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EFFECT OF GINGKO BILOBA SUPPLEMENTS ON CARDIOPROTECTIVE AND HAEMATOLOGICAL PARAMETERS OF CYCLOSPORINE-INDUCED WISTAR RATS

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Abstract: **Cyclosporine A is an immunosuppressant used in the prevention of immune denunciation of organ transplantation and it has been reported to induce cardiotoxicity and hematological alterations. Herbal remedy has been reported to possess a potential therapeutics efficacy due to their rich antioxidant properties and Gingko Biloba supplement has been reported for its antioxidant potentials. Hence, the present study was carried out to evaluate the effect of Gingko Biloba Supplements on cardioprotective and haematological parameters of cyclosporine-induced Wistar rats. The male Wistar rats were randomly allotted to four treatment groups (n=5), the group I-II was treated with normal saline and Gingko Biloba supplement respectively, and group III-IV was administered cyclosporine alone and Gingko Biloba supplement + cyclosporine respectively for fifteen days. The Wistar rats were euthanized after treatment and the blood sample was collected for hematological and biochemical analysis. The levels of cardiac biomarkers and antioxidant enzymes such as malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT) were evaluated along with gross histopathological examination of the heart and pancreas. The P< 0.05 was considered to be statistically significant. The result obtained from these present shows that GBS significantly improved haematological parameters. GBS showed a protective impact in terms of improved heart and pancreas histology, as well as the antioxidant enzyme activity (CAT, SOD, GSH, and MDA). In conclusion, the Ginkgo biloba supplementation attenuated cyclosporine toxicity by stabilizing haematological alterations and cardiac dysfunction.**

Keywords: **Oxidative Stress, Gingko Biloba Supplement, Cardiotoxicity and Cyclosporine.**

1. INTRODUCTION

Cardiovascular diseases are the leading cause of death in both developed and developing countries (Naghavi *et al.*, 2015). CVD is an atherosclerotic disease which is inflammatory in nature (Ross, 1999), manifested by stable angina, unstable angina, myocardial infarction (MI), or sudden cardiac death (Álvarez-Álvarez *et al.*, 2017). From genome-wide association studies (GWAS), several genetic variants have been found to be robustly associated with CVD (Lieb & Vasan, 2013). A recent study on the genetic differences in CVD risk among populations worldwide was reported to be due to demographic processes (Adebayo *et al.*, 2021). Genetic and environmental factors have been found to interact with one another to determine the clinical phenotype of cardiovascular diseases (Adebayo *et al.*, 2021). Moreover, lifestyle has been found to play an important role in the development of such cardiovascular diseases. Approximately 6 million deaths occurred in the American populations in the year 2005 due to CVD (Naghavi *et al.*, 2015). In the year 2007, it has been reported that

approximately one in 30 patients with CVD experiences death each year (Steg *et al.*, 2007). According to the World Health Organization report, submitted in the year 2009, 17.3 million deaths occurred due to cardiovascular diseases (Álvarez-Álvarez *et al.*, 2017) and the mortality of cardiovascular disease is increasing on yearly bases. *Ginkgo biloba* supplement (GBS) has been recognized for its therapeutics efficacy in Chinese ethnomedicine. The constituents of these supplements *Ginkgo biloba* includes; bilobalide, ginkgolide A, ginkgolide B, and ginkgolide, which is responsible for its multiple pharmacological effects (Naghavi *et al.*, 2015). However, although *G. biloba* leaves are widely prescribed as alternative herbal medicine for memory improvement, as well as for dementia (Naghavi *et al.*, 2015). The wild populationsof G. biloba have only few remaining trees, which place it in the endangered category according to the International Unionfor Conservation of Nature and Natural Resources (Pietri *et al.*, 1997). The anti-inflammatory effect of G. biloba extract has been recorded with downregulation of nitric oxide (NO) and PGE2production along with mRNA expression of iNOS and COX-2 enzymes and proinflammatory cytokinins (IL-1β, IL-6,and TNF-α) and up regulation of NF-kB factor (Pietri *et al.*, 1997). The effect of G. biloba leaf extract on the chronic inflammatory condition found in the colons of mice showed that the extract effectively suppresses the activation of macrophages and down regulates inflammation (iNOS, COX-2, and TNF-α) and inflammatory stress markers (p53and p53-phospho-serine 15). Also the numbers of T cells (CD4 +/CD25−/FOXp3) were reduced during this treatment (Pietri *et al.*, 1997). In a similar study, G. biloba extract was found to be effective in helping rats recover from colitis by significantly reducing macroscopic and histological damage, elevating the activity of antioxidant enzymes, and reducingMDA content (Naghavi *et al.*, 2015). This colon tissue was also examined for inflammatory markers and revealed that G.biloba extract inhibited mRNA expression of TNF-α, NF-kBp65, and IL-6. Up regulation of anti-inflammatory markers (IL-10 and IL-20R) in an atherosclerosis rat model was also recorded with administration of 100 mg/kg per/day of *G.biloba* leaf extract for 8 weeks along with a down regulation of the mRNA expression of IL-1β and TNF-α in comparison to a control group (Pietri *et al.*, 1997).

