ORIGINAL ARTICLE

Ameliorative effect of aqueous extract of bitter leaf on egg yolk induced kidney toxicity in adult wistar rats

ABSTRACT

The aim of the study is to assess the effect of aqeueous extract of bitter leaf on egg yolk induced kidney toxicity in adult wistar rat. A total of 44 (forty four) adult wistar rats weighing between 220 g to 250 g were separated into 4 groups of 11 rats per group. Group A rats were placed on normal diet only while Group B rats received 300 mg/ kg body weight / day (BWT/D) of egg yolk. Group C rats received 250mg / kg BWT/D of bitter leaf. Group D rats received 300 mg/ kg BWT/D of egg yolk and 250mg / kg BWT/D of bitter leaf. The dosage was given for 32 days via oro-gastric method. The biochemical outcome showed that group B revealed marked elevation in the urea, SOD, cholesterol level when compared with the other groups. Histologically, group B showed a mild vascular obstruction and patchy tubular necrosis, while group A, C and D revealed normal architecture of the kidney. We therefore concluded that the aqueous extract of bitter leaf have antioxidative and neproprotective properties against egg yolk induced kidney toxicity in wistar rat.

KEYWORD: Bitter leaf, Egg yolk, Cholesterol, Urea, Superoxide dismutase

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INTRODUCTION

Vernonia amygdalina is a shrub common to tropical Africa [1]. It is a very small tree of about 9 to 11m in height, with many broad leaves of varying colours which depend on the time of the year you are seeing the color. Nowadays, the leaf is used to make vegetable soup without washing the bitter taste or content away.

Bitter leaf soup is a very popular soup among the Owan people in the south – southern part of Nigeria[2].

A lot of scientists have opined that the flavanoids and its saponins are the active principles which confer antioxidant and anti-tumor activities on the plant [3]. It has been reported to contain antioxidants (luteolin, luteolin 7-O- _-glucuronoside and luteolin 7-O-_-glucoside flavonoid) isolated from the leaves [4].

A lot of herbalists and native doctors in Africa have recommended its aqueous extracts for their clients as treatment for a lot of ailments ranging from emesis, loss of appetite, nausea, dysentery and other gastrointestinal tract problems to sexually transmitted diseases and diabetes mellitus [1]. Previous work have shown that it as pharmacological activities such as anticancer effect, hepatoprotective effect, antioxidant effect, hypolipidaemic agent and nephroprotetive activities, antiviral effect.[5,6,7,2,8].

The aim of the study is to ascertain the nephron-protective nature of bitter leaf on egg yolk induced kidney toxicity in adult wistat rats.

MATERIAL

The leaves of *Vernonia amygdalina* were harvested from a farm in Ikhin town in Owan East Local Government Area of Edo State, Nigeria. It was identified by Mr. Sunny Nweke, a curator in the department of Pharmacognosy, University of Benin, Benin City.

METHOD

The plant material was dried in the oven at 50^{o}c after air drying for about a week. The dried leaf material was then grounded using the milling machine. The grounded powder was then weighed and taken for maceration. The cold maceration was done by soaking 700g of powdered Vernonia amygdalina leaf in 4 liters of water which was acting as solvent for 24 hours. The soaked *Vernonia amygdalina* was then filtered using cotton wool as filter and the filtrate was then concentrated over hot water bath using evaporating dishes. The residual solid extract of *Vernonia amygdalina* leaf after concentration was weighed to be 15g and was transferred into sample bottles for stored inside a refrigerator at 4^{0}C. A little amount of the extracts of *Vernonia amygdalina* leaf was dissolved in a measured volume of distilled water any time the extract was needed for the studies. The concentrated *Vernonia amygdalina* extract was then given to the wistar rats at 250 mg/kg body weight for 32 days.

All the preparations were done at the department of Anatomy, Faculty of Basic Medical Science, College of Medical Sciences, University of Benin, Benin City, Edo State.

