



Keywords

Antioxidant,
Enzymes,
Free Radicals,
Gongronema latifolium,
Oxidative Stress

Received: April 24, 2017

Accepted: May 8, 2017

Published: August 3, 2017

Protective Effects of Ethanolic Leaf Extract of *Gongronema latifolium* on Antioxidant Indices of Acetaminophen-Induced Toxicity in Albino Rats

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Citation

Chinedu Imo. Protective Effects of Ethanolic Leaf Extract of *Gongronema latifolium* on Antioxidant Indices of Acetaminophen-Induced Toxicity in Albino Rats. *Health Sciences Research*. Vol. 4, No. 2, 2017, pp. 6-9.

Abstract

This study examined the protective effects of ethanolic leaf extract of *Gongronema latifolium* on selected antioxidant indices of acetaminophen-induced toxicity in albino rats. Fifty healthy male albino rats were used in this study and were randomly placed into five groups with ten rats in each group. The leaf extract was administered accordingly to groups 3, 4 and 5 through gavage intubation. At the end of the administration of the extracts, blood was collected by cardiac puncture for biochemical analysis. The results show that antioxidant enzymes: Catalase, SOD, GST and GSH reduced, while MDA increased in the group administered acetaminophen only, but the antioxidant enzymes increased, while MDA decreased in all groups administered the leaf extract. Catalase and SOD increased from 2.35 ± 0.12 to 6.86 ± 0.29 $\mu\text{mol}/\text{mg}$ protein and 1.46 ± 0.09 to 3.59 ± 0.23 $\mu\text{mol}/\text{ml}$ respectively, while GST and GSH increased from 0.40 ± 0.02 to 0.97 ± 0.04 $\mu\text{mol}/\text{ml}$ and 0.61 ± 0.02 to 1.64 ± 0.02 $\mu\text{mol}/\text{ml}$ respectively. MDA reduced from 4.64 ± 0.33 to 1.57 ± 0.23 $\mu\text{mol}/\text{ml}$. These results indicate that the leaf extract of *Gongronema latifolium* has protective effect on the indices evaluated and can be used as an antioxidant agent against the toxicity induced.

1. Introduction

Many medicinal plants are reported to have potential anti-inflammation activity [1]. Tiwari and Rao [2] reported that the different composition of the active components in plants give medicinal plants an edge as better therapeutic agents than chemotherapy in management of different ailments such as atherosclerosis, hypertension and diabetes. In this study, a medicinal plant *Gongronema latifolium* Benth which has potent anti-inflammatory activity was used.

Gongronema latifolium Benth belongs to the family of Asclepiadaceae. It is an edible plant with soft and pliable stem and bitter leaves. It is widely used in most part of African region for a number of acclaimed medicinal and nutritional purposes. Its common name is amaranth globe. *Gongronema latifolium* is commonly called "utazi" and "arokeke" in South Eastern and South Western Nigeria respectively. It is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine [3, 4]. The leaves are used to prepare food for mothers that have recently put to bed, where it is

believed to stimulate appetite, reduce post-partum contraction and enhance the return of the menstrual cycle. *Gongronema latifolium* has been reported not only to possess hypoglycemic activity, but also hypotensive, hepatoprotective and hypolipidemic effects [3, 5].

In an earlier study into the phytochemicals present in the leaf of *Gongronema latifolium*, we (Imo and Uhegbu, [6]) reported the presence of different types of Alkaloids, Flavonoids, Total phenolic compound, Lignan, Terpenes, Sterol, Allicin, Hydroxycinnamic acids, Saponin and Carotenoid. *Gongronema latifolium* leaf possess an appreciable level of phytochemicals. It could be a good raw material for the production of some medicinal drugs and can be used in folk medicine for the treatment of some diseases [6]. Egbung *et al.* [7] reported the presence of mineral elements (Cr, Cu, Se, Zn and Fe) and vitamins (A, C, riboflavin, niacin and thiamine) in the root, bark and twig extracts.

The rate at which acetaminophen bought over the counter is used for self-medication without prescription is increasing every day. Overdose of acetaminophen may result to different biochemical changes in a patient [4]. Aerobic organisms developed a complex network of antioxidant defense system as a protection against harmful effects of reactive oxygen species in order to maintain tissue homeostasis. The antioxidant defense system primarily includes the antioxidant defense enzymes such as: superoxide dismutases, catalase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase. When reactive oxygen species generation exceeds the antioxidant capacity of cells, oxidative stress develops, potentially causing tissue damage [8]. With the chemical antioxidants, cells are protected against oxidative stress by an interacting network of antioxidant enzymes. Therefore, the use of *Gongronema latifolium* as an antioxidant protecting agent against acetaminophen toxicity warrants research.

