Original Research Article

THE AMELIORATIVE EFFECT OF *Ginkgo biloba* SUPPLEMENT ON CYCLOSPORINE-A INDUCED HEPATO-RENAL TOXICITY IN MALE WISTAR RAT

ABSTRACT

Ginkgo biloba supplememt is a flavonoid-rich herbal supplement with several therapeutic potentials. However, the possibility of *Ginkgo biloba* supplement to protect the kidney and liver against cyclosporine-A induced toxicity is not fully understood. This study is to investigate the effect of *Ginkgo biloba* supplement on cyclosporine-induced hepato-renal toxicity. Twenty animals were randomly selected into four groups (n=5);group 1 were given water (vehicle 10ml/kg b.wt) and feed, group 2, were induced with Cyclosporine (CsA) (25mg/kg b.wt),GBS (50mg/Kg b.wt) was administered to group 3 and group 4, were administered with CsA (25mg/kg b.wt) and GBS(50mg/kg b.wt) co-administration for 15 days respectively. The animals were then anaesthetized with ketamine (70mg/kg b.wt). The blood samples were collected into EDTA bottles for plasma biochemistry while Liver and kidney tissues were also harvested and preserved in 10% formalin for histological examination using standard methods. The resulting data obtained were subjected to descriptive statistics using one-way ANOVA and $p<0.05$ was considered significant. ALT, AST, ALP, Urea and Creatinine were significantly increased when compared with control. Treatment with *Ginkgo biloba* supplement showed reduction of these liver and kidney functional markers. Total protein was significantly reduced in Cyclosporine induced animals relative to *Ginkgo biloba* supplement treated animals. The weight of the liver and kidney were not significantly different in the animals that received both cyclosporine and *Ginkgo biloba* supplement. There were histological alterations in liver and kidney tissues following cyclosporine induction. However, *Ginkgo biloba* supplement restored normalcy to these tissues. In conclusion, this study suggests that *Ginkgo biloba* supplement protected the liver and kidney against cyclosporine induced hepato-renal toxicity and this is attributed to flavonoids present in it.

Keywords: *Ginkgo biloba Supplement*, Cyclosporine A, Kidney functional markers, Toxicity

INTRODUCTION

Cyclosporine-A (CsA), an active endecapeptide of fungal origin, is a potent immunosuppressive agent in widespread use for prolonging the survival of various allogenic organ transplants [1]. Its immunosuppressive action is predominantly mediated by the inhibition of T-helper cell production of interleukin 2, a T-cell growth factor essential for B cell and cytotoxic T cell proliferation [2,1]. While it has become the immunosuppressant of

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choice in clinical transplantation in the eighties, cyclosporine therapy can be complicated by several adverse effects including nephrotoxicity, hepatotoxicity, and hypertension [3]. Of all the known complications, nephrotoxicity is the most frequent and clinically important, and may ultimately limit the use of cyclosporine [4]. Cyclosporine-induced nephrotoxicity is often accompanied by decreased glomerular filtration rate and proximal tubular damage [3, 5]. The morphological changes range from toxic tubulopathy manifested by nonspecific vacuolization, swelling of endoplasmic reticulum and mitochondria in tubular epithelial cells, to capillary congestion and arteriolopathy [6]. In an animal model, cyclosporine treatment induced endothelial injury, capillary thrombosis, and glomerular infarction similar to that seen in a generalized Schwartzman reaction [7]. This and other reports have led some investigators to propose that cyclosporine inhibits the synthesis of a prostacyclin-stimulating factor in the kidney, thereby leading to decreased production of prostacyclin by endothelial cells [7]. Prostacyclin is a potent antagonist of thromboxane-mediated platelet aggregation and subsequent thrombosis; it also plays an important role in renal autoregulation by acting as a physiologic antagonist to the vasoconstrictive effect of angiotensin II [8]. Hence, decreased prostacyclin production may partly explain the hypertension and accelerated renal arteriolar lesions initiated by cyclosporine. It appears that endothelial injury is an important feature of cyclosporine induced nephrotoxicity, the mechanism of cyclosporine-induced nephrotoxicity clearly requires further elucidation. Cyclosporine-A has played an important role in the development of organ transplants, its therapeutic use has been severely limited due to the nephrotoxicity of CsA, a common andserious side effect [9]. In experimental animals, Cyclosporine A has been reported to cause acute renal vasoconstriction, followed by a decrease in glomerular filtration rate and renal blood flow. A down regulation of calbindin D 28 kDa, a vitamin D-dependent calcium binding protein, has been reported to be a critical factor for the renal side-effects of CsA [10].

