MANAGEMENT OF ALLOXAN INDUCED DIABETES IN MALE RATS WITH ETHANOL LEAF EXTRACT OF AFRICAN EGG PLANT AND BITTER LEAF SHOWING THEIR EFFECTS ON SOME KIDNEY INDICES

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ABSTRACT

The effects of ethanol leaf extract of African eggplant and bitter leaf plant, as well as their combination, on kidney histology and antioxidant enzymes activity, as well as selected biochemical and haematological parameters in male Wistar rats, were studied. The rats were divided into four groups: control group A, diabetic group B, group C treated with 1000 mg/kg African eggplant, group D treated with 1000 mg/kg bitter leaf, and group E treated with a combination of 1000 mg/kg African eggplant and bitter leaf. Level of significance set at $P< 0.05$, and groups C, D, and E were compared with groups B. Results showed that groups C and D showed improvement in sodium level and platelets count. Group D showed reduction in potassium level, with no positive changes in urea and creatinine concentration in groups C, D and E but red blood cell and haematocrit count increased. Group C showed increase in white blood cell. Groups C and E showed increase in haemoglobin content of the blood. Superoxide dismutase (SOD) activities and catalase (CAT) activities increased, while Malondialdehyde (MDA) decreased in groups C, D and E kidney tissue. Kidney histology in groups C and D showed regenerative changes, while group E had positive effect kidney tissue. The Results of this study showed that extracts were able to improve kidney function and haematological parameters in diabetic rats.

Keywords: Blood electrolyte, haematological parameters, kidney histology, antioxidant enzymes.
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INTRODUCTION

Medicinal plants have been used in the treatment of a variety of disease, due to the fact they are cheap and available, potent and have little or no side effect [1]. Many plants have long been thought to be a major source of effective anti-diabetic activities. Medicinal herbs are utilized to treat diabetes in developing countries, particularly to ease the financial load of standard drugs on the patient [2].

Solanum macrocarporn L is another name for African eggplants. They belong to the Solanum genus and are known as "*efo Igbo*" among the Yoruba language tribes of south west Nigeria. They are mostly produced in Africa for their fruits and leaves and are indigenous to Africa [3]. All plant parts are nutritious; the leaves are used in vegetable soups, and the fruits are consumed in Indonesia when cooked with rice. The plant's root is used to treat viral infections, itching, body pain, bronchitis, as well as to promote wound healing, its seeds are being used to treat toothaches. Gout, rheumatism, and angina are all treated with the juice from the leaves. It's also utilized as a labour anaesthetic agent, in the management of inflammation, and Parkinson's disease [4]. Different research studies done with African eggplant shows that its methanol extract has anti-diabetic activity [5], has antioxidant properties in the brain and liver of rats [6], African eggplant also shows anti- hypercholesterolemia activity [7].

Vernonia amygdalina also known as bitter leaf plant is an important shrub found in tropical Africa, it is a member of the Asteraceae family. It grows predominantly in tropical Africa especially in Nigeria, Zimbabwe and South Africa and it is domesticated in parts of West Africa [8]. It has a bitter-sweet taste when consumed, its bitter taste is caused by the presence of biologically active compounds like saponins, alkaloids, tannins, and glycosides [8]. Traditionally, the leaves of bitter leaf are used as a vegetable (parboiled leaves in soup) or as tonics (aqueous extracts) to treat a variety of ailments [9]. Many herbalists and naturopathic doctors prescribe aqueous extracts to their patients for anaemia, nausea, diabetes, loss of appetite, dysentery, and other gastro intestinal problems. Extracts of *V. amygdalina* have also been shown to help suppress, delay, or kill cancerous cells [10].

Diabetes mellitus is a metabolic disorder that is marked by a loss of glucose homeostasis due to a deficiency in insulin secretions and insulin action, resulting in impaired metabolism of glucose and other energy-yielding body fuels such as lipids and proteins [11]. Nephropathy is one of the major complications of

diabetes and is the leading cause of chronic kidney disease and end-stage renal failure in diabetic patients [12]. In diabetes mellitus there is elevated blood glucose which will in turn affect blood parameter [13]. The bioactive components present in plants, work together to give each particular plant its potential useful properties, and since medicinal plant contains different bioactive component, the combination of two medicinal plant may prove beneficial in the treatment and management of disorders, thus this study will evaluate the anti-diabetic potential of the ethanol leaf extract of two common medicinal plant; African eggplant and bitter leaf showing their effects on the kidney structure, antioxidant enzymes activity of the kidney with some selected biochemical and haematological parameters.