2. MATERIALS AND METHODS

Sample Collection

This research work was conducted at the Department of Biomedical Technology, University of Portharcourt, and Pamo University of Medical Science, Rivers State, Nigeria.

Male wistar rats (120-150g) were purchased from the Animal House, University of Portharcourt. The rats were acclimatized for one week in standard plastic cage at standard environmental conditions before the experiments. The animals had free access to standard rodent pellet food and water *ad libitum* for through the study.

Drug and chemicals

Gingko Biloba supplement (Mason natural chemicals, China) and Cyclosporine A (Sigma St.louis, Mo, USA) were used for the study.

Experimentation

Twenty male Adult wistar rats (20) were distributed into 4 groups with five rats in each group and the treated as follows;

Group 1 received normal saline (10 ml/kg)

Group 2 received Cyclosporine-A (25 mg/kg)

Group 3 received Cyclosporine A (25 mg/kg) plus *Gingko biloba* supplement (50 mg/kg)

Group 4 received *Gingko biloba* supplement (50 mg/kg)

Animal Sacrifice and Sample Collection

After the fifteen day (15) experimentation, the rats were weighed before anaesthetised by ketamine (70 mg/kg) and euthanized by cervical dislocation. The abdominal cavity was opened and the blood samples were collected via cardiac puncture into vials containing EDTA as an anticoagulant. The plasma samples were separated from the blood cells by centrifugation at 3000 rpm for 5min. The blood obtained was used for the hematological analysis. The heart and the pancreas were fixed in Phosphate buffer formalin for histological examination using H & E staining procedure. Analysis was carried out at Pamo University of Medical Sciences, Port Harcourt, Rivers State, Nigeria.

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Hematological Variables

Determination of Haemoglobin Concentration

Haemoglobin concentration was determined using the Spectrophotometric method involving the use of Hemoglobin Cyanide (HiCN) and spectrophotometer adopted by International Committee for Standardization in Haematology (ICSH).

Procedure:

- 25ul of the blood sample was added to 5mL of the reagent (HiCN).
- (HiCN) is a standard solution containing a mixture of Potassium ferricyanide (200mg) and Potassium cyanide (50mg) dissolved in 1000mL of distilled water.
- The mixture was allowed to stand for 3 minutes.
- The absorbance of HiCN and HiCN standard was measured at 540nm.

Determination of Red blood cell count

Red blood cell (RBC) count were analyzed using the improved Neauber Haemocytometer method using Hayem's diluting fluid.

Procedure:

- The dilution pipette with red mixer from the haemocytometer kit would be used to draw blood up to the "0.5" mark.
- Hayem's dilution fluid was then drawn up to the "101"mark (dilution ratio 1:200) and mixed gently.
- The first part of the pipette content containing the unmixed diluting fluid would be ejected then the mixture introduced into both chambers of the counting chamber of the haemocytometer (but counting will be done on one chamber).
- The counting chamber will be placed under the microscope and the RBC counted under x40 magnification.
- Only RBCs in each of five fields (top right & left, bottom right & left, center) would be counted.
- The counted cells would then be multiplied by the dilution factor (10,000) to obtain the RBC count per cu.mm of blood.

Determination of White blood cell count

White blood cell (WBC) count was analyzed using the improved Neauber Haemocytometer method using 2% acetic acid tinted with methyl violet.

Procedure:

- Blood sample was drawn up to the "0.5" mark of the white cell pipette then diluting fluid up to the "11"mark and mixed gently.
- The unmixed portion was ejected, and then the counting chamber was filled with the mixture and allowed to stand for 3minutes.
- The WBCs in the four fields (the four corner big squares) are counted and multiplied by the dilution factor (i.e 50) to obtain the WBC count per cu.mm of blood.

Determination of Platelet count

Platelet counts were done using the Direct Rees and Ecker's method as described by Sloan (1951).

Procedure:

Diluent used – Rees Ecker (Wintrobe modified) dilution fluid:

Sodium citrate	3.8g
40% formaldehyde	0.2ml
Brilliant cresyl blue	0.05g

Distilled water 100ml

- The diluent was drawn up to the 0.5mark in a red cell haemocytometer pipette.
- Sample blood was drawn into the pipette until the column of diluent and blood reaches 1mark, and then more diluent was drawn in until the mixture reaches the 101mark.
- The pipette was rolled gently against the palms to mix thoroughly for 2 to 3minutes
- About one third of the content (unmixed diluent at initial portion of pipette) was discarded.
- Both sides of the double Neauber counting chamber were filled with the remaining mixture.
- The Neauber counting chamber was placed in a moist chamber and left for about 20minutes to allow the platelets to settle.
- All the platelets in both sets of small squares were counted (a total of 800 small squares corresponding to a volume of 0.2 c.mm of diluted blood)
- The reading gives the platelet count in thousands per cubic millimeter.