The kidney is a critical part of the metabolic machinery, saddled with central role of homeostasis as well as excretion of metabolic waste. Over production of intermediate toxic radicals, however, can disturb the innate antioxidant guard mechanism, leading to several pathological disorders of this organ [11, 12]. Furthermore, overwhelming levels of free radicals may cause the depletion of thiols and result in lipid peroxidation, leading to cell membrane damage and hepatic injury [13,14]. End stage renal disease is best managed by kidney transplant. This increases the rate of survival when compared to dialysis. Prevention

of acute or chronic rejection necessitates the use of immunosuppressants. However, nephrotoxicity, hepatotoxicity, cardiovascular disease, post transplantation diabetes mellitus, chronic graft dysfunction and dyslipidemia may manifest as complications of immune-suppressive therapy [15].

Ginkgo biloba supplement is one of the amazing herbal medicines considered by scientists all over the world with its immense source of bioactive compounds and medicinal importance. It possesses many bioactive compounds such as terpenoids (e.g., ginkgolides, bilobalide), flavonoids (e.g., kaempferol, quercetin, isorhamnetin), biflavonoids (e.g., sciadopitysin, ginkgetin, isoginkgetin), and organic acids (e.g., ginkgolic acid), among others, this broadens its use in different biological systems [16]. The standard extract of *G. biloba* leaves (EGb 761), is widely used for treating neurological and cardiovascular disorders [17,18]and it is positioned as one of the most widely used therapeutic plant [19, 20].*Ginkgo biloba* supplement (GBS) exhibits promising biological activities against neurodegenerative and vascular disorders [21,22]. Besides, flavonoids possess the ability to attenuate the majority of enzymes integrated into inflammatory cascades. Flavonoids also exert beneficial effects in cardiovascular diseases, possibly by inhibiting coagulation, thrombus formation, and platelet aggregation [23]. Terpenoids have been shown to suppress the nuclear factor-kB signaling in inflammation and cancer pathogenesis [24]. *Ginkgo biloba supplement* has been reported to show nephroprotective effect against methotrexate [25], gentamicin, and cisplastin-Induced renal damage and nephrotoxicity [26, 27]. It enhanced blood flow [28], and showed protective effect against doxorubicin-Induced cardiotoxicity [29]. It shows protective action against oxidative stress and nephrotoxicity induced by vancomycin [30].

The purpose of the present study would be to investigate the protective effect of *Ginkgo biloba* supplement on cyclosporine-induced renal toxicity in male Wistar rats.

MATERIALS AND METHOD

Animals

Twenty (20) male Wistar rats were used for this experiment with their weight ranging between 120g to 150g. The animals were procured from the Animal House; University of Port-Harcourt and was transported to Animal House, PAMO University of Medical Science, Port-Harcourt, Rivers State Nigeria and fed on standard pelleted rat chow and drinking tap

water *ad libitum*. The animals were kept and maintained under convectional laboratory conditions of temperature, humidity and light. This study was approved and the protocol adhered to the University Ethical Committee on Animal Experimentation (PUMS-AREC/043) guidelines, which are in accordance with the 'Principle of Laboratory Animal Care' (NIH Publication N0.85-23).

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Chemicals

Cyclosporine-A (CsA) was gotten from Sigma (St. Louis, MO, USA), *Ginkgo biloba* supplement was gotten from Mason Natural chemicals (China). Creatinine, Urea and Total Protein-Randox kits for biochemical assays was gotten from Randox Laboratories Limited, Crumlin, United Kingdom.

