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Effect of Adedironic Herbal Mixture on Kidney, Electrolyte, Urea and Creatinine of Albino Rats

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Abstract
Background
Adedironic mixture is an anti-ulcer herbal mixture used in south western Nigeria as a herbal therapy for peptic ulcers it contains Whole plants of Ageratum conyzoides, roots of Vernonia amygdalina and Citrus aurantifolia and has proven to be a potent herbal therapy for ulcer
Aim and Objective
This study was designed to investigate the effect of Adedironic herbal mixture on the Kidney architecture, Electrolyte, Urea and Creatinine of Albino rats
Materials and Methods
20 rats divided into four groups were used. The control group received 2ml/kg of distilled water while the other three (3) groups were orally administered 0.5, 1.0 and 2.5g/kg/daydoses of Adedironic herbal mixture respectively for 28days. The animals were anesthetized and blood samples were collected for biochemical analysis. The kidneys were harvested and processed for histopathological examination.
Results
Results showed kidney vascular congestion and interstitial nephritis at dose 2.5g/kg and normal histology at dose 0.5g/kg of Adedironic herbal mixture administered. There was significant reduction ($p<0.05$) in the value of sodium at 0.5g/kgof the concoction, while Potassium, Urea and Creatinine levels were normal when compared with the control group.
Conclusion
With the results of this study it may be concluded that Adedironic herbal mixture has no deleterious effect on the Kidney architecture, Electrolytes, Urea and Creatinine values of albino rats

Keywords: *Adedironic herbal mixture, kidney, electrolyte, urea, creatinine*

1. Introduction

Adedironic mixture is an anti-ulcer herbal mixture used in south western Nigeria as an herbal therapy for peptic ulcers it contains; whole plants of Ageratum conyzoides, roots of Vernonia amygdalina and Citrus aurantifolia and has proven to be a potent herbal therapy for ulcer.

Ageratum conyzoides

Ageratum conyzoides is called goat weed in English, Imi-esu among Yorubas, Alkaura-tuturuwa in Hausa and Obiarakara among Igbo speaking people of Nigeria. It belongs to the family of Asteraceace. It is a pan-tropical herb with a long history of traditional medicinal uses. Its toxicity has not been well studied but the extracted oil has a powerful nauseating odour (Sood, 1973). Ageratum conyzoides is an erect annual herbaceous plant, 30-80cm tall with a long history of traditional medicinal uses in several countries of the world.

Figure 1: Ageratum Conyzoides (Sood, 1973)