MATERIALS AND METHODS

Animal care and grouping

Twenty adults apparently healthy male Wistar rats weighing between 160 g – 200 g were used for this experiment. The rats were bred in plastic and wire guaze cages in the animal house of the Obafemi Awolowo College of Health science, Sagamu campus. Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. The rats were allowed to acclimatize for a period of two weeks and were maintained on Growers feed from Joyful Feed and Flour Mill Ltd, Nigeria. Food and water were provided ad libitum. Animals were grouped according to table 1 below

Plants material

Matured leaves of bitter leaf plant were collected from an indigenous farm in Ikenne/Sagamu area of South West Nigeria, while matured leaves of African eggplant were bought from a local market in Ikenne/Sagamu area of South West Nigeria. These samples were identified and authenticated at the Department of plant science, Olabisi Onabanjo University, Ago-Iwoye, Ogun state, Nigeria.

Preparation of the ethanol extract of African eggplant and bitter leaf plant

Leaves of African eggplant and bitter leaf plant were air dried and powdered with the use of a blender. 150 g of the powdered leaf was soaked in 750 ml of ethanol (70% ethanol and 30% water) for 3 days inside a refrigerator. The resultant liquid was filtered using a funnel plugged with glass wool. After filtration the filtrate was heated at a temperature of 40°C for 5 min to allow ethanol to evaporate.

Administration of plant extracts

Ethanol extract of the leaves of African eggplant and bitter leaf plant (100mg/100g) were administered orally to the rats using an oral cannula. Treatment was done in the morning every day before the animals were fed and in the evening for the combination treatment for over a period of two weeks (14 days).

Determination of percentage yield of African eggplant and bitter leaf plant

The percentage yield of African eggplant extract was determined by calculating the percentage of the weight of the extract to the original weight before drying the sample, using the formula;

$$percentage\ yield = \frac{weight\ of\ extract}{weight\ of\ sample} \times \frac{100}{1}$$

Weight of African eggplant= 150 g
Weight of dried extract of African eggplant= 73.4g
Weight of extract = 150 g — 73.4g= 76.6 g

$$Percentage\ yield = \frac{76.6g}{150g} \times \frac{100}{1} = 51.0\%$$

The percentage yield for African eggplant is 51.0%
The percentage yield for bitter leaf plant was also calculated using the same formula stated above;

$$percentage\ yield = \frac{weight\ of\ extract}{weight\ of\ sample} \times \frac{100}{1}$$

Weight of bitter leaf plant – 150 g
Weight of dried extract of bitter leaf plant- 70.10g
Weight of extract = 150g—70.10g= 79.9g

$$percentage\ yield = \frac{79.9g}{150g} \times \frac{100}{1} = 53.3\%$$

The percentage yield for bitter leaf is 53.3 g

Measurement of fasting blood glucose

Fasting blood glucose was determined in a drop of blood from the tail using a glucometer (ACCU-CHECK, Roche, Germany), after an overnight fast of 14 hours every 48 hours.

Induction of diabetes

This was done using a single intra-peritoneal injection of newly prepared alloxan monohydrate (150 mg/kg body weight) dissolved in distilled water, before this, the rats were fasted overnight and their fasting blood glucose levels were measured. Then, 48 h after induction of alloxan, the animal's fasting blood glucose was evaluated and baseline blood glucose concentration was taken with the aid of Accu-check glucometer and rats with fasting blood glucose level ≥200 mg/dL were documented to be diabetic and used in this study

Histological examination

The kidney tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and 5 µm thick sections were prepared and stained with haematoxylin and eosin using standard procedures. The slides were viewed under light microscope and photomicrographs were taken (400×)

Procedure for determination of antioxidant enzymes
The kidney tissues to be assessed for oxidative studies were homogenized in phosphate buffer in ratio four to one. Superoxide dismutase (SOD) activities, glutathione reductase (GSH) activities, catalase (CAT) activities and malondialdehyde (marker of lipid peroxidation (MDA) were determined. Superoxide dismutase activities were determined according to the method of Valerino and McCormack (1971) [14]. Increased absorbance was monitored with a UV spectrophotometer at 480 nm every 60 seconds for 180 seconds. One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during one minute. The activity of SOD was expressed as µg/mg protein. Reduced glutathaione was determined using the methods of Sedlak and Lindsay (1968) [15]. The absorbance of the yellow color formed upon the addition of Ellman's reagent was read within 5 minutes at 412 nm with a UV spectrophotometer. A plot of absorbance versus concentration of reduced GSH was then obtained from a serial dilution of the stock GSH prepared by adding 1.5 mL of phosphate buffer and 1.5 mL of Ellman's reagent. The amount of GSH was expressed as µg/mg protein [16]. Catalase activity was determined with the method described by Shina (1972) [17]. Proper dilution of the serum samples was done at ratio one to ten dilution in series. Catalase was expressed as mmoles of H_2O_2 consumed per minute per mg protein. It used the principle that dichromate in acetic acid as an unstable intermediate. The chromic acetate produced was measured colorimetrically at 570 nm.