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Study Design and Experimental Procedure

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The animals were acclimatized for two weeks under normal laboratory condition and they were randomly selected into four groups (n = 5) as follows; Group 1 served as normal control and was treated with water, Group 2 was treated with Cyclosporine-A at 25mg/kg according to the studies of [31], Group 3 was treated with GBS (50mg/kg), the dosage was according to a previous study by [32] and Group 4 was treated with Cyclosporine-A + GBS simultaneously. All treatments were done through intraperitoneal route. At the end of the treatment, animals were anaesthetized with ketamine (70mg/Kg) and thereafter euthanized by cervical dislocation. Bloods was collected through cardiac puncture into EDTA coated bottle and thereafter were centrifuged and plasma was separated for biochemical assay. The kidney was harvested and preserved in 10% phosphate-buffered formalin for histological examination.

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Biochemical Assay

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The concentrations of creatinine and urea and total protein for renal function in the serum were determined using Randox test kit according to the protocol described by Reitman and Frankel [33].

Histology

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The kidney was fixed in 10% phosphate-buffered formalin, dehydrated in increasing concentration of ethanol, cleared with xylene and now embedded in paraffin. Two micrometer (2µm) sections was prepared from the kidney paraffin blocks and stained with hematoxylin and eosin (H&E). The stained slides were captured at 400x magnifications using a light microscope and the resulting photomicrographs were evaluated for alterations in morphology.

Statistical Analysis

Data was expressed as Means ± Standard Error of Mean. All data were subjected to one-way analysis of variance (ANOVA) and comparison within groups was performed with *Post hoc Newman-Keuls* test with GraphPad prism 7.0 (San Diego, CA, USA). P-value of less than 0.05% was considered significant.

RESULT

Right kidney

There was no significant (p>0.05) difference in CsA (0.25±0.01), GBS (0.33±0.02) CsA+ GBS (0.28±0.03) when compared with control (0.29±0.02) group as shown in figure 1.

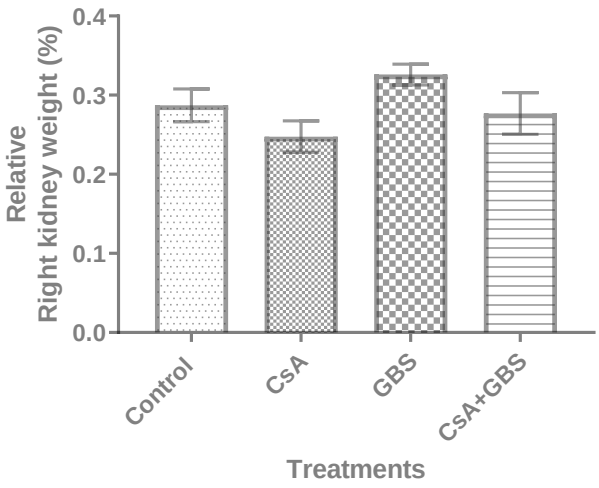

Fig. 1: Weight of Right kidney.

Values are expressed as mean + SEM (n=5).

CsA = Cyclosporine-A and GBS= *Ginkgo biloba* Supplement

Left kidney

There was no significant (p>0.05) difference in CsA (0.28±0.01), GBS (0.33±0.02) and CsA +GBS (0.28±0.02) when compared with control (0.3±0.02) group as shown in figure 2.

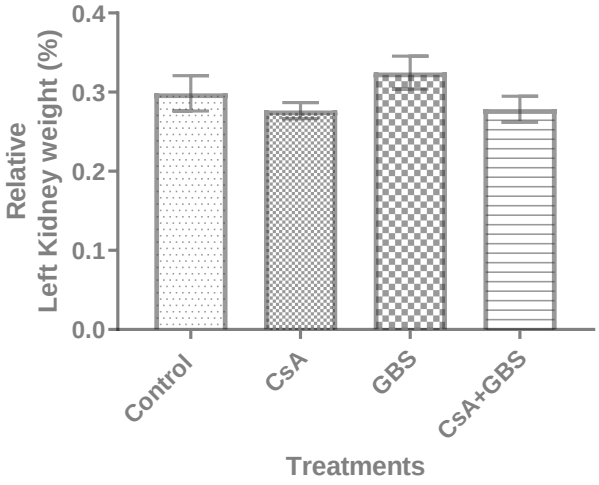

Fig. 2: Weight of Left kidney

Values are expressed as mean + SEM (n=5).

CsA = Cyclosporine-A and GBS= *Ginkgo biloba* Supplement

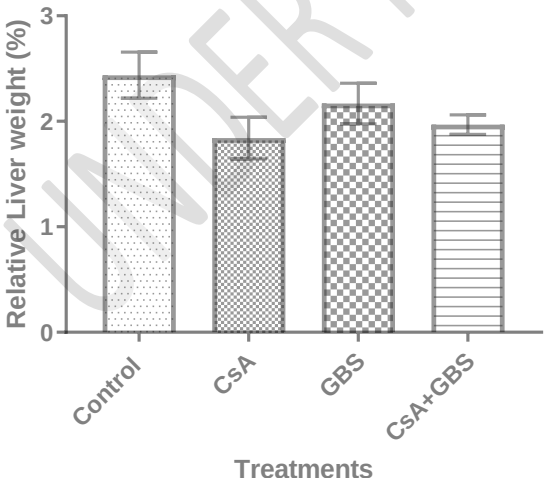

Fig. 3: Weight of Liver

Values are expressed as mean + SEM (n=5).

CsA = Cyclosporin-A and GBS= *Ginkgo biloba* Supplement

Total protein (TP)

There was a significant (p<0.05) decrease in total protein concentration in CsA group (26.7±0.85) when compared with control group (41.7±5.95). following GBS treatment, GBS (74.5±3.4) and CsA + GBS (81.7±3.66) significantly (p<0.05) increased when compared with CsA group as shown in figure 4.

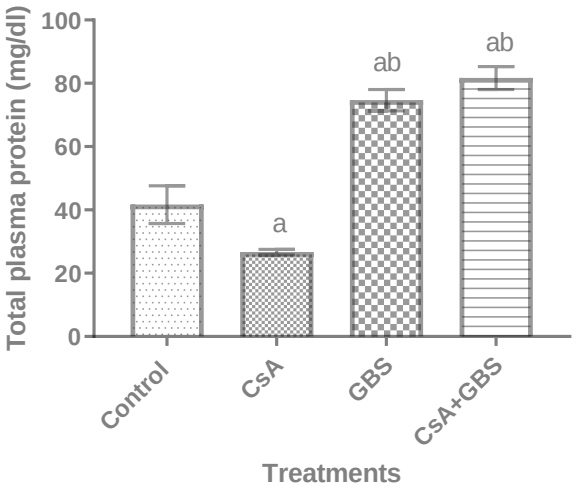

Fig. 4: Total protein

Values are expressed as mean + SEM (n=5). [a]*p<0.05* was significant when compared with control while [b]*p<0.05* was significant when compared with CsA treated group. CsA = Cyclosporine-A and GBS= *Ginkgo biloba* Supplement

Creatinine

Creatinine significantly ($p<0.05$) increased in CsA group (276±7.79) when compared with control (154±8.57). However, GBS (169±7.19) and CsA + GBS (207±6.57) were significantly ($p<0.05$) reduced when compared with CsA group as shown in figure 5.

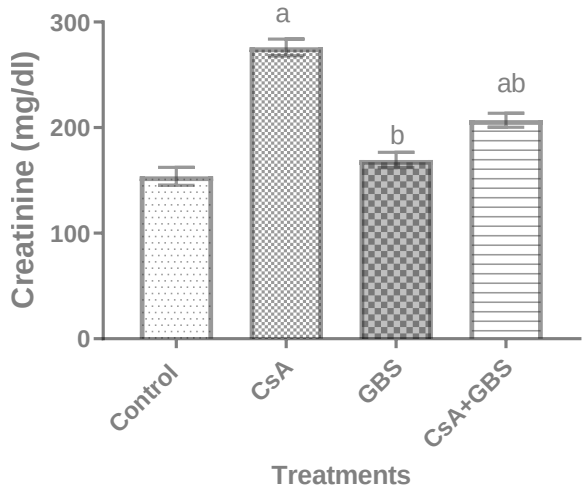

Fig. 5: Creatinine level

Values are expressed as mean + SEM (n=5). [a]*p<0.05* was significant when compared with control while [b]*p<0.05* was significant when compared with CsA treated group. CsA = Cyclosporine-A and GBS= *Ginkgo biloba* Supplement

Urea

Figure 6 shows that plasma concentration of urea in CsA group (41.6±1.89) increased significantly ($p<0.05$) when compared to control (20±1.44). However, GBS (28.1±2.48) and CsA +GBS (34.3±2.12) decreased significantly ($p<0.05$) when compared with control and CsA group.

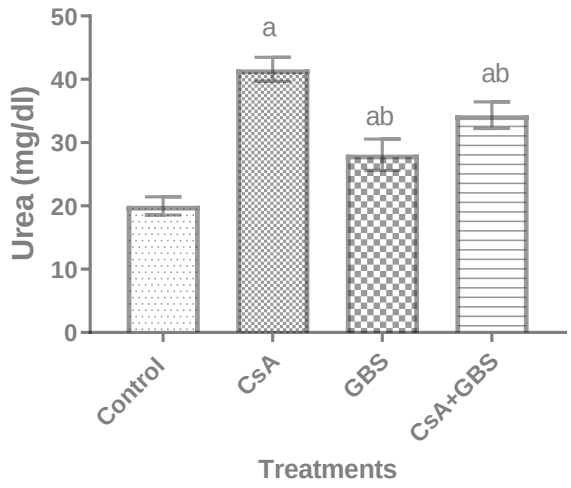

Fig. 6: Urea level

Values are expressed as mean + SEM (n=5). [a]*p<0.05* was significant when compared with control while [b]*p<0.05* was significant when compared with CsA treated group. CsA = Cyclosporine-A and GBS= *Ginkgo biloba* Supplement

