Protective effect of aqueous root extract of Gongronema latifolium against paracetamol induced hepatotoxicity and chloroquine induced nephrotoxicity in rats

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Abstract

The hepato- and nephro-protective potentials of aqueous root extract of Gongronema latifolium against paracetamol-induced hepatotoxicity and Chloroquine induced nephrotoxicity in adult albino rats, were studied. Thirty six (36) albino rats weighing between 200±34g were used for this study. They were randomly divided into nine groups (1-9), where group 1 served as the zero control (baseline). Hepatotoxicity was induced in groups 2, 6, and 7 via oral administration of paracetamol 600mg/kg body weight, while group 3, 8 and 9 were induced with nephrotoxicity via oral administration of chloroquine 69mg/kg body weight. These were treated for 14 days, except for group 2 and 3 which served as the hepatotoxic and nephrotoxic controls, respectively. Group 4 and 5 which served as extract controls were given100mg/kg and 200mg/kg of Gongronema latifolium root extract, respectively. Serum liver enzymes (Alanine aminotransferase, Aspartate aminotransferase and Alkaline phosphatase), urea and creatinine levels were evaluated spectrophotometrically in accordance with the methods provided by the diagnostic kits (Teco Diagnostic, Anaheim, USA). The outcome of this study showed that the mean±SEM serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels of the Paracetamol + Extract groups, as well as the mean±SEM serum urea and creatinine levels of the Chloroquine + Extract groups were significantly decreased (P<0.05) than that of the hepatotoxic and nephrotoxic control, respectively, in a dose-dependent manner. This study indicates that aqueous root extract of Gongronema latifolium exerted significant protection against Paracetamol induced hepatotoxicity and chloroquine induced nephrotoxicity.

Keywords: Gongronema latifolium, paracetamol, chloroquine, hepatotoxicity, nephrotoxicity

1. Introduction

The liver and kidneys are among the vital organs of the body as they play crucial role including detoxification and removal of toxic wastes from the body [1]. Humans are exposed intentionally and unintentionally to a variety of diverse chemical agents that harm target organs of the body including the liver and kidney. Injury to such vital organs of the body poses serious medical problems which must be properly managed. Some of the liver and kidney injuries are caused by the use and abuse of drugs. Prescription-drugs like paracetamol and chloroquine can cause serious side effects, even toxic effects on target organs like the liver and kidneys, especially when used in excess and for prolonged periods of time [2,3].

Paracetamol (Acetaminophen) is a widely used over-the-counter (OTC) non-narcotic analgesic and antipyretic. It is as potent as aspirin especially in the central nervous system [4]. While, chloroquine on the other hand, is a member of an important series of chemically related anti-malarial agents (the quinolone derivatives). Being a 4-aminoquinoline, it is a rapidly acting blood schizontocide with some gametocytocidal activity [5]. Chloroquine is effective for the treatment of malaria and has been the anti-malarial drug of choice for many years in most parts of the world, including Nigeria in spite of increasing prevalence of resistance of the malaria parasite to the drug [6,7].

However, evidences abound that over dosage and prolonged use of paracetamol and chloroquine are known to cause hepatotoxicity [8, 9, 10] and nephrotoxicity [5, 11] respectively. The commonest biochemical parameters regarded as indicators of liver damages are aspartate amino-transminase (ASAT), alanine amino-transminase (ALAT), alkaline phosphatase (ALP) and bilirubin [12, 13]; while urea and creatinine are important renal indicators for kidney damage [14].

The damage to the hepatocellular cells and renal cells results in the increase in these biochemical parameters which are roughly proportional to the extent of tissue damage [15]. Against the backdrop, that liver and kidney diseases are major global concerns and still have extremely poor prognosis and high mortality, natural remedies from traditional plants are been sought for as safe and effective alternative treatments for hepatotoxicity and nephrotoxicity. Many research efforts are now been directed towards the discovery and development of agents of plant origin, which might protect cells from oxidative reactions with potential hepatoprotective and nephroprotective effects [5, 16].
Gongronema latifolium is one of such medicinal plants, which is fast gaining recognition. Gongronema latifolium is an herbaceous shrub, with flowers usually yellow and the stem yields characteristic milky exudates. It is commonly grown in Nigeria and is locally called Utazi by the Efiks, Ibibios and Quas, Utazi by the Igbos and Arokeke or Madunmaro by the Yorubas [17].