Its methanolic extracts were reported by Oladejo et al. to have healing properties (Oladejo et al., 1973). Excision was made on dorsolateral flank of rats. The wounds were packed with Ageratum conyzoides dressing. Ageratum conyzoides treated wounds showed fewer inflammatory cells histologically, more fibrosis, greater wound contraction and significantly fewer fibroblasts than honey treated wounds. Adebayo et al. revealed the in-vitro activity of ethanol, petroleum ether, ethylacetate butanol and water extracts of Ageratum conyzoides on some cancer cell lines (Adebayo et al., 2010). They include human non-small cell lung carcinoma (A-549), human colon adenocarcinoma (HT-29), human gastric carcinoma (SGC-7901), human glioma (U-251), human breast cancer (MDA-MB 231), human carcinoma (DU-145), human hepatic carcinoma (BEL-7402), and mouse leukaemia (P-388).The researchers elucidated that ethylacetate extract exhibited highest cytotoxicity on A-549 and P-388 cancer cells. Durodola revealed range of chemical compounds analyzed from Ageratum conyzoides as alkaloids, flavonoids, chromenes, benzofurans and terpnoids (Durodola, 1977). He further reiterated medicinal use to include treatment of pneumonia, wounds and burns. Borthikur and Baruah expantiated its use as a bacteriocide, anti-dysentric and antilithic (Borthakur and Baruah, 1987) while Ekundayo et al. reported the use of aqueous extract of the plant as a bacteriocide in Asia, South America and Africa (Ekundayo et al., 1988). Guthens before 1948 highlighted the uses of Ageratum conyzoides to include the use as purgative, febrifuge, colic treatment of ulcers and wound dressing (Guthens, 1948). Vernonia amygdalina (bitter leaf) Vernonia amygdalina, a member of the Asteraceae family, is a small shrub that grows in tropical Africa. V. amygdalina typically grows to a height of 2–5 m (6.6–16.4 ft). The leaves are elliptical and up to 20 cm (7.9 in) long. The leaves are green with a characteristic odour and a bitter taste. No seeds are produced and the tree has therefore to be distributed through cutting. Its bark is rough andit is commonly called bitter leaf in English because of its bitter taste, Ewuro in Yoruba,Shakwa shuwaka in Hausa and Onugbu among Igbo speaking people of Nigeria. Other African common names include grawa (Amharic), Etidot (Ibibio), Ityuna (Tiv), Oriwo (Edo), Mululuza (Uganda), Labwori (Acholi), Olusia (Luo) and Ndoleh (Cameroon).In Nigeria, V. amygdalina is used for food and medicinal purposes. The roots and twigs are used for abdominal and other gastrointestinal problems in humans while the decoctions from the leaves are used as anti-malaria in Guinea and as cough remedy in Ghana (Akinpelu, 1999; Masaba, 2000). It is widely described by livestock farmers as a potent anti-helmitic (Alawa et al., 2008). The cooked leaves are a staple vegetable in soups and stews of various cultures throughout equatorial Africa. There are about 200 species of Vernonia. Ofori et al.described Vernonia amygdalinaas plants that are naturally found along rivers and lakes, in forest, wood and grassland where mean annual rainfall is 750- 800mm. it thrives on all soil types but prefers humus-rich soils (Ofori et al., 2013).

Figure 2: Vernonia Amygdalina (Ofori Et Al., 2013)

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Oluwatosin et al. investigated the lipid-lowering effects of methanolic extract of Vernonia amygdalina leaves in rats fed with a high cholesterol diet (Oluwatosin, 2008). The researchers confirmed and concluded from the result of their investigation that Vernonia amygdalina could serve as a new potential natural product for the treatment of hyperlipidemia. Anibijuwon et al. assessed the physiologically active principles found in Vernonia amygdalina extract (Anibijuwon, et al., 1979). Their results showed the presence of terpenoids, tannins, alkaloids, saponins and glycosides. They confirmed that the aqueous extract of Vernonia amygdalina had very high value of minimum inhibitory concentration (MIC) on both Staphylococcus aureus and Streptococcus mutans.

Citrus aurantifolia (lime tree)

It belongs to the family Rutaceae. It is used for nausea, indigestion and constipation (Bent and Nko, 2004). It exhibits activities for cold fevers, sore throats, sinusitis and bronchitis as well as helping asthma (Khanc, 2007).The Key lime (Citrusaurantiifolia) is a citrus hybrid (C. micrantha x C. medica) with a globose (spherical shaped) fruit, 2.5–5 cm in diameter (1–2 in), that is yellow when ripe but usually picked green commercially. It is smaller and seedier, with a higher acidity, a stronger aroma, and a thinner ring, than that of the Persian lime (Citrus latifolia). It is valued for its unique flavour compared to other limes. The name comes from its association with the Florida Keys, where it is best known as the flavouring ingredient in Key lime pie. It is also known as West Indian lime, bartender's lime, Omani lime, or Mexican lime, the last classified as a distinct race with a thicker skin and darker green colour. Philippine varieties have various names, including dayap and bilolo. It is aromatic, juicy, and highly superior to the lemon." C. aurantiifolia is a shrubby tree, to 5 m (16 ft), with many thorns. Dwarf varieties exist that can be grown indoors during winter months and in colder climates. Its trunk, which rarely grows straight, has many branches, and they often originate quite far down on the trunk. The leaves are ovate, 2.5–9 cm (0.98–3.54 in) long, resembling orange leaves (the scientific name aurantiifolia refers to this resemblance to the leaves of the orange, (Citrus aurantium). The flowers are 2.5 cm (0.98 in) in diameter, are yellowish white with a light purple tinge on the margins. Flowers and fruit appear throughout the year, but are most abundant from May to September in the Northern Hemisphere. When in contact with the skin, the Key lime can sometimes cause phytophotodermatitis, in which a chemical reaction makes the skin extra sensitive to ultraviolet light (Bisen et al., 2012).