***Ginkgo biloba* Supplement Protects Against Cyclosporine-A Induced Kidney Toxicity in Male Wistar Rats**

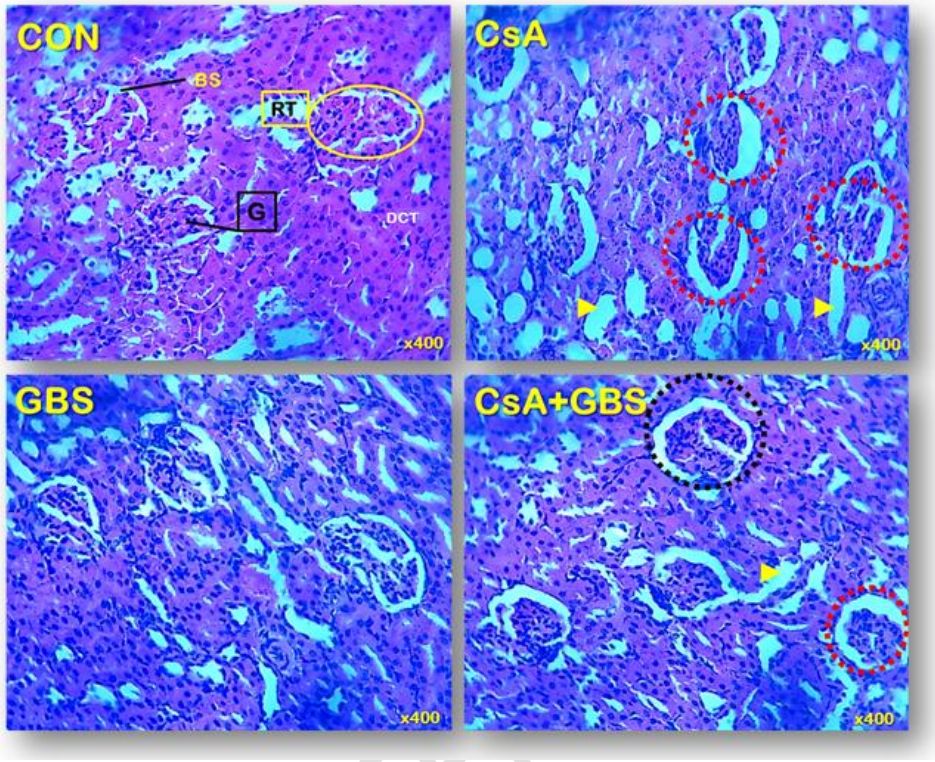

Fig. 7: Histological presentation of the kidney (H&Ex400) showing the cortex.

Glomerulus (G), Renal tubules (RT), Distal convoluted tubules (DCT), Bowman's space (BS), Proximal and distal tubular necrosis (arrow head).

Mild dilatation and atrophy of the glomerular tubules (dotted circle),

CsA = Cyclosporine-A and GBS= *Ginkgo biloba* Supplement

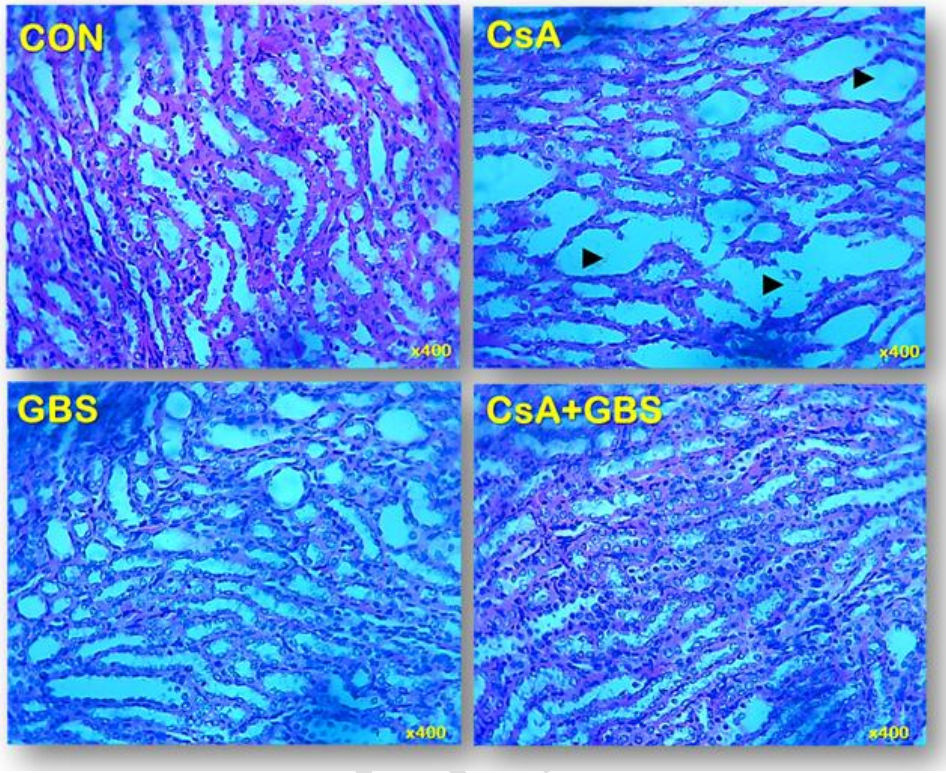

Fig. 7: Histological presentation of the kidney (H&Ex400) showing the medulla.