Malondialdehyde (MDA) was determined spectrophotometrically from the pink color product of thiobarbituric acid (TBA) reactive substances complex. 0.1 mL of the test sample was mixed with 0.5 mL of 10% TCA and 0.5 mL of 75% TBA was added to it. The mixture was then placed in a water bath at 80^{o}C for 45 minutes. The resulting pink solution's absorbance was measured against a reference blank of distilled water at 532 nm. The test sample was calibrated using the MDA as a standard and the result was expressed as the amount of free MDA produced. The MDA level was calculated according to Adam-Vizi and Sergi (1982) [18] The Lipid peroxidation was expressed as µg/mg Protein.

Procedure for blood collection
Blood was collected from the orbital venous sinus, the rat was restrained, the neck gently scruffed and the eye made to bulge. A capillary tube was inserted dorsally into the eye and blood was allowed to flow by capillary action into the capillary tube. Blood sample were collected into sample bottles (EDTA and Lithium heparinized bottle) until needed.

Determination of full blood count
White blood cell (WBC), red blood cell (RBC), haemoglobin level (HGB), haematocrit level (HCT), and platelet count level (PLT), were estimated using an automated Beckman coulter haematological analyser.

Determination of serum elctrolytes
Potassium ion (K^+), sodium ion (Na^+), chloride ion (Cl^-) and pH were determined using an electrolyte analyser (SFRI ISE6000- France

Statistical analysis
The statistical analysis of data collected from twenty-five (25) male Wistar rats was done using the SPSS-8.0 statistical software package (Marija and Norusis 1998) for analysis of data. The data was presented as Mean± Standard Error of Mean (SEM) and statistical analysis was carried out using the student t-test and a standard software package (ANOVA). Values were considered to be statistically significant when $p<0.05$

RESULTS AND DISCUSSION

Effect of combined leaf extract of African eggplant and bitter leaf on blood electrolytes and haematological parameters in alloxan induced diabetes male Wistar rats
When compared with the normal control and with level of significance set at $p<0.05$, Na+ was observed to reduce across all group but was only significant in AEP and BLP treated rats, Cl^-, urea and creatinine levels were also observed to increase across all groups but only showed significance in DC (Cl^-), DC,AEP, BLP, and combined treatment (urea and creatinine), values of K+ shows increase in DC, AEP and combined treatment and decrease in BLP groups, these changes seen in K^+ were not significant when compared to the control group as observed from table 2. Across all test group, the changes in the haematological parameters were compared to the normal control group, In the diabetic control rats value of WBC, RBC, and HCT decreased while HGB and PLT increased, all the changes in DC rats were not significant, In rats treated with AEP, PLT and WBC showed decrease values, while other parameters increased, only WBC, RBC and HGB had significant changes. In BLP treated group, there was decrease in RBC value while other parameters increased; all changes in this group were not significant. In the combined treatment group, RBC and HCT value decreased, while the value of other parameters increased; only PLT had changes that were significant in the combined group according to observation from table 3. The analysis of haematological and biochemical parameters could show the harmful effects of metabolic diseases and foreign chemicals, such as plant extracts,