Figure 3: Citrus Aurantifolia [14]

During the last two decades, Citrus aurantifolia has been subjected to extensive phytochemicals, pharmacological and clinical investigation in the area of insecticides (Loius), cardiac diseases, anti-cancer, eye conditions, inflammatory bowel disease and improved lung function. Rafi et al. evaluated the antimicrobial efficacy of Citrus aurantifolia leaves against some micro-organisms-bacteria and fungus were Staphylococcus aureus Esherichia coli, Klebsiella pneumonia, Pseudomonas spp, Aspergillus Niger Aspergillus fumigates, Mucor spp and Penicillium. It was indicated by these researchers that the hydro-alcoholic extract of Citrus aurantifolia leaves possess good antibacterial and antifungal activity (Rafi et al.,2012). This confirms the presence of bioactive compounds and the great possibility of being used in primary health care.

2. Materials and Methods

Rats of either sex were assigned to four groups. Each group contained five rats. The first group was used as control and received 2ml/kg of distilled water for 28days orally. The remaining three (3) groups in each drug model were administered doses of 0.5, 1.0 and 2.5g/kg/day respectively for 28days orally. The dose of the extract was chosen based on the method described by Nora et al.(Nora et al., 2016). On the 28th day, the animals were anesthetized with chloroform vapour in a chamber and blood samples were collected from the abdominal aortas for biochemical analysis. The kidneys were harvested and placed on absorbent paper for 5minutes to drain the blood and transferred into neutral buffered formal for fixation. The organs were processed for histopathological examination.

2.1. Histopathological Investigation

The tissue specimens were fixed in neutral buffered formalin and processed in Thermo Scientific Spin Tissue Processors STP 120. The tissues were treated for half hour each in three baths of 50% alcohol and were transferred into three baths of 80/20 Ethanol/IPA for half hour each and later treated in three baths of neat Isopropyl alcohol (IPA) for 1

hour each. The samples were allowed to drain for 2 hours before they were immersed in two baths of cell path wax at 56°C for 1½ hours each (Wallington and Drury, 1979).
The tissues were embedded in cell path paraffin wax, sectioned and stained using Haematoxylin and Eosin stains and special stains using methods described by Wallington and Drury (Wallington and Drury, 1979).

2.2. Biochemical Evaluation

About 5ml of blood sample was drawn with needle and syringe from the abdominal aortas of the rats into lithium heparin bottles and mixed well. Plasma was separated into labelled plain bottles by using Pasteur pipettes. Samples were analysed using Randox kits for Urea, Jaffe's reaction method for Creatinine and flame emission photometry for Sodium and Potassium (Doumas, 1981).

2.3. Statistical Analysis

Data are presented as mean ± standard deviation and where statistically analysed using Microsoft excel for T test and significance level using alpha error level (95% confidence level). P<0.05 where considered statistically significant.

3. Results

Plates 1 to 8 are photomicrographs showing the histopathological analysis of the effect of Adedironic herbal mixture on the kidney of albino rats. The highest dose (2500mg/kg) of the concoction (plates 3 and 7) showed vascular congestion and interstitial nephritis with the least dose (500mg/kg) showing a normal histology (plates 1 and 5).
Table 1 shows biochemical evaluations done in this study. There was significant reduction (p<0.05) in the value of sodium at 500mg/kg of the herbal mixture when compared with the control group. All other biochemical parameters carried out in this study were normal when compared with their respective controls.

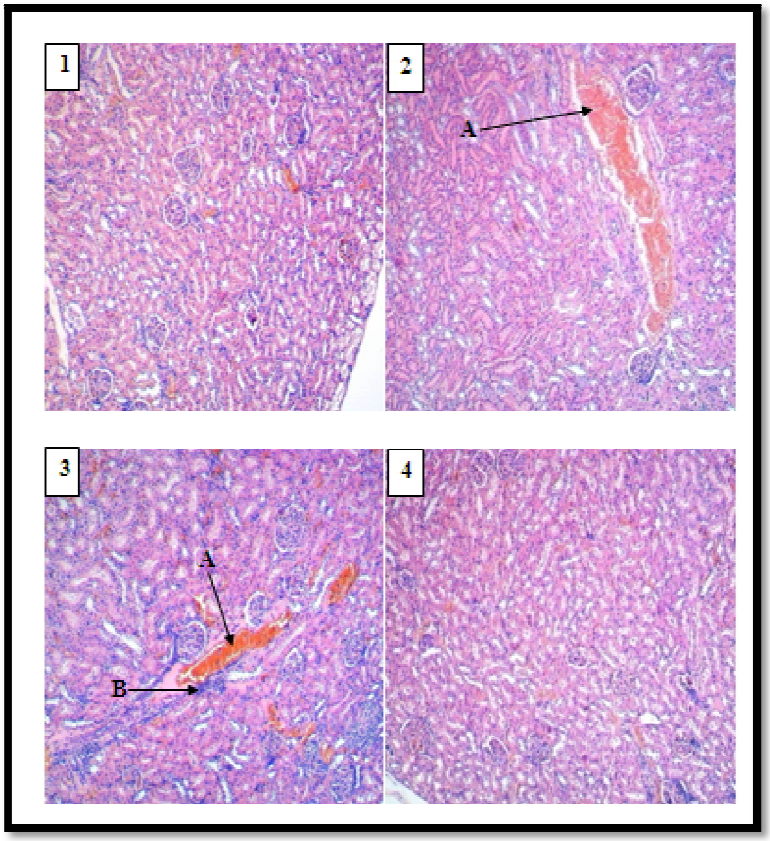

Figure 4: Kidney (1) Normal (2) A, Vascular Congestion (3) A, Vascular Congestion B, Interstitial Nephritis (4) Normal 1-4 (500, 1000, 2500mg/Kg Adedironic Herbal Mixture and CONTROL Groups Respectively), H&E X400