Mild dilatation and atrophy of the medulla tubules (arrow head)

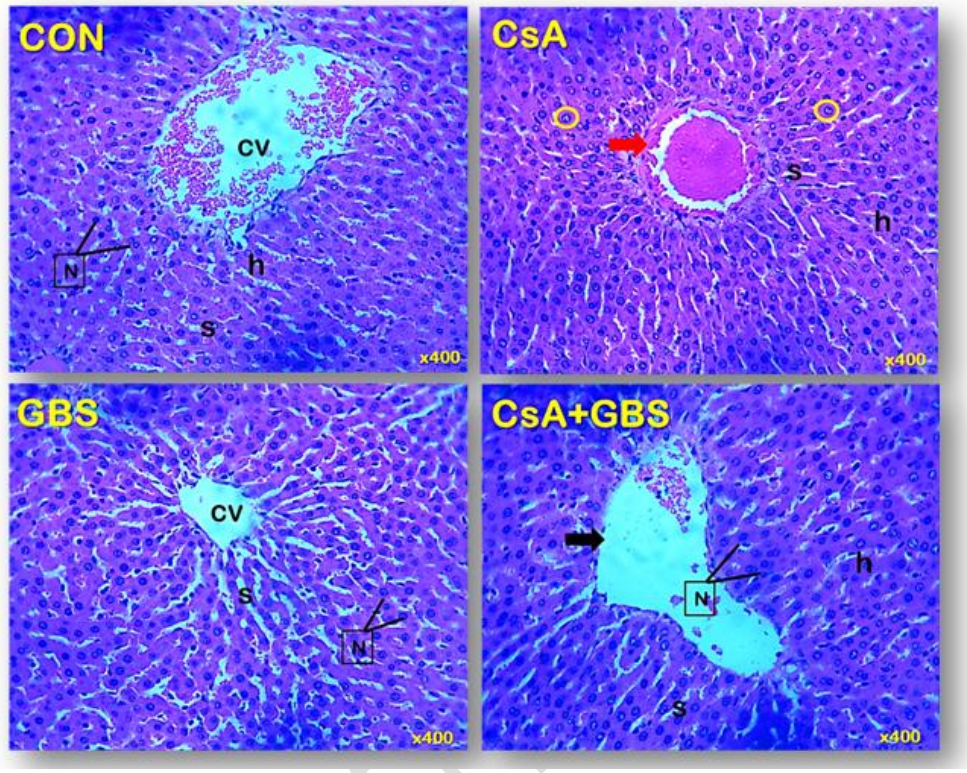

Fig. 8: Histological presentation of the liver (H&Ex400) showing the central vein.

Central vein (CV) and surrounding hepatocytes (h), sinusoids (s) and nucleus (N);

Increased infiltration of inflammatory cells in pericentral areas (red arrow),

Marginated chromatin in some nuclei (yellow circle)