Determination of Packed Cell Volume

The haematocrit was expressed as the percentage volume of the blood that was occupied by red blood cells. The capillary tube method which was based on the principles of centrifugation was used to determine the PCV.

Procedure:

- Took a capillary tube which has already been rinsed to prevent blood from coagulation.
- Drew out 1ml of the blood sample and allow it to enter the capillary tube by capillary action until the tube was about 2/3 filled with blood. The tube contains heparin (an anti-coagulant).
- Sealed off both ends of the tube with plasticine.
- Then spun the tube in a haematocrit centrifuge for about 5 minutes at 11,000rpm
- Read the height of the column of packed cells and expressed the result in percentage.

Assessment of oxidative stress markers

The heart homogenates was prepared from frozen samples in 0.1 M Tris–EDTA buffer (pH 7.4). The supernatant fractions were used for the spectrophotometric determination of malondialdehyde (MDA), Superoxide dismutase (SOD), Glutathione (GSH) and Catalase (CAT) activity were performed as reported in the literature.

Measurement of MDA content

The level of MDA was measured according to the method previously described (Okhawa *et al.*, 1979). Briefly, an aliquot of 0.4 mL of the sample was mixed with 1.6 mL of Tris-potassium chloride (Tris-KCl) buffer to which 0.5 mL of 30% trichloroacetic acid (TCA) was added. Then, 0.5 mL of 0.75% TBA was added and heated in a water bath for 45 min at 80°C. This was then cooled in ice and centrifuged at 1200 g for 15 min. The absorbance of supernatant was measured at 532 nm. The brain MDA content was calculated and the value was expressed as nanomole of MDA per mg protein (nmol MDA/mg protein).

Catalase (CAT) activity

The activity of CAT was determined using the procedure earlier. The supernatant (1 mL) of the sample was mixed with 19 mL of distilled water to give a 1:20 dilution. Then, 1 mL of this mixture was added to 5 mL of phosphate buffer (pH 7.0) and 4 mL of H_2O_2 solution (800 µmoles). Dichromate/acetic acid reagent (2 mL) was added to 1 mL of the reaction mixture. The absorbance was measured using spectrophotometer at 570 nm and change in absorbance at 60 s interval. CAT activity was expressed as µmoles of H_2O_2 decomposed per min per mg protein (Unit/mg protein).

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Heart concentration of Glutathione (GSH)

The GSH concentration was measured using the method of Jollow *et al.*, 1974. Briefly, 0.4 mL heart tissue suppernant was added to 0.4 mL of 20% trichloroacetic acid (TCA) and then centrifuged at 5400 g for 20 min 4 °C. Then, 0.25 mL of the supernatant was added to 2 mL of 0.6 mM DTNB. The solution was made up to 3 mL with phosphate buffer (0.2 M, pH 8.0) an the absorbance was read at 412 nm against blank reagent (2 mL of 0.6 mM DTNB + 1 mL phosphate buffer (0.2 M, pH 8.0) using a spectrophotometer. It was expressed as nanomoles per milligram protein (nmol/mg protein).

Measurement of Superoxide dismutase activity

Superoxide dismutase activity was measured. Briefly0.1 mL sample supernatant was applied to 2.6 mL carbonate buffer (0.05 M) (pH 10.2). The mixture was then easily combined by inversion with 0.3 mL of freshly prepared 0.3 mM adrenaline. For 3 minutes, the rise in absorbance at 480 nm was measured at 60 s intervals. The activity of superoxide dismutase (SOD) was measured in units of adrenaline consumed per minute per mg of protein.

Estimation of Protein Content

This protein content estimate was done using the Biuret method. The diluted sample (1 mL) of the homogenate was added to 3 mL of Biuret reagent in triplicate. The mixture was incubated at room temperature for 30 min and the absorbance was then read at 540 nm using distilled water as blank.

Tissue Processing

Grossing: The tissues were observed and cut into small pieces of not more than 4mm thick into pre-labeled cassettes. These were further immersed in 10% formal saline for 24 hours to fix.

Tissue Processing: This was done automatically using automatic tissue processor (Leica TP 1020). The tissue were allowed to pass through various reagents including; stations 1 & 2 containing 10% formal saline, station 3 to station 7; alcohol (70%, 80%, 90%, 95%, absolute 1 & absolute 11) for the purpose of dehydration. The tissues continued to pass through station 8 and station 9 containing two changes of xylene for the purpose of clearing and finally transferred into three wax baths for infiltration/impregnation. The machine has been programmed to run for 12 hours, tissues stayed in each station for 1hour.