2. Materials and Methods

2.1. Acetaminophen (Paracetamol)

Acetaminophen (product of Emzor Pharmaceutical Industries Ltd., Nigeria) was purchased from a pharmacy shop (Ndukwe Family Chemist Nig. Ltd.) in Umuahia, Abia State, Nigeria.

2.2. Plant Material and Extraction

The leaves of *Gongronema latifolium* were harvested at Itaja-Amaegbu Olokoro in Umuahia, Abia State, Nigeria. The plant material was identified at the Department of Plant Science and Biotechnology, Abia State University, Uturu. The plant material was rinsed and then sun-dried. The dried leaf was milled to powder. Two hundred and fifty gram (250g) of the powder was extracted with 625 ml of 70% ethanol by cold maceration for 48 hours and filtered. The ethanol in the filtrate was eliminated and the concentration of the extract was made in normal saline for the experiment.

2.3. Experimental Animals

Fifty healthy male albino rats aged 7 weeks were used in this study. The rats were bought and kept in the Animal House, Department of Biochemistry, Faculty of Biological and Physical Science, Abia State University, Uturu. The animals were allowed to acclimatize for 7 days under standard laboratory conditions with free access to commercial rat feed and water.

2.4. Experimental Design

The fifty male albino rats (between 140g-160g body weight) were randomly placed into five (5) groups with ten (10) rats in each group. Group 1 served as the normal control (it received a placebo of normal saline for 21 days). Group 2 received acetaminophen (1000 mg/kg b.w.) only: as negative control. Group 3 received 200 mg/kg of leaf extract of *G. latifolium* and acetaminophen (1000 mg/kg b.w.). Group 4 received 400 mg/kg of leaf extract of *G. latifolium* and acetaminophen (1000 mg/kg b.w.). Group 5 received 600 mg/kg of leaf extract of *G. latifolium* and acetaminophen (1000 mg/kg b.w.).

The test animals (groups 3, 4 and 5) received the leaf extract as stated above for twenty-one consecutive days. Groups 2, 3, 4 and 5 animals received acetaminophen one hour after each administration of the leaf extract. In the test groups, the drug and extract were administered through oral route using a gavage intubation. All animals were allowed free access to feed and water *ad libitum* throughout the study. All processes involved in the handling of animals and the experiment was carried out according to the standard protocols approved by the Animal Ethics Committee of the Faculty of Biological and Physical Sciences, Abia State University, Uturu, Nigeria.

2.5. Blood Collection

After administration of the leaf extract and acetaminophen, the animals were starved overnight, anaesthetized with chloroform and sacrificed. Blood was collected by cardiac puncture from each animal into dry test tubes. The blood sample was allowed to stand for about 15 minutes to clot and further spun in a centrifuge at 4000rpm for 10min. Serum was separated from the clot with Pasteur pipette into sterile sample tubes for the measurement of the antioxidant activities.

2.6. Biochemical Analysis

Malondialdehyde (MDA) was measured by the method described by Onkawa *et al.* [9]. Reduced glutathione (GSH) level was determined using the method described by Ellman [10] with slight modifications, while Glutathione transferase was determined according to Habig *et al.* [11]. Superoxide dismutase (SOD) activity was determined using the method of Sun and Sigma as described by Ogbunugafor *et al.* [12]. Catalase activity was determined according to the method of Sinha [13].

2.7. Statistical Analysis

Statistical analysis was carried out with the use of Analysis of Variance (ANOVA) and standard Student-T-distribution

test: using Statistical package for Social Sciences (SPSS) version 21 and group mean were compared for significance at $p \leq 0.05$.

Data were presented as mean \pm standard deviation (n=10).

3. Results and Discussion

Table 1. Antioxidant enzymes activity.

Parameters	Group 1 (Normal control)	Group 2 (APAP 1000mg/kg bw (negative control))	Group 3 (200mg/kg bw of <i>G. lat.</i> + APAP 1000mg/kg bw)	Group 4 (400mg/kg bw of <i>G. lat.</i> + APAP 1000mg/kg bw)	Group 5 (600mg/kg bw of <i>G. lat.</i> + APAP 1000mg/kg bw)
CATALASE ($\mu\text{mol}/\text{min}/\text{mg}$ protein)	8.21 \pm 0.20	2.35 \pm 0.12 ^a	4.09 \pm 0.26 ^b	5.78 \pm 0.30 ^c	6.86 \pm 0.29 ^d
SOD ($\mu\text{mol}/\text{ml}$)	3.52 \pm 0.24	1.46 \pm 0.09 ^a	2.20 \pm 0.13 ^b	2.60 \pm 0.17 ^c	3.59 \pm 0.23 ^d
GST ($\mu\text{mol}/\text{ml}$)	0.96 \pm 0.03	0.40 \pm 0.02 ^a	0.65 \pm 0.01 ^b	0.87 \pm 0.02 ^c	0.97 \pm 0.04 ^d
GSH ($\mu\text{mol}/\text{ml}$)	1.83 \pm 0.03	0.61 \pm 0.02 ^a	1.14 \pm 0.01 ^b	1.47 \pm 0.02 ^c	1.64 \pm 0.02 ^d

Results represent mean \pm standard deviation of group serum results obtained (n=10).