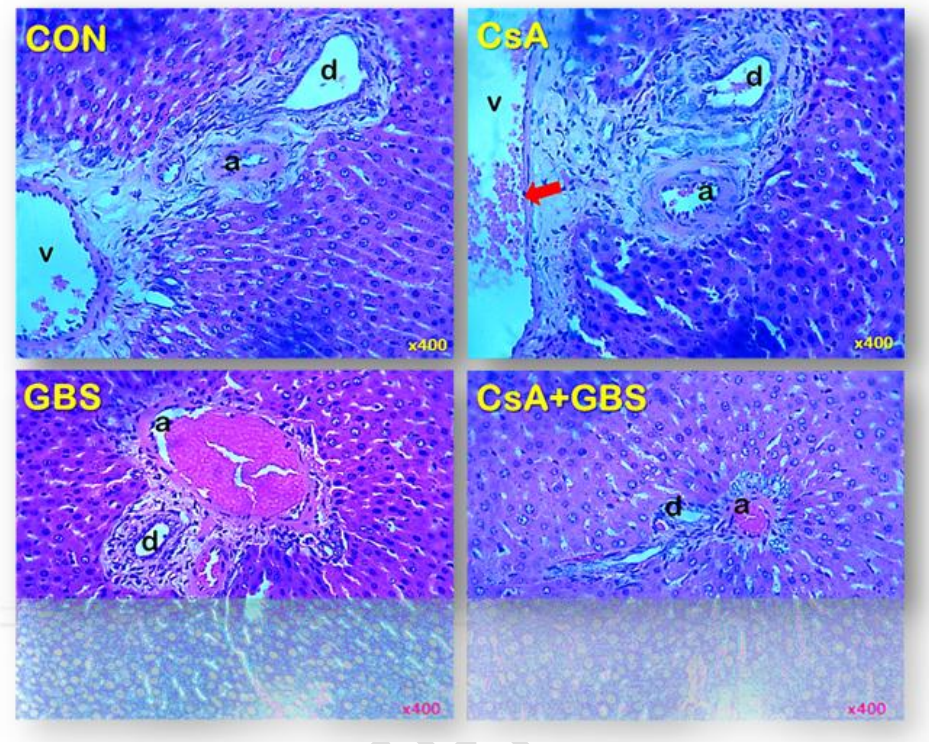

Fig. 9: Histological presentation of the liver (H&Ex400) showing the porta tried.

d= Duct, a=Hepatic artery, v=Hepatic vein

DISCUSSION

Cyclosporine-A (CsA) is an immunosuppressive drug commonly used to treat autoimmune problems after organ transplantation [1]. Cyclosporine toxicity has been observed in various organs, including the kidney, brain, and heart [3]. The kidneys are a vital component of the metabolic machinery, playing a key role in homeostasis and metabolic waste excretion. Over production of intermediate toxic radicals, however, can disturb the innate antioxidant guard mechanism, leading to several pathological disorders of these organs [11]. The use of plant supplements or herbal remedies; the treatment of several diseases and the ethno medicinal efficacy of plant supplements in several kidney malfunctions have been reported in literatures [32]. In this study, the weight of the kidney was not affected this might have been as a result of the duration of the study. In Cyclosporine intoxicated animals, observation was made of a significant increase in serum urea and creatinine levels, this was also reported in previous

studies [34,35]. Increased protein breakdown is caused by oxidative stress, which boosts ammonia levels and, as a result, serum urea concentrations [36]. The brush border epithelia of renal cells were broken down by free radicals, rendering the cells impermeable to urea and creatinine [37]. Due to restricted or no tubular absorption of urea by the renal tubules, the levels of this kidney function indicators in the blood rise. In the current study, *Ginkgo biloba* supplement significantly lowered urea and creatinine levels. *Ginkgo biloba's* renal protective effect in cyclosporine poisoning may be related to its antioxidant capacity, counteracting the oxidative attacks that occur in the kidney as a result of cyclosporine-induced toxicity. Histological examination showed that *Ginkgo biloba* supplementation repaired tissue structural abnormalities caused by cyclosporine treatment. Cyclosporine induces oxidative stress in the kidneys, resulting in pathological changes as previously established by [35] and [38]. According to studies on cyclosporine transit in the kidney, it is absorbed by proximal tubular cells [39]. High levels of cyclosporine can also damage the renal tubules, causing glomerular and tubular atrophy and accidental tubular epithelial coagulative necrosis [40,41]. *Ginkgo biloba* supplement has been proven to protect histological integrity in injured renal tissue with parenchymal necrosis, tubular dilatations, and hyperaemic conditions [42], these tallies with the result obtained from the histological studies. The histological type of kidney injury was revealed to be greatly reduced after *Ginkgo biloba* supplement consumption. Findings in this study matched those of [38], who discovered similar effects from ginger therapy. The histological studies also showed that treatment with *Ginkgo biloba* supplements resulted in the restoration of kidney structure.

CONCLUSION

According to the findings of this study, *Ginkgo biloba* supplements significantly reduced serum urea and creatinine levels in response to cyclosporine increases. As a result of biochemical assay, *Ginkgo biloba* appeared to play an important role in protecting kidneys from oxidative damage and changes in tissue structure.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is

absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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