Embedding: Eachprocessed tissue was given a solid support medium (paraffin wax) and this is done using a semi-automatic tissue embedding center. The molten paraffin wax was dispensed into a metal mold and the tissue was buried and oriented in it, a pre labeled cassette was placed on this and was transferred to a cold plate to solidify. The tissue block formed was separated from the mold.

Microtomy: The blocks were trimmed to expose the tissue surface using a rotary microtome at 6micrometer. The surfaces were allowed to cool on ice before sectioning. The tissues were sectioned at 4 micrometer (ribbon section)

Floating: The sections were floated on water bath (Raymond lamb) set at 55^0C and these were picked using clean slides. The slides were labeled.

Drying: The slides were dried on a hotplate (Raymond lamb) set at 60^0c for 1hour.

Staining: The staining technique used was Haematoxylin and Eosin technique

Procedure for Haematoxylin and Eosin (H&E) Technique

De-waxed in Xylene for 15mins.

Took through Absolute Alcohol, 95% and 70% Alcohol,

Rinsed the section to water.

Stained in Harris haematoxylin for 5mins.

Rinsed in water

Differentiate in 1% acid alcohol briefly.

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Rinsed in water.

Blue under running tap water for 10mins

Counter stained with 1% aqueous Eosin for 2min.

Rinse in water.

Dehydrate in ascending grades of alcohol.

Clear in xylene and Mount in DPX.

3. RESULTS AND DISCUSSION

Effect of *Ginkgo biloba* Supplement on Hematological parameter of cyclosporine-A treated rats.

The effects of *Ginkgo biloba*supplements on the cyclosporine treated rats on hematological parameter are shown in Table 1. We observed significant ($P \leq 0.05$) decreases in blood parameters (RBCs, HB, PCV and platelets) in CSA-treated rats compared to thecontrol group respectively. Contrary to these results, asignificant ($P \leq 0.05$) increase in WBCs and platelet was also observed.

Effect of *Ginkgo biloba* Supplement on Malondialdehyde concentration of cyclosporine-A treated rats.

The MDA content in CsA (1.72±0.35) group was significantly ($p<0.05$) increase when compared with control (0.78±0.14) group. However, GBS (0.79±0.09), CsA + GBS (0.99±0.16) was significantly ($p<0.05$) reduced when compared with CsA group as shown in figure 10.

Effect of *Ginkgo biloba* Supplement on glutathione level of cyclosporine-A treated rats

The effect of GBS on reduced GSH in CsA (1.12±0.07) group was significantly ($p<0.05$) reduced when compared with control (2.19±0.45) group. However, GBS (2.69±0.50), CsA + GBS (2.49±0.32) was significantly ($p<0.05$) increase when compared with CsA group as shown in figure 11.

Effect of *Ginkgo biloba* Supplement on superoxide dismutase activity of cyclosporine-A treated rats.

The effect of GBS on superoxide activity in CsA (0.11±0.03) group was significantly ($p<0.05$) reduced when compared with control (0.25±0.03) group. However, GBS (0.18±0.02), CsA + GBS (0.19±0.02) was significantly ($p<0.05$) increase when compared with CsA group as shown in figure 12.

Effect of *Ginkgo biloba* Supplement on Catalase activityof cyclosporine-A treated rats

The effect of GBS on catalase activity in CsA (4.06±0.46) group was significantly ($p<0.05$) reduced when compared with control (7.67±0.64) group. However, GBS (5.42±0.45), CsA + GBS (5.29±0.92) was significantly ($p<0.05$) increase when compared with CsA group as shown in figure 13.

Table 1: Effect of *Ginkgo biloba* Supplement on Hematological parameter concentration of cyclosporine-A treated rats.

	PCV (%)	HB (g/dl)	RBC ($\times 10^6$/µl)	WBC ($\times 10^3$/µl)	PI($\times 10^3$/µl)
NORMAL SALINE	44.7±4.5	14.9±1.49	4.87±0.46	8.73±1.25	105±2.68
CSA	33±2.55[a]	9.33±0.85[a]	3.53±0.23[a]	9.6±0.48	224.3±7.98[a]
GBS	45±2.04[b]	15±0.69[b]	4.97±0.21[b]	6.33±0.85	109.7±9.39[b]
CSA + GBS	43.3±1.18[b]	14.4±0.40[b]	4.6±0.08[b]	9.4±0.46	170.7±10.87[ab]

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

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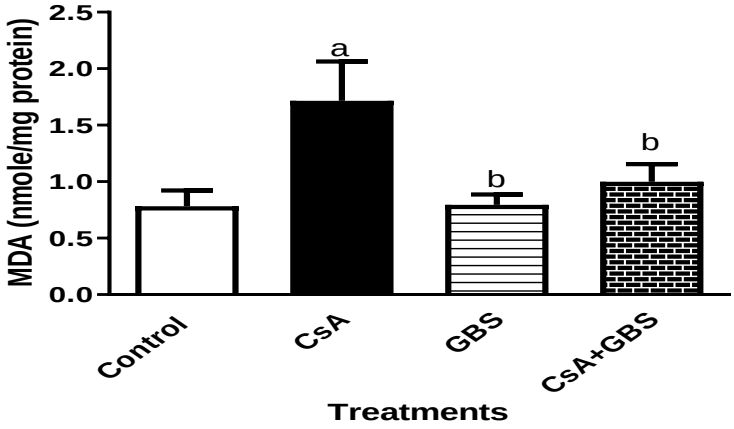

Fig.1. Effect of *Ginkgo biloba* Supplement on Malondialdehyde concentration of cyclosporine-A treated rats.

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

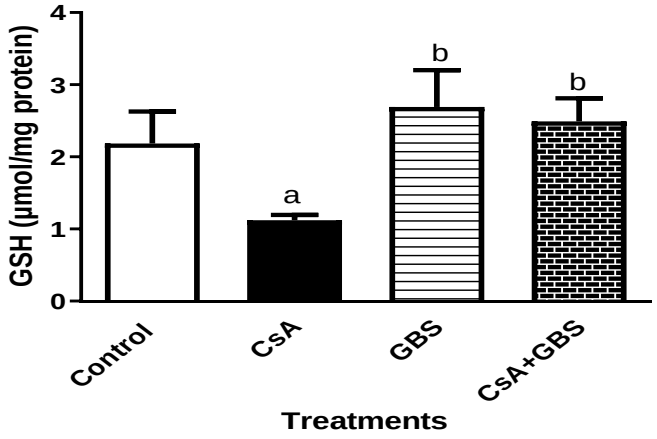

Fig.2. Effect of *Ginkgo biloba* Supplement on glutathione level of cyclosporine-A treated rats

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

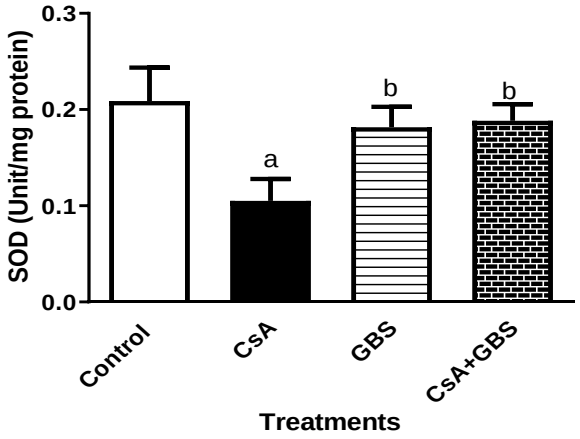

Fig.3. Effect of *Ginkgo biloba* Supplement on superoxide dismutase activity of cyclosporine-A treated rats

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

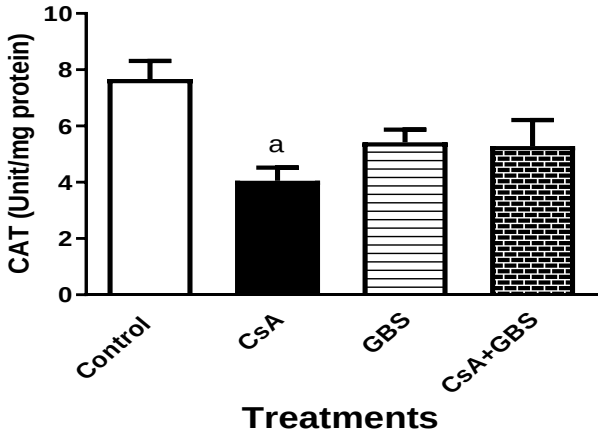

Fig.4. Effect of *Ginkgo biloba* Supplement on Catalase activityof cyclosporine-A treated rats

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

Histology of the Heart

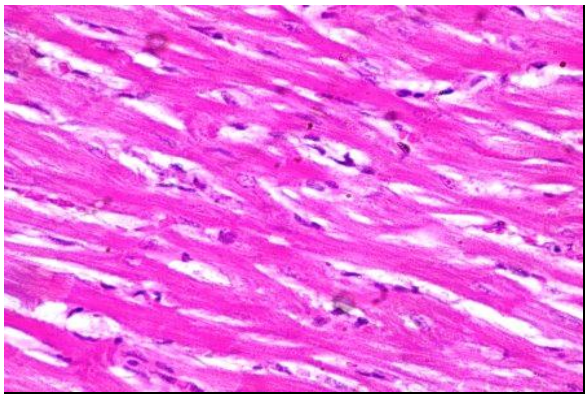

PLATE 1. Heart control (H&E X400). Normal cardiac histoarchitecture. No significant lesion seen.

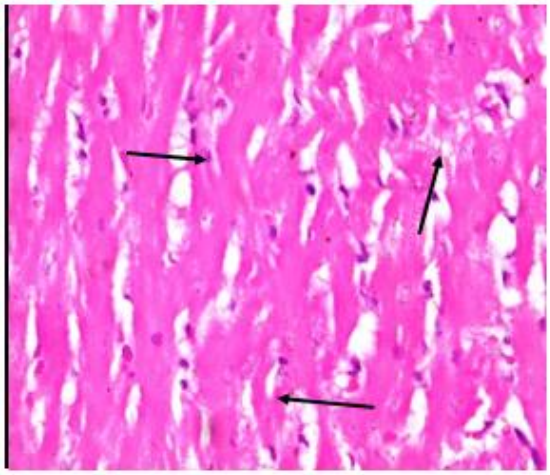

PLATE 2. Heart CsA (H&E X400). Arrows indicates slight tissue infiltration of inflammatory cells into the myocardium.

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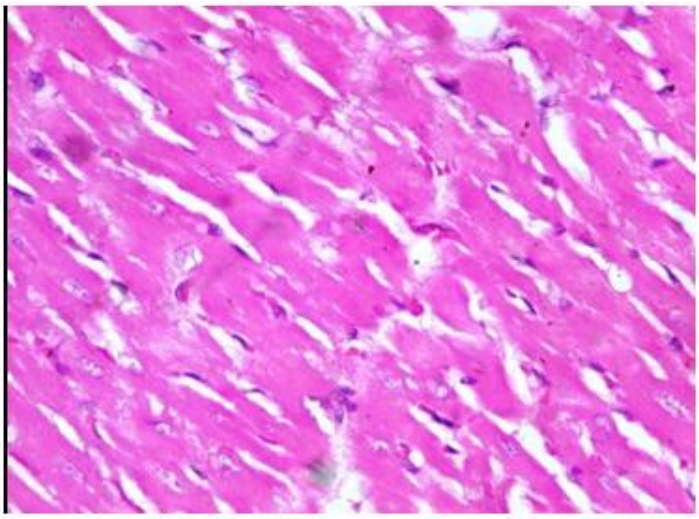

PLATE 3. HEART GBS (H&E X400).Normal Cardiac histoarchitecture. No significant lesion seen

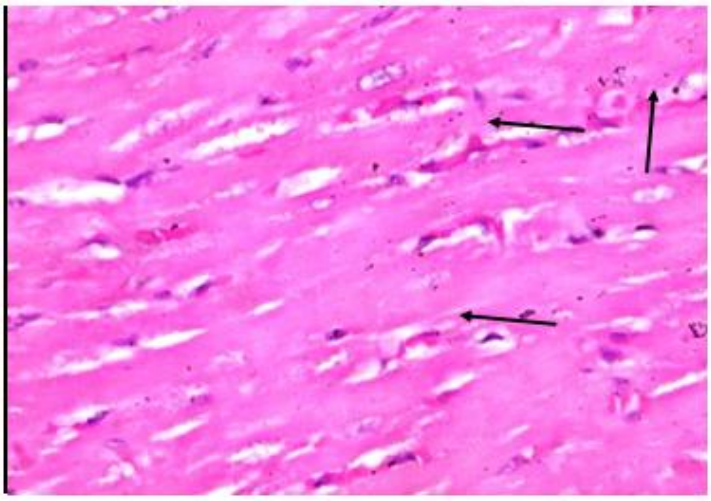

PLATE 4. Heart CsA +GBS (H&E X400). Arrows indicates mild presence of eosinophilic substances within the myocardium.

HISTOLOGY OF PANCREAS

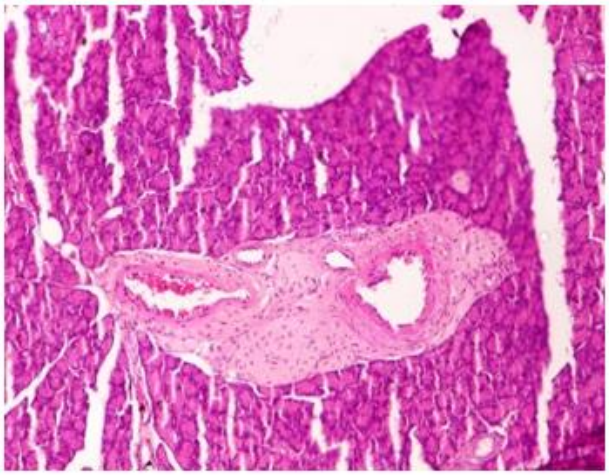

PLATE 5. Pancreas control (H&E X400). Normal Pancreatic histoarchitecture. No significant lesion