on the blood components. They're also assessed to look for changes in the quantities of biomolecules like enzymes and metabolic products, as well as haematology, proper organ function, and histo-morphology [19]. One of the main symptoms of diabetes is hyperglycaemia which will in turn cause electrolyte abnormalities, the balance between hyperglycaemia-induced water transport out of the cells, which lowers Na+ concentration, is reflected in the serum concentration of Na+ in diabetes mellitus [20], there was improvement in serum Na+ across the groups that received treatment, when compared with the DC, The increase in K+ in DC, is supported by another study Khadouri *et al.* (1987) [21] which concluded that serum sodium decreased significantly while serum potassium increased in diabetic patients. In rats that received BLP treatment there was a decrease in serum K+ level which is supported by Atangwho *et al.* (2007) [22], the changes in serum K+ was not significant. From table 2 above there was a decrease in the Cl^- of rats after induction of diabetes, this is as a result of osmotic diuresis with subsequent loss of water and electrolytes induced by glycosuria [23], but Cl^- increased after administration of treatment as noticed in table 2. In the DC and across all treatment group there was increase in serum urea which is common among diabetic patient, urea, acts directly on pancreatic β cells and impair insulin secretion, and several research has shown that there is a link between insulin deficiency and increase in urea levels in the body [24, 25]. Impairment of urea and creatinine level due to increased blood glucose level indicates reduction in kidney function in diabetic patients [26], the increment observed across all treatment groups shows the leaf extract administered at 1000 mg/kg for 14 days are not really effective in combating insulin deficiency and preventing diminished renal function due to the short period of the administration of treatment.

In the levels of WBC, there was a decrease in DC rats, this decrease is caused by the damage alloxan monohydrate causes the WBC and the organ of the body and may also be due to the suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection, which will in turn have effects on the immune system and phagocytic activity of the animals [27, 28], but there was improvement in BLP and combination treated rats which also correspond with the study of Akah *et al.* [29], only AEP treated group did not show improvement in WBC levels.

The increase in non-enzymatic glycosylation of RBC membrane proteins in DC rats causes a decrease in RBC and HCT levels. In diabetes mellitus, oxidation of these proteins and hyperglycaemia produce a rise in the formation of lipid peroxides, which leads to RBC haemolysis [30, 31], across all treatment groups there was improvement in the level of RBC and HCT when compared to the DC, there was increased haemoglobin across all groups when compared to the normal control, only BLP and combination treated rats showed decrease when compared to the DC, the increase of Hb in DC may be a response to hypoxia secondary to vascular diseases [32], the decrease In BLP and combination treated groups may indicate improvement and diminished vascular disease, in diabetes rats there was an increase in PLT count, which is as a result of Hyperglycaemia which contributes to greater platelet reactivity through direct effects and by promoting glycation of platelet proteins [33], AEP and BLP treatment groups, shows reduction in the value of PLT when compared to the DC.

Effect of combined leaf extract of African eggplant and bitter leaf on antioxidant enzymes activity in the kidney of alloxan induced diabetes male Wistar rats

Table 4 shows the effect of the combination of African eggplant and bitter leaf on antioxidant enzymes activity in the kidney, with the level of significance set at $P<0.05$ and changes in comparison with the normal control, Across all groups there was reduction in the level of CAT, only AEP and combination treatment showed significant difference when compared to the normal control group, in the GSH activity only the DC group showed significant increase, while the other treatment groups showed significant decrease, SOD activity increased significantly in BLP, DC and combined groups , MDA activity increased significantly only in DC and AEP. The diabetic state is associated with a generalized increase in tissue oxidative stress, which might be reflected in the changes in the tissue antioxidant system [34]. Superoxide dismutase (SOD) is one of the most important enzymes in the enzymatic anti-oxidant defence system [35]. The superoxide anion has been known to inactivate CAT, which is involved in the detoxification of H_2O_2 [36]. Superoxide dismutase (SOD) scavenges the superoxide anion, hence diminishing the damaging effects of this free radical, Wohaieb *et al.* (1987) [37] had suggested that the reactive oxygen free radicals could inactivate and reduce the hepatic SOD and CAT activities [37]. In the present study, it was observed that there was an increase in CAT activity across all treatment group, while BLP and combined treatment groups showed increase in SOD activity, this means that the extract can reduce ROS and improve the activities of the anti-oxidant enzymes in the kidney. In our studies an increase in GSH activity was observed in the kidney in contrast to the reported decrease GSH activity in diabetic state [38, 39, 40] the decrease noticed across all treatment groups may be due to the utilization of GSH in the kidney [41], Increase in MDA activity indicate increase in lipid peroxidation and oxidative stress, The quantity of malondialdehyde is a frequent indicator of oxidative stress and antioxidant status [42], In this present study, DC rats showed

increase in MDA concentration while combined and BLP treatment groups showed significant decrease in MDA activity, the decrease in MDA shows that our treatment has the ability to prevent lipid peroxidation.

Effect of combined leaf extract of African eggplant and bitter leaf on histology of kidney of alloxan induced diabetes male Wistar rats

Figure I below show the effect of diabetes on the weight of the kidney and the effect of the administered BLP, AEP and combined extracts treatment on the kidney, DC, AEP and BLP showed increase in the weight of the kidney, only the combined treatment showed decrease when compared to the normal control.