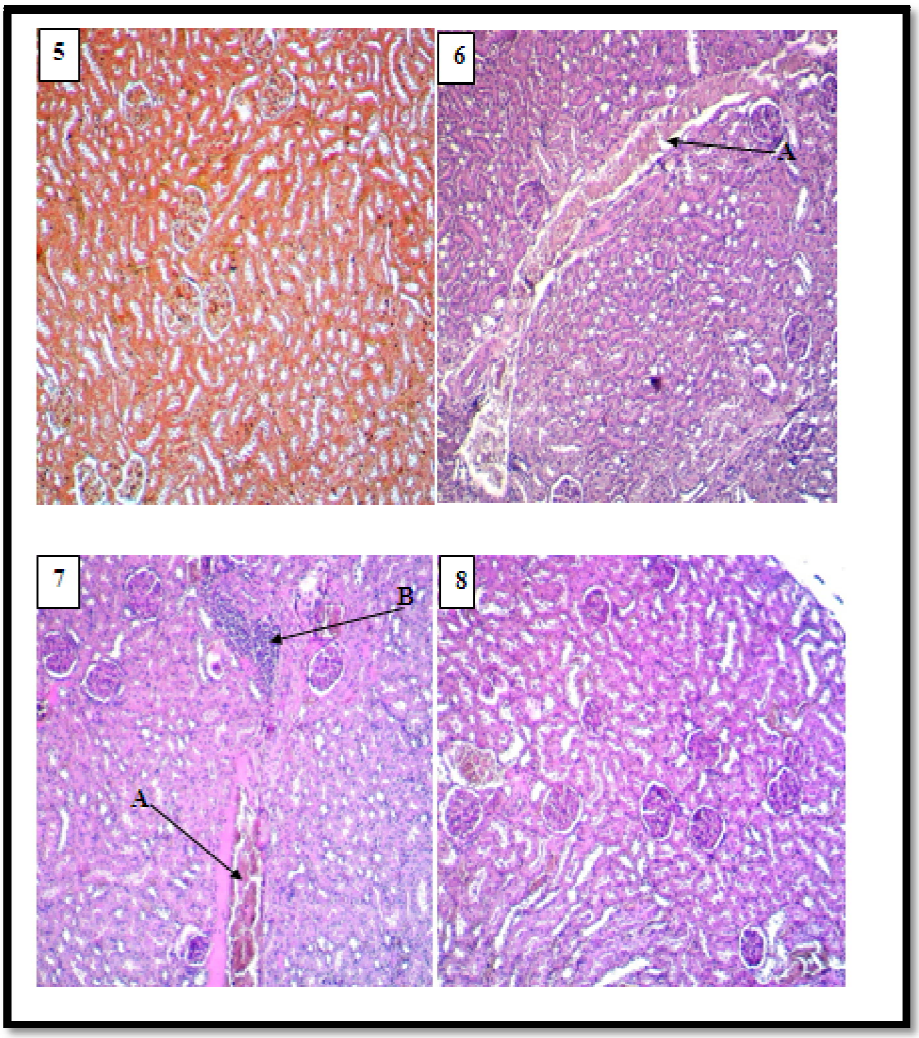

Figure 5: Kidney (5) Normal MASSON TRICHROME X400 (6) A, Vascular Congestion PAS X400 (7) A, Vascular Congestion B, Interstitial Nephritis PAS X400 (8) Normal PAS X400 93-96 (500, 1000, 2500mg/Kg Plant Mixture and CONTROL Groups Respectively)

	Na+	K+	UREA	Cr
CONTROL	134±2	8.7±1.7	34.4±4.6	0.1±0.04
(0.5g/kg)	130.2±2.9*	7.3±1.2	39±5.9	0.2±0.1
(1g/kg)	131.8±2.9	8.8±1.7	32±2.8	0.2±0.2
(2.5g/kg)	129.6±5.5	7.9±0.9	37±7.7	0.2±0.1

Table 1: Biochemical Parameters of Test and Control Rats – Pure Plant Mixture

4. Discussion

Plants have been selected and used as drugs over centuries and have great potential to treat both human and livestock diseases (Alawa, 2008).Some herbal medicines have been demonstrated to be efficacious (Doumas, 1981; MacDonald and Vancrey 2004).Although, herbal medicines are natural, they must be subjected to necessary investigation to dissect valuable herbal drugs from harmful and toxic ones (Phlomena, 2011; Efferth and Kaina, 2011).

In this study, Adedironic herbal mixture -An anti-ulcer herbal concoction used for the treatment of peptic ulcers is under focus for its effect on the Kidney, Electrolytes Urea and Creatinine of Albino rats.

Findings in this study shows that Adedironic herbal mixture has little effect on the kidney of albino rats. The highest dose (2500mg/kg) of the concoction showed a mild vascular congestion and interstitial nephritis while the least dose (500mg/kg) showed normal histology. There was significant reduction ($p<0.05$) in the value of sodium at 500mg/kg of the herbal mixture when compared with the control group with a normal sodium value for all other doses. The potassium, urea and creatinine levels were normal when compared with their respective controls. This shows that the ingestion of the herbal concoction has no toxic effect on the kidney architecture, Electrolytes, Urea and Creatinine levels. Adedironic herbal mixture can therefore categorised as a nontoxic substance in accordance to the Hodge and sterner scale for toxicity (Hodge and Sterner, 1956) and can also be classified as a substance with low toxicity in reference to OECD (OECD, 2001). Effect on diuretic action due to enhanced excretion could be responsible for the significant reduction of sodium levels and vascular congestion in the kidneys, but it is not very clear if both are related. Similar results were seen

in the work of Nora et al who studied the acute and sub-acute evaluation of aqueous leaf extract of nauclea latifolia in albino rats (Nora et al., 2016).

5. Conclusion

With the results of this study it may be concluded that Adedironic herbal mixture at recommended doses has no deleterious effect on the Kidneys and Biomedical indices such as Sodium, Potassium, Urea and Creatinine levels of albino rats

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