It is important to know that Gongronema latifolium is a climbing shrub with broad, heart-shaped leaves that has a characteristic sharp, bitter and slightly sweet taste, especially when eaten fresh. The stems have soft/hairy that yields milky latex or exudates [18]. It belongs to the family of plants known as Asclepiadaceae and it is widespread in tropical rainforest of West African countries, such as Nigeria, Côte d'Ivoire, Sierra Leone, Ghana and Senegal, etc. Research has shown that the whole plant exhibits several herbal actions including: analgesic, antitumor, broad spectrum antimicrobial, antipyretic, antioxidant, anti-inflammatory, anti-ulcer, anti-sickling, anti-asthmatic, mild expectorant, hypoglycemic, hypolipidemic, hepatoprotective, digestive tonic and laxative properties [19].

In southern part of Nigeria, especially among the people of South-East and South-South, the leaves of Gongronema latifolium are used commonly for nutritional purposes, including: as a spice and vegetable to garnish some special local delicacies, such as Isiewu, Nkwobi, Abacha/Ugba (African salad) Ofé nsala (white soup), unripe plantain porridge, etc because of its sharp-bitter and sweet taste. In many local “joints,” where people enjoy Isiewu, Nkwobi and Abacha/Ugba (African salad) with palm wine or beer, the leaves are usually added to these delicacies to help prevent drunkenness or hangover [20]. The leaves are believed to neutralize the intoxicating properties alcohol and its harmful effects on the liver. An infusion or decoction of the whole plant (the leaves and stems) is used in the home treatment of digestive problems such as: loss of appetite, dyspepsia, colic and stomach ache, constipation, dysentery and intestinal worms [21].

To the best of our knowledge, no study has been carried out to show that regular intake of aqueous root extract of Gongronema latifolium can attenuate paracetamol induced hepatotoxicity and chloroquine induced nephrotoxicity in animal model. Previous related works carried out by researchers [3, 10, 22] were on the leaf extracts of Gongronema latifolium and not on the root extracts. Scarcity of information in this regard, therefore necessitates the need for this present work. It is hoped that the aqueous root extract of Gongronema latifolium will show similar protective attenuation role like its leaf counterpart previously studied. This study is therefore designed to assess the protective attenuation role of aqueous root extract of Gongronema latifolium in paracetamol induced hepatotoxicity and chloroquine induced nephrotoxicity using animal model.

2. Materials and Methods
2.1 Area of study
The study was carried out at the Experimental Animal House, Babcock University, Ilishan-Remo, Ogun State; a Seventh-day Adventist Institution of higher learning located in the Southwest region of Nigeria, coordinates: 6.8862° N, 3.7055°E.

2.2 Duration of study
The study lasted for a period of 2 months (June-August, 2016).

2.3 Ethical clearance
Ethical clearance was sought for and obtained from the Babcock University Health Research Ethics Committee (BUHREC).

2.4 Plant material

Fresh root of Gongronema latifolium was collected from Irolu village in Ikenne Local Government Area of Ogun-State. The plant was authenticated by a Botanist, Dr. J. S. Ashidi, of the Department of Plant Sciences, Obalási Onabanjo University, Ago Iwoye, Ogun State. The root was washed with distilled water and dried at room temperature (25±2°C) for 14 consecutive days. The air-dried root was crushed in a mortar with a pestle and further ground into powder using an electric blender. Extraction was done as follows: 300g of the coarse powder of the root was soaked in 3 litres distilled water and the mixture was allowed to stand for 24 hrs before filtration. The mixture was filtered three times through sterile cheese cloth and then filtered into sterile container using a funnel containing sterile Whatman No. 1 filter paper for clarification. The filtrate was concentrated following removal of the solvent using Rotary evaporator. Yield of extract was obtained and the extract stored in the dessicator until use.

2.5 Drugs
Paracetamol (500mg) and chloroquine (250mg) made by Emzor Pharmaceutical Industries Ltd., (Lagos, Nigeria) was purchased from the Pharmacy unit of Babcock University Teaching Hospital (BUTH), Ilishan-Remo, Ogun State. The tablets were dissolved in appropriate volume of sterile distilled water according to the required concentrations needed for administration to the rats on the basis of their body weight.

