Gongronema latifolia* indicating that saponins and flavonoids are most abundant of all the phytochemicals reportedly present, while glycosides and phenols are the least abundant phytochemicals in the leaf of *G. latifolia*. Table 2 shows the lipid profiles of rats administered with the aqueous methanol leaf extract of *G. latifolium* indicating that the oral administration of thioacetamide (TAA) increased the levels of triacylglyceride (TG), total cholesterol (TC), high density lippoprotein (HDL), and low density lippoprotein (LDL). However, administration of 400 mg/kg of the methanol leaf extract of *G. latifolium*, resulted in a significant ($p<0.05$) reduction in the levels of the aforementioned lipids, although they were still significantly ($p<0.05$) higher than those reported for the normal control. The ability of the extract to maintain a stable lipid profile could be attributed to its phytochemical constituents some of which may possess antioxidant properties. This result is

consistent with the result of a research conducted by Rosemary et al. [12] which demonstrated the hypoglycemic and hypolipidemic effect of *Gongronema latifolium* extract on healthy subjects. Biochemically, a pronounced elevation in the levels of renal parametres is suggestive of renal alterations Table 3 presents the renal function markers of rats administered with *G. latifolia* leaf extract, showing that serum urea and creatinine levels were significantly (P<0.05) increased in Group II following the induction of renal damage with oral administration of TAA. However, a contrary observation was made on Groups III and IV pretreated with the extract prior to administration of TAA. The observed decrease in serum creatinine and urea levels in groups III and IV could be attributed to the effect of the reactive oxygen species (ROS) generated by TAA. This finding is consistent with the outcome of the study by Omodale et al. [13], which demonstrated that *G. latifolium* root extract protected against kidney damage. Table 4 shows body weight changes in rats administered with the methanol leaf extract of *G. latifolia* indicating that the body weight of rats at the 4th week of feed intake was significantly (p<0.05) higher than that reported at week 1 for Groups I, III and IV. However, a contrary observation was made in Group II.

Table 1. Qualitative phytochemical composition of *Gongronema latifolium* (Utazi)

Phytochemicals	Abundance
Saponins	+ ++
Tannins	+
Flavonoids	+ ++
Alkaloids	+ +
Glycosides	+
Phenol	+

+ [abundant], ++[more abundant], +++[most abundant]

Table 2. Lipid profile of rats administered aqueous stem bark *Gongronema Latifolium* (Utazi)

Treatment	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Normal Ctrl (2 ml of distilled H_2O)	200.00±5.22^{a}	60.00±5.51^{a}	41.32±3.01^{a}	127.34±5.02^{a}
Negative control (induction without treatment)	270.00±6.64^{d}	100.00±1.15^{e}	70.23±5.60^{d}	203.76±2.08^{c}
MEGLU $_{200}$ mg/kg TAA_{100} mg/kg	226.37±0.89^{cd}	77.01±4.80^{d}	63.03±2.82^{c}	132.32±2.42^{bc}
MEGLU $_{400}$ mg/kg+TAA_{100} mg/kg	223.86±5.28^{c}	73.42±2.30^{c}	60.00±3.72^{b}	129.76±4.82^{b}

Results are expressed as mean ± standard deviation from five determinations. Values with the same superscript in a column are not significantly different (P<0.05)

Table 3. Renal function markers of rats administered with *G. Latofolia* (Utazi)

Groups	Urea	Creatinine
Normal Ctrl (2 ml of distilled H_2O)	5.63 ±0.73^{a}	74.22±6.280^{a}
Negative control (induction without treatment)	6.34±0.70^{c}	88.20±4.3^{c}
MEGLU $_{200}$ mg/kg+TAA_{100} mg/kg	5.92±0.91^{b}	77.00±3.52^{b}
MEGLU $_{400}$ mg/kg+TAA_{100} mg/kg	5.88±0.61^{ab}	77.01±5.541^{b}

Results are expressed as mean ± standard deviation from five determinations. Values with the same super script in a column are not significantly different (P<0.05)

Table 4. Body weight changes in rats administered with methanol leaf extract of *Gongronema latifolium*

	Body weight			
Groups	WK 1	WK 2	WK 3	WK 4
Control	150.0±3.48^{a}	159.0±5.83^{ab}	165.6±6.82^{b}	168.4±6.73^{b}
Negative control	148.3±2.56^{c}	147.2±4.78^{c}	144.6±3.45^{b}	140.3±5.34^{a}
MEGLU $_{200}$ mg/kg TAA_{100} mg/kg	156.2±4.23^{a}	164.1±7.18^{b}	172.0±4.34^{c}	175.2±6.62^{c}
MEGLU $_{400}$ mg/kg+TAA_{100} mg/kg	156.4±3.39^{a}	158.0±2.72^{a}	160.6±4.21^{a}	166.0±3.37^{b}

Results are expressed as mean ± standard deviation from five determinations. Values with the same superscript in a column are not significantly different (P<0.05)

4. CONCLUSION

The study evaluated the nephroprotective and anti-hyperlipidemic potential of methanol leaf extract of *Gongronema latifolium* (utazi) in Wistar rats. The finding revealed that saponins and flavonoids are the most abundant of the phytochemicals reportedly present. The study concludes that *Gongronema latifolium* leaf is nephroprotective and anti-hyperlipidemic.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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