In the figure above A; normal control group having normal renal histology with all structures well defined, distal and proximal convoluted tubules (DCT&PCT), Glomerulus (black thick arrow), simple squamous epithelia cells (black thin arrow) and capsular space (CS). B; alloxan induced diabetic control group shows thickening of the basement membrane altering the epithelial cells (black thin arrow), hyper-cellularity of the proximal and distal convoluted tubules (PCT&DCT). C; alloxan Induced diabetes + BLP treatment shows preventive changes of the renal histo-morphology of the capsular space (CS), epithelial cells (black thin arrow) and the proximal and distal convoluted tubules (PCT&DCT) D; alloxan Induced + AEP treatment shows preventive effect of the extract on the histo-morphology of the renal tissue, the capsular space (CS), epithelial and basement membrane (black thin arrow) and the proximal and distal convoluted tubules (PCT&DCT) are intact. E; alloxan Induced + combined treatment shows thickening of the epithelial and basement membrane (black thin arrow), the capsular spaces are well defined (CS) and hyper-cellularity of the proximal and distal convoluted tubules (PCT&DCT). In the micro-structure of the kidney, rats treated with BLP and AEP extract showed preventive and regenerative changes on the kidney tissues of diabetic rats this corroborates the study of Atangwho *et al*. [2007] [22]. A comparison to the histo- architecture of the kidney tissue between the DC rat and combined treatment rats showed pathological changes, the presence of hyper-cellularity and thickening of the basement membrane, When the histology of the PCT is damaged, the reabsorption of water from the glomerulus is disrupted. This is the reason why diabetes patients suffer excessive urination. Distal convoluted tubules necrosis affects sodium reabsorption as well as hydrogen and potassium secretion, both of which have negative consequences for diabetics' overall health, several studies had also shown that diabetes mellitus affect the histology of the kidney [43, 44, 45]

CONCLUSION

The administration of AEP, BLP and combination treatment showed ameliorative effects on selected haematological and biochemical parameters of alloxan-induced diabetic, increase in antioxidant enzymes activity and reduction in lipid peroxidation of the kidney, AEP and BLP showed preventive and regenerative changes in the histo- architecture of the kidney, Further studies are to be carried out to evaluate the positive effect of higher dose of combination treatment on the kidney.

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Table 1: Animal grouping and treatment administered

Groups	Treatment administered	No. Of rats per group
A	Normal control; distilled water only	5
B	DC; distilled water only	5
C	100mg/kg bw of AEP	5
D	100mg/kg bw of BLP	5
E	100mg/kg bw of BLP and 100mg/kg of AEP	5

DC; diabetic control, AEP: African eggplant, BLP: bitter leaf plant, bw: body weight

Table 2: Effect of the administration of combined leaf extract of African eggplant and bitter leaf on blood electrolyte in alloxan induced diabetes male Wistar rats

Grps	Treatment	Na^+(Mmol/L)	K^+(Mmol/L)	Cl^-(Mmol/L)	Urea(mg/dl)	Creatinine (mg/dl)
A	Normal Control	136.67±1.22	4.67±0.58	102.6±3.71	24±6.14	0.970.11
B	DC	134.8±2.93	4.79±0.62	96.4±2.51[a]	42.2±13.39[a]	1.46±0.46[a]
C	AEP	135±1.22[a]	4.82±0.16	108±19.01	84.6±23.45[ab]	1.88±0.74[a]
D	BLP	134.8±0.45[a]	4.16±0.74	100.8±0.45[b]	44.6±12.26[a]	1.54±0.41[a]
E	Combination treatment	135.6±2.88	4.86±0.47	104.4±9.89	51.8±7.16[a]	1.78±0.27[a]

Each value is an expression of mean ± SEM. (P <0.05)
[a] - Values were significant when compared to group A
[b] - Values were significant when compared to group B

Table 3: Effect of the administration of combined leaf extract of African eggplant and bitter leaf on selected haematological parameter in alloxan induced diabetes male Wistar rats

Grps	Treatment	WBC (10^3/µL)	RBC (10^6/µL)	HGB (g/dL)	HCT (%)	PLT (10^3/µL)
A	Normal Control	9.08±1.58	9.7±0.51	14.7±0.55	55.5±2.26	698.3±245.4
B	DC	5.03±0.74[a]	9.26±0.91	15.42±1.13	52.66±5.19	837.4±93.66
C	AEP	7.84±0.63[ab]	10.14±0.29[**ab**]	15.66±0.47[a]	56.7±1.4	483±235.5[**b**]
D	BLP	11.42±3.72[b]	9.43±0.64	15.24±1.03	55.84±3.51	**788.2**±14.79
E	Combination treatment	9.18±2.88 [b]	9.63±0.56	14.82±0.88	54.8±2.92	1400.2±233.95[ab]

Each value is an expression of mean ± SEM. (P <0.05)
[a] - Values were significant when compared to group A
[b] - Values were significant when compared to group B

Table 4: Effect of combined leaf extract of African eggplant and bitter leaf on antioxidant enzymes activity in alloxan induced diabetes male Wistar rats

Grps	Treatment	Glutathione (µmol/ml)	Super oxide dismutase (µmol/ml/min/mg pro)	Catalase (µmol/ml/min/mg pro)	Malondialdehyde (µmol/ml)
A	Normal Control	137.10 ±8.54	2.85 ±0.37	7.82 ±1.72	1.51 ±0.81
B	DC	145.34 ±2.15 [a]	3.1 ±0.13 [a]	9.04 ±0.35	2.21 ±0.91 [a]
C	AEP	114.4±11.76 [a,b]	2.82 ±0.43	10.95 ±0.80 [a,b]	2.69 ±0.35 [a,b]
D	BLP	128.8 ±2.15 [a,b,c]	3.39 ±0.49 [a,c]	10.73 ±3.69	1.30 ±0.98 [b,c]
E	Combination treatment	129.72±8.64 [b,c]	3.74 ±0.10 [a,b,c]	15.76 ±0.21 [a,b,c,d]	1.09 ±0.21 [b,c]

Each value is an expression of mean ± SEM. (P <0.05)
[a] - Values were significant when compared to group A
[b] - Values were significant when compared to group B

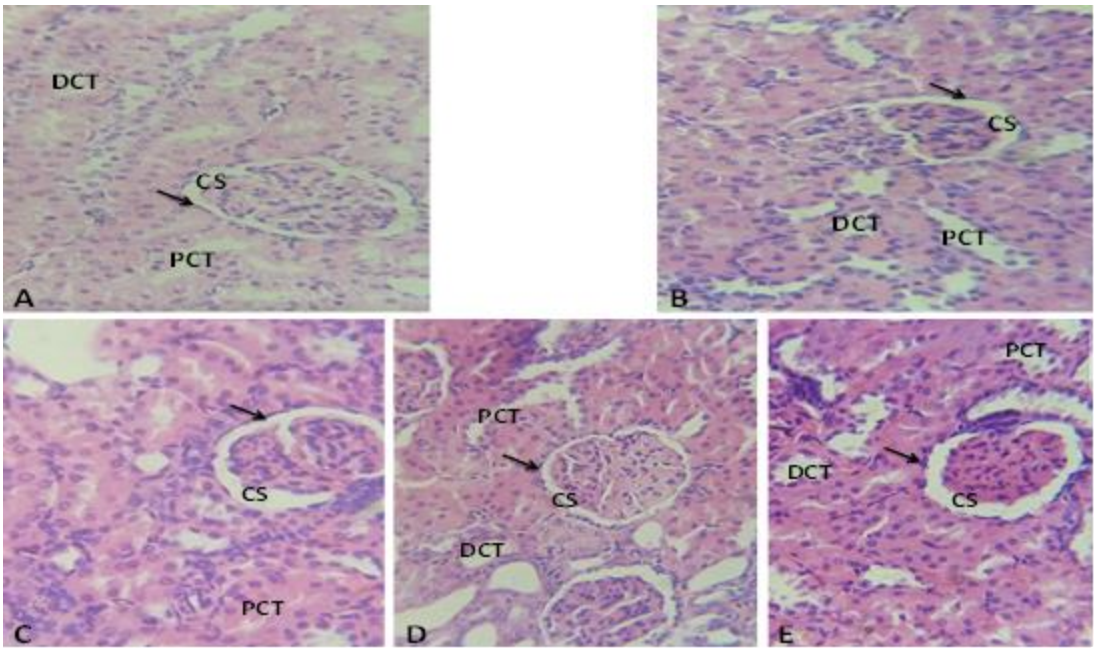

Plate I: Sections showing the microarchitecture of the kidney H/E X400
PCT; proximal convoluted tubules, DCT; distal convoluted tubules, CS; capsular space