2.6 Experimental animals
Animal use protocol is in accordance with international standard on the care and use of experimental animals [23], Wistar albino male rats weighing 200±34g (mean±SD) were purchased from the small animal house, University of Ibadan (Oyo State, Nigeria) and were clinically examined upon arrival and those that shows signs of abnormality or disease were excluded from the study. Only symptom-free animals were used. They were housed in the Experimental Animal
A total of 36 rats were randomly assigned into 9 groups (n=4/group) and were treated as follows: group 1 received sterile distilled water (zero control), group 2 received 600 mg/Kg/d paracetamol only (Hepatotoxic control), group 3 received 69 mg/Kg/d chloroquine only (Nephrotoxic control), group 4 received 100 mg/Kg/d Gongronema latifolium only, group 5 received 200 mg/Kg/d Gongronema latifolium only, group 6 received 600 mg/Kg/d paracetamol plus 100 mg/Kg/d of Gongronema latifolium, group 7 received 600 mg/Kg/d paracetamol plus 200 mg/Kg/d of Gongronema latifolium, group 8 received 69 mg/Kg/d chloroquine plus 100 mg/Kg/d of Gongronema latifolium, while group 9 received 69 mg/Kg/d Chloroquine plus 200 mg/Kg/d of Gongronema latifolium as shown in Table 1.

The volume of extract (10ml/1kg body weight) to be administered to individual rat in each group using intragastric tube were calculated, recorded and adjusted weekly with changes in body weight throughout the treatment period which lasted for 14 days (once daily). They were observed daily for any observable change.

2.9 Euthanasia
Overnight prior to euthanasia, the animals were starved of food and were sacrificed by cervical dislocation as described by [24].

Table 1: Experimental Pharmacological Protocol for Test and Control Rats

<table>
<thead>
<tr>
<th>Groups (n=4)</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>G1</td>
<td>Sterile distilled water (Zero control)</td>
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<tr>
<td>G2</td>
<td>600 mg/Kg/d Paracetamol only (Hepatotoxic control)</td>
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<tr>
<td>G3</td>
<td>69 mg/Kg/d Chloroquine only (Nephrotoxic control)</td>
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<tr>
<td>G4</td>
<td>100 mg/Kg/d of Gongronema latifolium</td>
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<tr>
<td>G5</td>
<td>200 mg/Kg/d of Gongronema latifolium</td>
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<tr>
<td>G6</td>
<td>600 mg/Kg/d Paracetamol + 100 mg/Kg/d of G. latifolium</td>
</tr>
<tr>
<td>G7</td>
<td>600 mg/Kg/d Paracetamol + 200 mg/Kg/d of G. latifolium</td>
</tr>
<tr>
<td>G8</td>
<td>69 mg/Kg/d Chloroquine + 100 mg/Kg/d of G. latifolium</td>
</tr>
<tr>
<td>G9</td>
<td>69 mg/Kg/d Chloroquine + 200 mg/Kg/d of G. latifolium</td>
</tr>
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2.10 Specimen collection
2.10.1 Blood specimen
Cardiac blood specimen was taken from each rat by terminal bleeding from the heart. The blood was transferred into an anticoagulant-free test tube and allowed to clot at room temperature (25°C) and subsequently centrifuged at 750 g for 15 min to obtain serum component ready for evaluation of hepatotoxicity and nephrotoxicity indicator parameters. Serum were assayed on the same day, thaw cycles were avoided.

2.11 Laboratory Analyses
2.11.1 Evaluation of hepatotoxicity indicator parameters
The serum level of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and alkaline phosphatase (ALP) were evaluated spectrophotometrically in accordance with the methods provided by the diagnostic kits (Teco Diagnostic, Anaheim, USA).

2.12.2 Evaluation of nephrotoxicity indicator parameters
The serum level of urea and creatinine were also similarly evaluated spectrophotometrically in accordance with the methods provided the diagnostic kits (Teco Diagnostic, Anaheim, USA).

2.13 Statistical Analyses
Data for the serum ALAT, ASAT, ALP, Creatinine and Urea were presented as means of 4 rats using tables and were analyzed using one way analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test using SPSS-18.0 (Statistical packages for social Scientists – version 18.0) statistical program. P values <0.05 was considered significant [25].

3. Results and Discussion
This present study examined the protective attenuation role of aqueous root extract of Gongronema latifolium in paracetamol induced hepatotoxicity and chloroquine induced nephrotoxicity using animal model. The effect of aqueous root extract of G. latifolium on hepato-protective indicator parameters is presented in Table 2. The mean±SEM serum Alanine aminotransferase (ALAT), Aspartate aminotransferase (ASAT) and Alkaline phosphatase (ALP) levels of the control and treated groups were as follow:
The main value of the **Aminotransferases** and **Alkaline phosphatase** is in detecting hepatocellular damage and monitoring the patient's progress as the levels rapidly return to normal following resolution of the factors causing hepatocellular damage. The observed increase in enzyme activities in the hepatotoxic control is thought to be due to a leakage of cytoplasmic enzyme into circulation as a result of inflammation of the liver cells following induction. Following simultaneous administration of the extract along with Acetaminophen, a rise in the serum level of the liver enzymes was noted, indicating liver cell damage following ingestion. Gentamicin in the nephrotoxic control caused a rise in serum creatinine and urea levels, whereas in the Paracetamol + extract, a decrease was noted. This decrease was statistically significant compared to the Paracetamol only group. Serum creatinine levels in the Paracetamol + extract group were lower than the nephrotoxic control at 5 percent level (P<0.05) when the serum urea and creatinine levels of the Zero control were compared with other groups, except for the nephrotoxic control which was significant higher (P<0.05). There were no significant differences (P>0.05) when the serum ALAT, ASAT and ALP levels of the Zero control was compared with other groups, except for the hepatotoxic control which was significant higher (P<0.05). Still, there were no significant differences (P>0.05) between the mean±SEM serum ALAT, ASAT and ALP levels of the Extract only and Paracetamol + Extract groups. Also the mean±SEM serum ALAT, ASAT and ALP levels of the Paracetamol + Extract groups were significantly decreased (P<0.05) than that of the hepatotoxic control in a dose-dependent manner. Furthermore, the effect of aqueous root extract of Gongronema latifolium on nephro-protective indicator parameters is presented in Table 3. The mean±SEM serum urea and creatinine levels of the control and treated groups were as follow: Zero control (21.25±4.89 mg/dl and 0.20±0.41 mg/dl, respectively), Nephrotoxic control (128.00±16.37 mg/dl and 6.83±1.03 mg/dl, respectively), 100 mg/kg/d aqueous extract of *G. latifolium* (41.00±8.30 mg/dl and 0.44±0.16 mg/dl, respectively), 200 mg/kg/d aqueous extract of *G. latifolium* (46.25±24.01 mg/dl and 0.48±0.95 mg/dl, respectively), 69 mg/kg/d Chloroquine + 100 mg/kg/d aqueous extract of *G. latifolium* (42.75±13.68 mg/dl and 0.37±0.90 mg/dl, respectively). There were no significant differences (P>0.05) when the serum urea and creatinine levels of the Zero control was compared with other groups, except for the nephrotoxic control which was significant higher (P<0.05). Still, there were no significant differences (P>0.05) between the mean±SEM serum urea and creatinine levels of the Extract only and Chloroquine + Extract groups. Also the mean±SEM serum urea and creatinine levels of the Chloroquine + Extract groups were significantly decreased (P<0.05) than that of the nephrotoxic control in a dose-dependent manner.

The outcome of this study revealed that the mean±SEM serum levels of ALAT, ASAT and ALP were significantly lower (P<0.05) in the treated group (Paracetamol + extract) when compared to the hepatotoxic control (Paracetamol only) in a dose-dependent manner. Plasma concentration of liver enzymes is normally low, but is elevated in the event of hepatotoxicity, which may occur in alcohol, drug or herb-induced liver damage leading to necrosis of hepatocytes, increased permeability and leakage of cellular enzymes from the liver cytosol into the blood stream.

The main value of the **Aminotransferases** and **Alkaline phosphatase** is in detecting hepatocellular damage and monitoring the patient's progress as the levels rapidly return to normal following resolution of the factors causing hepatocellular damage. The observed increase in enzyme activities in the hepatotoxic control is thought to be due to a leakage of cytoplasmic enzyme into circulation as a result of inflammation of the liver cells following induction. Following simultaneous administration of the extract along with Acetaminophen, a rise in the serum level of the liver enzymes (i.e, *transminitis*) as was initially anticipated was absent in the treated groups. It can therefore be inferred that the extract possess hepato-protective potential at the concentrations tested.
Furthermore, the values of the liver enzymes of the zero control rats were within the normal range. This confirms no occurrence of hepatic alterations in this group of animals since they were not induced with hepatotoxicity at the first instance. Besides, there were no significant differences ($P>0.05$) when the mean±SEM serum ALAT, ASAT and ALP levels of the Zero control were compared with the treated group (Acetaminophen + extract). This further confirms the hepatoprotective potential of the extract.