Experimental animals: Adult Wistar rats numbering 44 (forty four) and weighing between 200g to 240g were purchased from the animal house in the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria and were used as experimental animal for this research .

The animals were kept in the animal house for 2 weeks prior to the experiment for acclimatization. The animals were fed with rat food and clean water were served *ad libitum*. They were placed on standard livestock feed (vital growers feed).

The wistar rat experimental usage was in conferring to the National Institutes of Health Guide for the care and use of laboratory Animals (NIH, 2002 publication, No. 83-23), (Revised 1978). The wistar rats were grouped into four (4) groups of eleven (11) animals per group.

Group A animals served as control that were neither administered with egg yolk nor bitter leaf.

Group B animals were treated daily with oral administration of 300mg/kg body weight of egg yolk only for 32 days.

Group C animals were treated with oral administration of 250mg/kg body weight of

Vernonia amygdalina leaf extract only for 32 days.

Group D animals were treated daily with oral administration of 300 mg/kg body weight of egg yolk followed by250mg/kg body weight of bitter leaf, for 32 days.

Administration of the egg yolk was performed orally once daily using metal cannula attached to a 1.0 ml syringe.

METHOD OF SACRIFICE AND TISSUE COLLECTION

After 32 days, the animals were anesthetized via chloroform inhalation, the anterior abdominal wall of the rats were exposed by midline incision and blood samples were collected which was later spined into serum and plasma. It was the spined serum that was used for biochemical analysis. Also, the kidneys were harvested and quickly fixed in 10% formal saline for 24 hour before the routine histological analysis.

The tissue sections were examined under Leica DM750 research microscope with a digital camera (Leica ICC50) attached. Digital photomicrographs of the tissue sections were taken at X100 magnifications

Biochemical assays:

SOD activity in kidney was determined according to the method described by Marklund and Marklund.

The activities urea was determined by the method of Fawcet and Scott, 1960.

Cholesterol level was estimated by standard procedure using an auto- analyzer with a recognized biochemical kit.

Statistical analysis: Data were expressed as the mean ± SEM. The data were analyzed by analysis of variance (ANOVA) followed by least square difference using the Statistical Package for the Social Sciences (S.P.S.S. 17). The level of significance was set at P<0.001.

RESULTS

The mean urea was found to be significantly high in egg yolk only group (90.87±3.41) but the urea level are within normal range in other groups (Control rats who received 1ml of sterile water daily (24.11±3.23), bitter leaf only group (30.61±2.13) and the combined group (34.10±0.41) respectively). The mean Cholesterol level was also found to be significantly elevated in egg yolk only group (260.13 ±12.44) when compared to control (130.22±2.43), bitter leaf only (142.56 ±1.31) and combined group (148.11±0.24). The mean SOD level was seen to be significantly increased in the egg yolk only group (150.30±4.12) while the control (50.19±2.54), bitter leaf only (58.12 ±2.32) and combined group (60.10±1.50) are within normal range.

TABLE 1

		Group A	Group B	Group C	Group D	P- Value
Urea mg/dl	mean±SD	24.11±3.23	90.87±3.41	30.61±2.13	34.10±0.41	p<0.001
Choles g/dl	mean±SD	130.22±2.43	260.13±12.44	42.56±1.31	148.11±0.24	P<0.001
SOD ng/mL	Mean±SD	50.19 ± 2.54	150.30 ±4.12	58.12 ± 2.32	60.10±1 .50	P<0.001

