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FLAVONOID-RICH EXTRACT OF *SOLANUM MACROCARPON* LEAVES SHOWS TISSUE PROTECTIVE AND AMELIORATE CERTAIN INFLAMMATORY AND OXIDATIVE INDICES ASSOCIATED WITH D-GALACTOSE EXPOSED RATS

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ABSTRACT

The present study assessed the protective potentials of Flavonoid-Rich Extract of *Solanum macrocarpon* Leaves (FRESML) against D-galactose-induced oxidative stress in rats. Animals were randomly selected into four groups of seven per group and treated for 28 days. Group A (control) were given distilled water, Group B (extract only) were administered 100mg/kg/day of FRESML, Group C (D-galactose only) were administered 500mg/kg/day of D-galactose and Group D (D-galactose + extract) received combined administration of 500mg/kg/day of D-galactose and 100mg/kg/day of FRESML. Total Protein (TP), Urea and Creatinine concentrations, Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Gamma Glutamyl Transaminase (GGT), Superoxide Dismutase (SOD) and Catalase activities, Reduced Glutathione (GSH), Malondialdehyde (MDA), Packed Cell Volume (PCV), White Blood Cell (WBC) and Fibrinogen levels were assessed using standard assay methods. D-galactose administration in rats triggered significant ($p<0.05$) increases in serum levels of ALT, AST, GGT, ALP, Urea and Creatinine and significant ($p<0.05$) decreases in serum, liver and

kidney TP concentrations. Significant ($p<0.05$) increases in percentage PCV, WBC counts and Fibrinogen concentration were obtained. In addition, significant ($p<0.05$) increases in liver and kidney MDA concentrations and depletion in liver and kidney SOD, Catalase and GSH levels were also obtained. Combined administration of D-galactose and FRESML significantly ($p<0.05$) modulated the levels of ALT, AST, GGT, ALP, Urea and Creatinine and significantly ($p<0.05$) increased serum, liver and kidney TP to nearly control levels. The extract significantly ($p<0.05$) reversed D-galactose-induced increases in percentage PCV, WBC counts, Fibrinogen and MDA concentration and increases liver and kidney SOD, Catalase and GSH to nearly control level. Group treated with the extract alone showed no adverse effects on the parameters. Results are possible indication of FRESML ability to protect against cellular oxidative stress in tissues, boost antioxidant status and ameliorate inflammation associated with oxidative stress.

KEYWORDS: Antioxidant, D-galactose, Oxidative Stress, Protective, *Solanum macrocarpon*.

INTRODUCTION

All living organisms are exposed to reactive oxygen species (ROS), reactive nitrogen species (RNS) and other highly reactive mediators such as Advanced Glycation End Products (AGEs) throughout their life cycle.[1] Though the body is equipped with the antioxidant system to protect it from excessive oxidant insults,[2] oxidative stress occur when there is imbalance between the systemic manifestation of these reactive species and the biological system's ability to detoxify the reactive intermediates.[3] Oxidative stress is among the largest known risk factors for human diseases.[4] Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process.[5,6] D-galactose administration is associated with multiple physiological and biochemical changes, the chief among which is formation of Advanced Glycation End Products.[7-9] Advanced Glycation End Products are a heterogeneous complex group of compounds that are formed when reducing sugar react non-enzymatically with amine groups of amino acids in proteins, lipids or DNA.[10,11] They are highly reactive mediator that causes mitochondrial DNA (mtDNA) common deletion (CD), alters the structure and function of molecules and increases oxidative stress in biological system.[1,12]

Prasad *et al.*[2] reviewed that any intervention that inhibits formation of AGEs is capable of putting generation of other oxidants (ROS and RNS) under check and consequently reduce

oxidative stress. However many researchers have suggested that the best way to neutralize free-radical mediated oxidative stress is to ingest diet rich in phytochemicals with antioxidant properties or to take dietary supplements of antioxidants.[13-16] These are widely found in nature, especially in plant products and are an extremely diversified group of chemicals.[17-21] One such naturally occurring antioxidant is flavonoids.[22-24] Flavonoids reportedly activate key enzymes in mitochondrial respiration and therefore protect cells by acting as antioxidants to break the vicious cycle of oxidative stress and tissue damage.[25] Flavonoids have been confirmed to be abundant in *Solanum macrocarpon* leaves,[13,26] an indigenous vegetable domesticated in Africa.[27-29] The uses of *Solanum macrocarpon* leaves in indigenous medicine ranges from weight reduction to treatment of several ailments including asthma, allergic rhinitis, nasal catarrh, skin infections, rheumatic disease and swollen joint pains, gastro-esophageal reflux disease, constipation and dyspepsia.[30,31]

Many flavonoid-rich extracts has been reported to revealed renal and hepato-protective effects in experimental animals.[32,33] However, few reports were available on the protective potentials of flavonoid-rich extract of *Solanum macrocarpon* leaves against experimentally induced oxidative stress in rats. Therefore, the present study was carried out to assess the protective potentials of Flavonoid-Rich Extract of *Solanum macrocarpon* