Protective Effects of Bi-Herbal Formulation of Aqueous Extracts of Vernonia amygdalina and Gongronema latifolium against Gentamicin Induced Nephrotoxicity and Liver Injury in Rats

Nwadike Constance1*, Dike-Ndudim Joy1, Oly-Alawuba Nkeiruka2, Ezekwe Ahamefula3, Akanazu Chidimma4 and Aloy-Amadi Oluchi1

1Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.
2Department of Nutrition and Dietetics, Imo State University, Owerri, Nigeria
3Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Rivers State University, Nkpou Ororo, Port Harcourt, Nigeria.
4Department of Public Health, Federal University of Technology, Owerri, Nigeria.

Authors’ contributions

This work was carried out in collaboration among all authors. Authors NCDNJ and EA designed the study. Authors OAN and AC performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AAO and NC managed the analyses of the study. Authors NCEA, OAJ and AAO managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

The study was carried out to investigate the protective effects of bi-herbal formulation of aqueous extracts of Vernonia amygdalina and Gongronema latifolium against gentamicin induced nephrotoxicity and liver injury in rats. Forty (n=40) male Wistar albino rats were procured and separated into five groups. Groups I and II served as normal control and experimental control respectively. Groups III to V served as test groups. Rats of experimental control (group II) and test groups were induced with lethal dose of gentamicin. Test groups III and IV were placed on herbal formulation of aqueous extracts of V. amygdalina and G. latifolium respectively, whereas rats in test
INTRODUCTION

For years now, the use of plants in the art of folkric medicine has been on the increase [1-7]. Since the acceptance of plants as agent to remedy disease conditions in primary and healthcare, greater part of the world has been relying on them [8-11]. The universal role of plants against diseases has been accepted and it is applicable to almost all major aspects of medicine and healthcare system [7,12-13]. The use of folkric medicine is prevalent in the traditional African Society and most of the Asian countries. The plants used in folkric medicine are known as medicinal plants [14-21]. Medicinal plants have been extensively defined by different authors [16-21]. It has been reported that medicinal plants are biologically and physiologically active against disease conditions due to their constituents [3-4,7,12,14,21-32]. These constituents dissolve in extracts, infusions, concoctions and decoctions made from medicinal plants, which are effective against disease inducing microorganisms.

V. amygdalina (Del.) and G. latifolium (Benth) are among those medicinal plants that are bioactive in nature and are physiologically active against diseases. V. amygdalina popularly known as bitter leaf, is a common shrub that grows in tropical Africa [10,18]. It belongs to the Asteraceae family. In Nigeria, the Igbofs of Southeast call it “Onugbu”, the Yorubas of Southwest call it “Ewuro”, while the Hausas of North call it “Chusar-doki”. V. amygdalina is also known as “Ilo”, “Itunya” and “Oriwo” in Igalia, Tiv and Edo respectively. The leaves of V. amygdalina have characteristic bitter taste and green colouration [10,18]. They are used in the preparation of the popular “bitter leaf soup” commonly consumed in Nigeria. They are also used as spice for “Ndole”, a Cameroon dish [33].

The plant is used in the production of extracts, infusion, concoction, decoction or tonic that are traditionally used in folkric medicine against ailments such as infertility, diabetes, malaria, gastrointestinal problems and sexually transmitted diseases [34-35]. The use of V. amygdalina is not limited to humans alone. Studies have also implicated its bioactivity to others animals such as horse, chimpanzee as well as minute organisms [36-37]. The fresh leaves of the plant contain high moisture, protein and carbohydrate, with low fibre and ash [38].

G. latifolium (Benth), known commonly as amaranth globe, is a glaborous plant that grows in tropical Africa. It is a climber with characteristics greenish yellow flowers [10]. It can inhabit secondary forest, deciduous forest, rainforest or mangrove forest. It can be propagated by seed, stem cutting or softwood [10,17-18]. The flower grows around July and August. Aside Nigeria, the plants can also be found in Senegal, Chad, and DR Congo [17-18,39-40]. In Nigeria, the Igbofs, Efik/Ibibios call G. latifolium “utasi”, the Yorubas call it “arokeke” or “madumaro”. According to Edim et al. [39], the Akan-asantes in Ghana, the Serers in Senegal and the Kissis in Sierra leone call it “kurutu nsurogya”, “gasub” and “ndono-polole” respectively. G. latifolium has sweetly bitter in taste. It is used as in soups, salads and sauces as spice [41]. It is also used in the local brewing of beer. The stem is sometimes used as chewing stick [10,17-18,39].

The plant has widespread application in folkric medicine against abnormal blood glucose levels, diarrhoea, and tussive [10,17-18,42]. Its bioactive constituents are effective against disease inducing microorganisms and certain diseases [43-45].

Gentamicin is an aminoglycoside antibiotic approved for use in the United States since

Keywords: Aminoglycosides; Gongronema latifolium; gentamicin; nephrotoxicity; Vernonia amygdalina.

group V received bi-herbal formulation of aqueous extracts of V. amygdalina and G. latifolium. Nephrotoxic indices such as urea reduced significantly (p<0.05) in test groups (III, IV, and V) when compared to experimental control (group II) and normal control (group I). Creatinine also reduced significantly (p<0.05) in test groups III and V against group II (experimental control), and increased insignificantly (p>0.05) in test group V when compared to normal control (group I). Rats induced with gentamicin had upsurge in liver enzymes indicating possible compromise of hepatocellular integrity but the ameliorating effects of the herbal formulations were seen clearly in test groups in this study as they tried to protect the hepatocellular integrity. The bi-herbal formulation of aqueous extract of 5% v/v each of V. amygdalina and G. latifolium offered the best protection as observed in this study. This study has revealed the protective effects of bi-herbal formulation of aqueous extract of V. amygdalina and G. latifolium against gentamicin induced nephrotoxicity and liver injury in rats.
1970. It has excellent against many Gram-negative bacterial organisms. Nicholas et al [46], noted that gentamicin in synergy with β-lactams, is effective against *Staphylococcus* and *Enterococcus*. The same authors noted that gentamicin remains a first-line antibiotic for many severe infections with low rates of resistance and inexpensive [47]. Gentamicin can be taken through the muscle or vein and has multiple generic parental formulations. The antibiotic acts by binding to bacterial ribosomes and inhibiting protein synthesis [47]. It has been reported that dose related adverse effects are common to all aminoglycosides include oto- and nephrotoxicity [46-51]. Different studies have reported as low as 1.2% to 56% of such adverse effects on patients post administered the drug [46-51]. Generally, the uptake of aminoglycosides by the hepatocytes is highly limited and they are rapidly excreted in the urine with high concentrations found in the urine though there are isolated cases reports of acute injury on the liver coupled with jaundice on associated aminoglycosides therapy [47].

Different studies have observed the aminoglycosides had oto- and nephrotoxicity [46-51], but not much has been done to seek a possible protection to avert such toxicity occurring following the therapy of aminoglycosides. This study looked at such area and evaluated the protective effects of bi-herbal formulation of aqueous extract of *V. amygdalina* and *G. latifolium* against gentamicin induced injury on kidney and liver in rats.

2. MATERIALS AND METHODS

2.1 Sample Collection, Identification and Preparation

*V. amygdalina* and *G. latifolium* used in the present study were collected in November-2019, from Imo State University School farm in Owerri. The samples were identified in the Department of Plant Science and Biotechnology, Imo State University and the voucher specimens deposited at the Herbarium unit of Imo State University, Owerri. The leafy parts of the freshly harvested and identified samples were cut, rinsed, and air-dried for seven days before they were milled and packaged in sterile well labelled polyethylene bags for storage and for further use.

2.2 Preparation of Aqueous Extracts

Two hundred gram (200 g) each of the milled sample was soaked separately in 2000 mL of distilled H$_2$O for a day, filtered, and freeze-dried to obtain the dried extract. 250 mL of each of the filtrate was freeze dried to yield approximately 12.72 mg/kg *V.amygdalina* and 13.87 mg/kg for *G.latifolium*. The extracts were re-dissolved in distilled water for further use.

2.3 Gentamicin

The gentamicin used in this study was commercially purchased and formulated into 80 mg/kg bod weight capable of inducing nephrotoxicity.

2.4 Experimental Design and Experimental Animals

Forty male Wistar albino rats weighing between 110 to 200 g were procured from the animal colony of Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria. The rats were placed on drinking water, food and room temperature of 25 °C for the initial acclimatization of four days, before they were separated into five groups of 8 rats each and induced with the gentamicin. The grouping was done on weight basis. The treatments of the rat groups were designated as follows.

Group I: Rats with no treatments with gentamicin, no aqueous extract of *V.amygdalina* and *G.latifolium* (Normal control 1).  
Group II: Rats treated with only 80 mg/kg body weight of gentamicin for 8 days (Experimental control 2).  
Group III: 10% v/v of aqueous extract of *V.amygdalina* and 80 mg/kg body weight of gentamicin for 8 days.  
Group IV: 10% v/v of aqueous extract of *G.latifolium* and 80 mg/kg body weight of gentamicin for 8 days.  
Group V: Bi formulation (5% v/v) each of aqueous extract of *V.amygdalina* and *G.latifolium* and 80 mg/kg body weight of gentamicin for 8 days.  
Groups III, IV and V were test rats. The extracts were given to the rats orally on daily basis as the experiment completed (8 days). The groups were placed on the same feed and water. The floors of the cages were constantly cleaned at on daily basis, their feed consumption and body weights were taken on daily basis.
Experimental handling of animals was in accordance with international guidelines on animal care and uses of National Institute of Health [52].

2.5 Sample Collection for Analysis

After 8 days of treatment, the rats were sacrificed after subjecting them to overnight fast and weighed. The rats were anaesthetized with chloroform and blood samples were collected by cardiac puncture into clean tubes for nephrotoxicity and liver enzyme studies.

2.6 Nephrotoxicity and Liver Enzyme Analysis

Urea, creatinine, sodium ion, potassium ion, chloride, and bicarbonate were analysed for nephrotoxicity. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin were for liver status. Urea was analysed using the Bethlot Searcy's method [53]. creatinine was determined by the method described by Larsen [54]. Sodium ion (Na\(^+\)) and chloride (Cl\(^-\)) ion levels were determined according to the instructions on their diagnostic kits purchased from Randox laboratories (UK). Potassium ion (K\(^+\)) was determined by direct spectrophotometric method. Bicarbonate (HCO\(_3\)-) was determined using Forrester et al [55]. method. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using Reitman and Frankel [56]. methods; alkaline phosphatase (ALP) was carried out using the phenolphthalein monophosphate method [57]. total bilirubin was measured using Doumas et al [58]. method.

2.7 Statistical Analysis

Results are presented as mean and standard deviations of triplicate determinations. Group comparisons were done using least significant difference (LSD). Significant difference was established at 5% level as described by Onu and Igwemma [59].

3. RESULTS AND DISCUSSION

Fig. 1 shows the weight changes in rats placed on bi-herbal formulation of aqueous extract of V.amygdalina and G. latifolium. From the results Group I rats (Normal control) had the highest increase in weight (8.81 g). It was followed by group III (6.38 g), group IV and group V. Group II rats (Experiment control) had reduced weight of -3.09 g. Gentamicin induced in the rats could be behind the reduction.

From Table 1, urea ranged from 34.66 to 71.20 mg/dl. Creatinine ranged from 0.89 to 2.06 mg/dl. Sodium (Na\(^+\)) ion values ranged from 135.02 to 153.31 mEq/L, Potassium (K\(^+\)) ranged from 3.98 to 4.87 mEq/L. Chloride (Cl\(^-\)) ranged from 81.29 to 104.00 mEq/L and bicarbonate (HCO\(_3\)-) ranged from 25.02 to 32.30 mmol/L. Urea is mainly a nitrogenous waste product of metabolism generated by protein break down [5,16]. It is normally eliminated exclusively through the urine by the kidneys [15,16,60]. Reduced urea excretion and consequent rise in blood concentration indicates possible kidney disease. Urea reduced significantly (p<0.05) in test rats (Groups III, IV and V) when compared to group II (Experimental control) rats but increased significantly (p<0.05) against group I (Normal control) rats. Creatinine has been described as the waste product of normal wear and tear on the muscles of the body. Different authors have noted that creatinine is a waste production of muscle metabolism [5,11,60]. It has important and interesting properties that make it very critical in assessing the failure of the renal system [5,27,60]. The creatinine levels of groups III to V rats reduced significantly (p<0.05) against group II rats. However, the increase observed in creatinine level of group V rats against group I rats was insignificant (p>0.05). Sodium ion, potassium ion and bicarbonate levels of groups III to V reduced significantly (p<0.05) when compared to group II rats but only groups II and IV had creatinine levels that increased significantly (p<0.05) against group I. Chloride in groups III to V increased significantly (p<0.05), when compared to the normal control (Group I) and experimental control (group II).

Aspartate aminotransferase (AST) ranged from 21.43 to 33.42 U/L. Alanine aminotransferase (ALT) ranged from 31.71 to 46.90 U/L. ALP ranged from 47.90 to 68.34 U/L while bilirubin total ranged from 0.75 to 1.51 mg/dl. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are excellent markers of liver damage brought about by exposure to toxic substances [16].

According to Duru et al [16]. Moss and Henderson [61]. when the integrity of the
hepatocellular membrane is comprised, there is extrusion of the enzymes into the plasma. Levels of AST and ALT in test rats reduced significantly (p<0.05) against the experimental control rats (group II) but test groups III and IV rats had significantly (p<0.05) increased AST levels against normal control rats (group I). Alkaline phosphatase (ALP) levels in test rats (groups III, IV, and V) reduced significantly (p<0.05) when compared to experimental control rats (group II) and increased significantly (p<0.05) against normal control (group I) rats. The observed enzyme reduction in test rats as the case maybe in this study against the control in the present study, could be indication of the extracts trying to salvage the integrity of the liver. Levels of bilirubin reduced significantly (p<0.05) in test groups (III, IV and V) when compared to the control. However, only test groups III and IV rats significantly increased (p<0.05) bilirubin against normal control rats (group I).

Fig.1. Weight changes in rats

Table 1. Nephrotoxicity indices

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Urea (mg/dl)</td>
<td>34.66±1.40^b</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.89±0.10^a</td>
</tr>
<tr>
<td>Na^+ (mEq/L)</td>
<td>137.68±0.80^b</td>
</tr>
<tr>
<td>K^+ (mEq/L)</td>
<td>4.28±0.75^c</td>
</tr>
<tr>
<td>Cl^– (mEq/L)</td>
<td>95.40±5.36^b</td>
</tr>
<tr>
<td>HCO_3^– (mmol/L)</td>
<td>26.20±3.00^b</td>
</tr>
</tbody>
</table>

Results are mean and standard deviation if triplicate determinations. Values with different letters of alphabets along the same row are statistically significant at 5% significant levels

Table 2. Liver enzyme of rats

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Groups</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>21.43±1.13^a</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>31.71±0.15^a</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>47.90±0.00^b</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.75±0.04^a</td>
</tr>
</tbody>
</table>

Results are mean and standard deviation if triplicate determinations. Values with different letters of alphabets along the same row are statistically significant at 5% significant levels
4. CONCLUSION
There was observed ameliorating effects on nephrotoxicity and liver damage in rats given bi-herbal formulation of aqueous extract of \textit{V. amygdalina} and \textit{G. latifolium} induced with gentamicin in this study. From the study, bi-herbal formulation of aqueous extract (5\% v/v) each of \textit{V. amygdalina} and \textit{G. latifolium} offered better protection than aqueous extract (10\% v/v) of \textit{V. amygdalina} and aqueous extract (10\% v/v) of \textit{G. latifolium}. This study has revealed the protective effects of bi-herbal formulation of aqueous extract of \textit{V. amygdalina} and \textit{G. latifolium} against gentamicin induced nephrotoxicity and liver injury in rats.

ETHICAL APPROVAL
This study was approved by Ethical Committee of the Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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