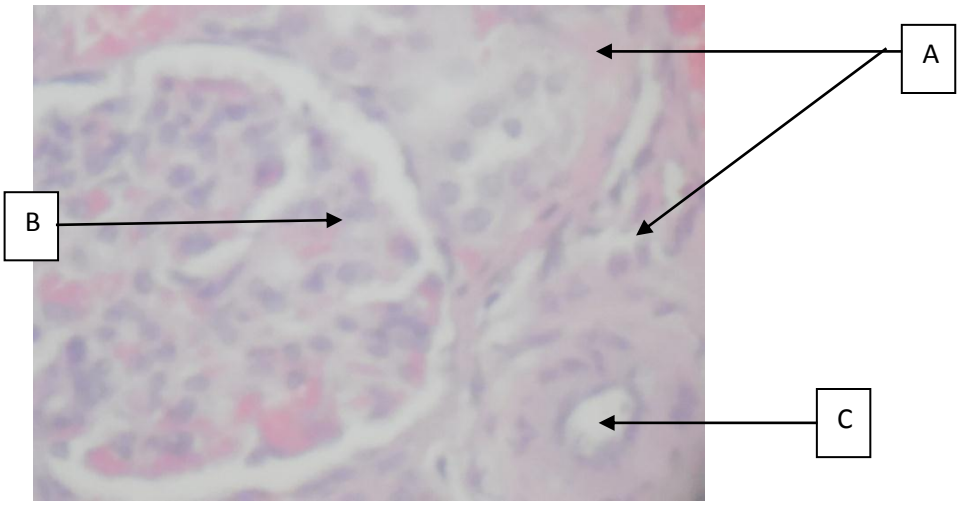

Plate. A Control: Rat kidney composed of A, tubules, B, glomerulus, C, arcuate artery (H&E x 100)

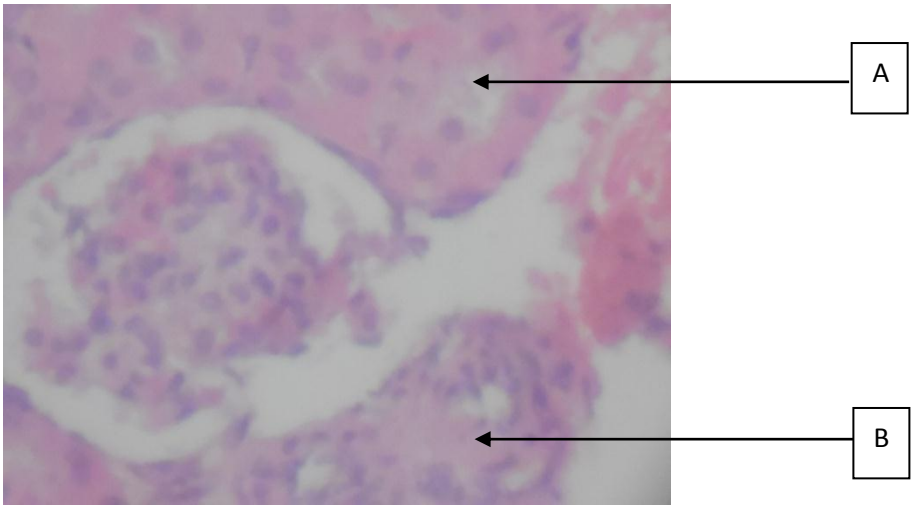

Plate B: Rat kidney given Egg Yolk only showing A, patchy tubular necrosis and B, vascular obstruction (H&E x 100)

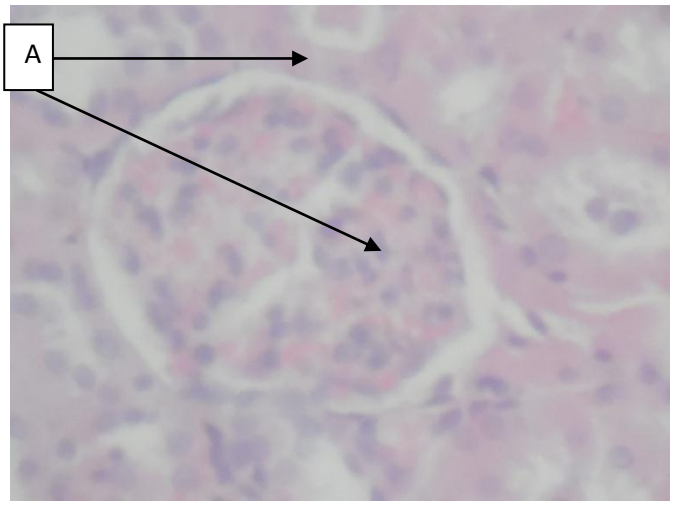

Plate C: Rat kidney given 250mg/kg of the *Vernonia amygdalina* extract showing A, normal renal architecture (H&E x 100)