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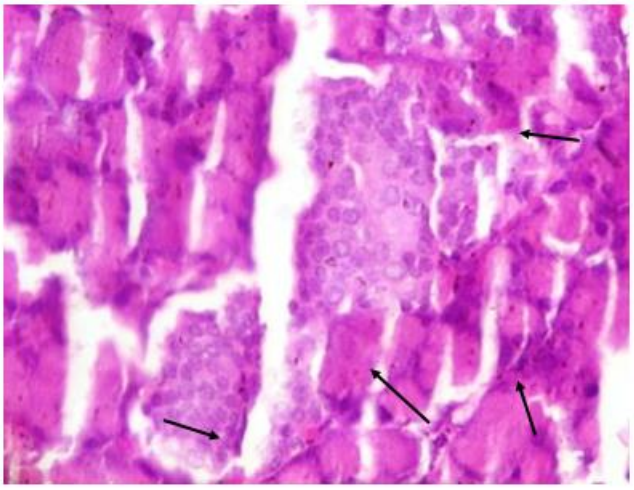

PLATE 6. Pancreas CsA (H&E X400). Mild vascular congestion and focal area of hyperplasia.

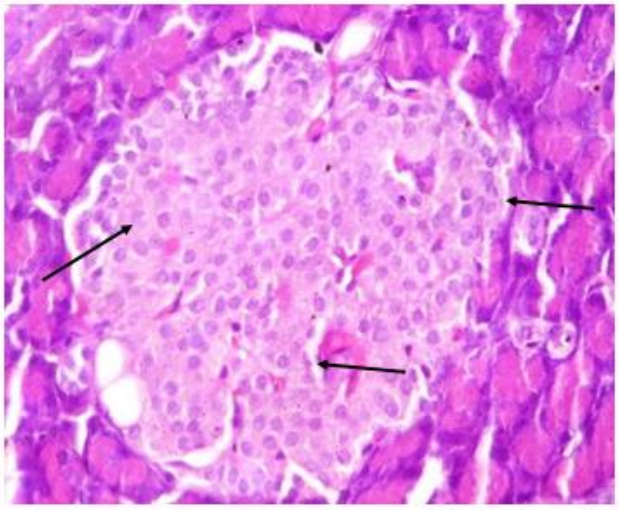

PLATE 7. Pancreas GBS (H&E X400). Arrows indicate area of hyperplasia

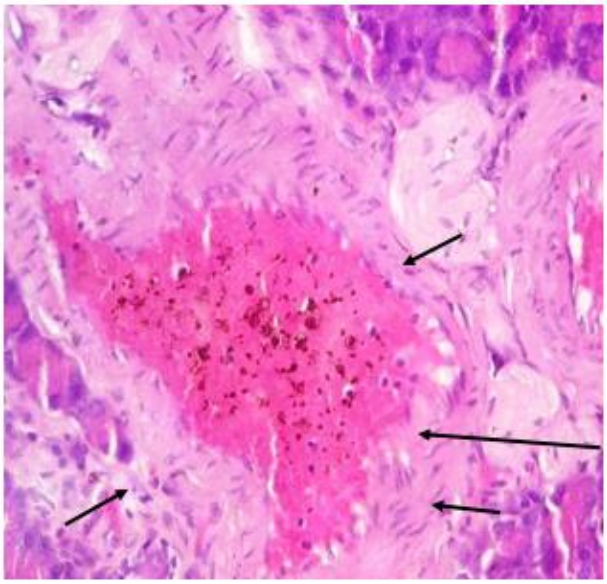

PLATE 8. Pancreas CsA+GBS (H&E X400). Arrows indicates inflammation and mild vascular congestion

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4. DISCUSSION

Cardiovascular disease is one of leading cause of death in both developed and developing countries (Naghavi *et al.*, 2015) and the mortality of these diseases keep increasing due to the several factors such as life style, diet and lack of regular exercise. Also, cyclosporine A which is an immunosuppressant that is widely used for treating autoimmune complications that arise during organ transplants, despite its beneficial effect in organ transplant, cyclosporine A has pose a great health challenge to human due to the side effect accompanying the usage such as heptatoxicity, neurotoxicity, cytotoxicity and nephrotoxicity. Hence, there is need to discovered adjunct therapeutics agent that can help to ameliorate the toxicity.