The outcome of this present study, appear to agree with the results of other similar studies like that of [22], who examined the protective role of $G$. latifolium in acetaminophen induced hepatic toxicity in Wistar rats treated with 200 and 400 mg/kg b.w of the aqueous leaf extract of $G$. latifolium for 14 days. The outcome of their work showed that the serum ALAT, ASAT and ALP levels dropped marginally in the extract treated rats.

Similarly, [26] investigated the hepatoprotective potential of Ethanolic leaf extract of $G$. latifolium in acetaminophen-induced hepatic toxicity in male albino rats. The extract was administered at the doses of 200, 400 and 600mg/kg b.wt. orally for 21 consecutive days. The result showed a significant decrease ($P<0.05$) in the serum liver enzymes (ALAT, ASAT and ALP) of all the test animals treated with the extract compared to the hepatotoxic controls.

Another study by [27], also showed that diabetic rat made to consume $G$. latifolium leaves had significant ($P<0.05$) reduction in the levels of ALAT, ASAT and ALP in the serum and liver tissue homogenate relative to diabetic control. Furthermore, on the other hand, the outcome of this study revealed that the mean±SEM serum urea and creatinine levels were significantly lower ($P<0.05$) in the treated group (Chloroquine + extract) when compared to the nephrotoxic control (Chloroquine only) in a dose-dependent manner. Plasma concentration of urea and creatinine is normally low, but is elevated in the event of nephrotoxicity, which may occur in drug or herb-induced kidney damage. The main value of the urea and creatinine is in detecting renal damage and monitoring the patient's progress as the levels rapidly return to normal following resolution of the factors causing renal damage.

Here, similarly, a rise in the serum level of urea and creatinine as was initially anticipated was absent in the treated groups. It can therefore, also be inferred that the extract possess nephro-protective potential at the concentrations tested. Furthermore, the values of the urea and creatinine of the zero control rats were within the normal range. This confirms no occurrence of renal alterations in this group of animals since they were not induced with nephrotoxicity at the first instance. Besides, there were no significant differences ($P>0.05$) when the mean±SEM serum urea and creatinine levels of the Zero control were compared with the treated group (Chloroquine + extract). This further confirms the nephro-protective potential of the extract.

Biochemically, significant elevation in the levels of renal parameters is indicative of renal alterations. These parameters were elevated in the nephrotoxic control group administered Chloroquine (69mg/kg/b.wt). The increase is roughly proportional to the extent of renal following anti-malaria administration as earlier reported by [28]. It is normal to have these parameters elevated in various renal disorders including nephritis, kidney failure, kidney stone and urinary tract infection. The renal cell damage may be associated with the generation of reactive oxygen species (ROS) by chloroquine overdose which are also partly responsible for their anti-malaria effects, hence the harmful effects were considered to be caused by ROS produced during peroxide formation [14, 29]. The level of hydroxyl and peroxide radical induced by chloroquine treatment may be responsible for the renal impairment in Albino rats [30].

Furthermore, the values of the renal parameters of the zero control rats were within the normal range. This confirms no occurrence of renal alterations in this group of animals since they were not induced with nephrotoxicity at the first instance. Significant reduction ($P<0.001$) in the extract treated rats in comparison with the nephrotoxic control proved that the treatments (at the concentrations tested) were nephro-protective. The outcome of this present study, also agree to agree with the results of other similar studies like that of [22], which shows that the aqueous leaf extract of $G$. latifolium at doses of 1ml once daily and 1ml twice daily showed a significant dose dependent reduction in urea and creatinine levels in rats induced with carbon tetrachloride (CCl4). [3] also reported that leaf extract of $G$. latifolium at 250 and 500mg/kg b.wt. produced a significant ($P<0.05$) decrease in urea and creatinine levels when compared with control in chloroquine induced nephrotoxic rats.

4. Conclusion

The present investigation indicates that the aqueous root extract of Gongronema latifolium exerted significant protection against paracetamol induced hepatotoxicity and chloroquine induced nephrotoxicity. This further complements previous studies on $G$. latifolium and also confirms the claim made by traditional healers that almost every part of the plant is medicinal. It can therefore be concluded that this study has scientifically justified the traditional uses of Gongronema latifolium in the management of various human diseases.

5. Competing Interests

Authors have declared that no competing interests exist.

6. References