Mean in the same row, having different letters of the alphabet are statistically significant ($p < 0.05$) compared with the negative control (group two).

LEGEND: SOD= Superoxide dismutase, GST= Glutathione transferase and GSH= reduced Glutathione.

Table 2. Malondialdehyde (MDA) assay.

Parameter	Group 1 (Normal control)	Group 2 (APAP 1000mg/kg bw (negative control))	Group 3 (200mg/kg bw of <i>G. lat.</i> + APAP 1000mg/kg bw)	Group 4 (400mg/kg bw of <i>G. lat.</i> + APAP 1000mg/kg bw)	Group 5 (600mg/kg bw of <i>G. lat.</i> + APAP 1000mg/kg bw)
MDA ($\mu\text{mol}/\text{ml}$)	1.34 \pm 0.22	4.64 \pm 0.33 ^a	3.17 \pm 0.32 ^b	1.50 \pm 0.20 ^c	1.57 \pm 0.23 ^d

Results represent mean \pm standard deviation of group serum results obtained (n=10).

Mean in the same row, having different letters of the alphabet are statistically significant ($p < 0.05$) compared with the negative control (group two).

The body has an effective defence mechanism to prevent and neutralize free radicals-induced damage. This is accomplished by a set of endogeneous antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase and catalase [14]. Decrease in enzyme activity of SOD is a sensitive index of hepatocellular damage and is the most sensitive enzymatic index in liver injury [15, 16].

The increased level of malondialdehyde (MDA) in the rats administered acetaminophen only (Table 2) may be as a result of membrane lipid peroxidation by free radicals generated and failure of antioxidant defence mechanisms to prevent formation of excessive free radicals [17, 18]. Also, the decreased activity of SOD, Catalase, GST and GSH in the acetaminophen-induced rats (group 2) may be due to high concentration of these free radicals generated by excess dose of acetaminophen which may lead to decreased level or inactivation of these endogenous antioxidant enzymes. Superoxide dismutase is the antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2 . Peroxide can be destroyed by CAT or GPX reactions. In animals, hydrogen peroxide is detoxified by CAT and by GPX. Catalase protects cells from hydrogen peroxide generated within them. Even though CAT is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells. Administration of ethanolic leaf extract of *Gongronema latifolium* significantly ($P < 0.05$) increased the levels of SOD, Catalase, GST and GSH activities (table 1) and a consequent significant ($P < 0.05$) reduction in MDA

level, when compared with the acetaminophen-induced group (group 2).

The result of this study confirms the antioxidant or free radical scavenging activities of *Gongronema latifolium* leaf. The antioxidant activities of *Gongronema latifolium* leaf is believed to be due to the phytochemicals (eg. polyphenolics) present in the plant, which could exert beneficial action against pathophysiological alterations caused by the presence of superoxide and hydroxide free radicals as well as hydrogen peroxide. The protective effect of the leaf extract of *Gongronema latifolium* on the antioxidant parameters measured was dose dependent. The stability of these enzymes and MDA level in the group administered 600mg/kg bwt. of the leaf extract indicates protection against the alterations exhibited by the acetaminophen. In this study, the antioxidant/protective effect against the induced-damage was better at the highest dose of *Gongronema latifolium* extract used. This result also suggests that *Gongronema latifolium* leaf could improve or assist the endogenous enzymatic antioxidants to mop up free radicals generated by the excess dose of acetaminophen thereby preventing oxidative stress.

4. Conclusion

This study shows the antioxidant stability effect of *Gongronema latifolium* leaf extract against excess dose of acetaminophen. Administration of *Gongronema latifolium* leaf extract prevented the alteration of the antioxidant indices caused by the excess dose of acetaminophen. *Gongronema*

latifolium ethanolic leaf extract exhibits a potent positive antioxidant regulating effect against acetaminophen-induced toxicity in male albino rats. It is possible that, the mechanism of these positive effects may be due to the reported phytochemicals present in the leaf which possesses antioxidant and free radical scavenging properties. I therefore encourage the use of *Gongronema latifolium* leaf as vegetable in sauce and food preparations as a possible protective and antioxidant stabilizing agent against certain toxic substances.

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