The *Gingko Biloba* supplements possess cardiovascular properties by dilating the blood vessel which results in increased blood flow. Previous study has shown that *Gingko biloba* supplements is rich in antioxidant and possess cardioprotective actions against adriamycin-induced cardiotoxicty via regulation of inflammatory and nitric oxide signaling pathways (Naghavi *et al.*, 2015). Also, the study conducted by Panda and Naik, 2008 show that treatment with *Ginkgo biloba* supplemented products led to protective action against the acute myocardial infarction and injury caused by ischemia-reperfusion. The haematological parameters in wistar rat can significantly change due to toxicological manifestation. Literatures has shown that the decrease in the RBC count and Haemoglobin concentration may be as a result of inhibition of erythropoiesis, haemosynthesis, or osmoregulatory dysfunction or due to an increased rate of erythrocyte destruction in the hematopoietic organ. The result obtained from this current study indicates that the white blood cell count was increased although not significantly in the group that was treated with cyclosporine-A alone. Briefly, *Ginkgo biloba* supplementation (Table 1), on the other hand, appears to have haematinic potential, as Hb and RBC counts were significantly increased, resulting in increased packed cell volume. The white blood cell and its differentials' main functions are to fight infections, defend the body against invasion by foreign organisms through phagocytosis, and produce or at the very least transport and distribute antibodies in the immune response. The haematological values of the groups treated with GBS were similar to the control group that was given normal saline alone and significantly different from the groups that were treated with cyclosporine-A alone. The result obtained in this present study suggest that GBS might be helpful in reducing the harmful effect or the common side effect that is associated with the immunosuppressive activities of cyclosporine-A via optimization of haematological values. *Ginkgo biloba* treatment, on the other hand, significantly boosted platelet count while decreasing clothing time, suggesting that Ginkgo biloba may have a function in platelet aggregation. Fibrinogen is a glycoprotein made in the liver that aids in the production of fbrin, a protein that forms blood clots. The discovery of hyperfbrinogenemia in cyclosporine-treated rats indicates that thrombosis is a possibility. GBS, on the other hand, improved this condition while also removing the risk of thrombosis and infection (Chang *et al.*, 2017). Oxidative stress and mitochondrial dysfunction are associated with disease and toxic process. The treatment of wistar rats with cyclosporine showed some effects in the heart mitochondria functions especially: the drug decreases therespiratory control rate and increases the superoxide anion and the MDA production. These biochemical abnormalities did not occur in heart mitochondria and cardiac tissue of animals treated with oral administration of GBS (50 mg/kg/day) alongside Cyclosporine (25mg/kg), we observed: a restoration of the RCR, a decrease of superoxide anion production and an inhibition of the lipid peroxides formation. These results suggested that lipid peroxidation, superoxide anion and decrease of RCR may play an important role in cyclosporine toxicity. These results permitted also to clarifythe prevention mechanism of GBS against cyclosporine inducedwistar rat. This study's histological analysis revealed distortion in the architectural formation of the heart and pancreas as a result of treatment with cyclosporine, an immunosuppressive drug. The findings reveal that GBS considerably reduces the cytoarchitectural changes generated by cysclosporine treatment (Plate 2 and 4). Briefly, in plate 1-4, histological alterations in the heart tissue of CsA-treated animals and following GBS injection were quantified. Control rats' hearts were normal in appearance, with striated muscle fibers and scant connective tissue. The muscle fibers were made up of cellular units linked end-to-end in a thin connective tissue network by specialized junctions termed intercalated discs. All animals treated with cyclosporine A showed pathological indications of cardiac injury. Inflammatory cell infiltration, cardiac muscle fiber disruption, and interstitial fibrosis were also observed in the cyclosporine A-treated group. The majority of the infiltrated cells were mature lymphocytes. Fibrosis was visible around cardiac fibers, and connective tissue was substantially enlarged (Plate 3). In the hearts of CsA-treated rats and *Gingko biloba* supplemented rats, the number of infiltrating cells, cardiac fiber disarray, and interstitial fibrosis were reduced (Plate 4). The group administered *Gingko biloba* alone showed no morphological abnormalities. .

5. CONCLUSION

In conclusion, the results of this study showed that treatment with *Ginkgo biloba* supplement significantly ameliorated cyclosporine induced toxicity by regulation of haematological indices and modulation of haemorheological factors as well as maintaining erythrocyte membrane integrity. It also improved heart and pancreas functions by protecting the tissues against oxidative damage and histoarchitectural alterations caused by administration of cyclosporine. However, the findings from this study therefore suggest that *Ginkgo biloba* supplementsmight be a viabletherapeutic approach for reducing cyclosporine-induced alterations and oxidative injury–induced haematological imbalance and organ damage in cyclosporine toxicity.

ETHICAL APPROVAL

Eternal approval was sought and was received from the Animal Committee of the Faculty.

CONFLICT OF INTEREST

There's no conflict of interest amongst the authors

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