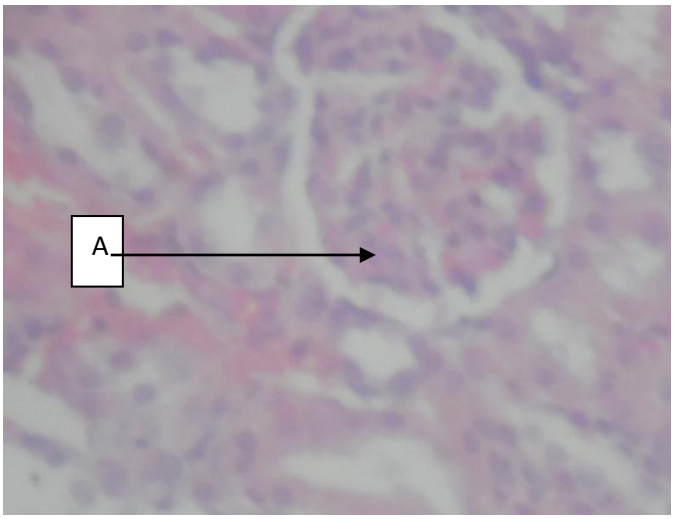

Plate D: Rat kidney given 250mg/kg of Egg Yolk plus 250mg/kg of Extract showing a mild interstitial congestion (H&E x 100)

Plate A shows normal histology of the kidney of the control group.

Plate B shows the histology of the kidney from the group given daily dose of 300mg/kg body weight egg yolk only, showing patchy tubular necrosis and vascular obstruction.

Plate C that was given 250mg/kg body weight of Vernonia amygdalina extract shows normal renal architecture.

Also, plate D that was given 300mg/kg body weight of egg yolk and 250mg/kg of body weight of Vernonia amygdalina leaf revealed mild interstitial congestion

DISCUSSION

Herbal leaves are widely accepted to be a blessing in the third world countries. (Ehimigbai 2015) Antioxidant enzymes like SOD and others naturally act via the free radical mopping mechanism by scavenging the excess free radicals like hydrogen peroxide in the system thereby preventing the oxidative action of the egg yolk on the tissue. Therefore, the antioxidant strength of the extract reduces the level of oxidative stress on the kidney and in that case recovered the normal renal function of the rat, which was before damaged by the egg yolk.

The urea, SOD and cholesterol level was statistically significant in egg yolk only due to toxic effect of excess egg yolk on the kidney leading to reduction in the elimination of the urea from the system and resulting in the accumulation of urea in the blood stream (TABLE 1) while the bitter leaf only group and bitter leaf plus egg yolk group was able to ameliorate these toxic effect because of the flavonoid content of the bitter leaf which is a strong antioxidant component. The bitter leaf is therefore enhancing the renal function making the kidney to liberate the toxic substance termed urea. The kidney is the medium where most toxic substances like plant and drugs are eliminated from the body. So therefore a very high intracellular accumulation do happened in this region, mainly in the medulla due to its poor blood supply when compared to the renal cortex(Aronson 2003)

The histological outcome is supported by earlier work done by Ehimigbai 2015. In their work, They showed the efficacious nature of bitter leaf. They revealed the ameliorative strength of bitter leaf on the kidney of adult rats. Therefore, revealing the nephro-protective power of bitter leaf.

CONCLUSION

It can be concluded that the aqueous extract of bitter leaf was able to ameliorate the egg yolk induced kidney toxicity in adult wistar rat.

RECCOMENDATION

Bitter leaf may act as an antidote in the management of hyperlipidemia and nephrotoxicity

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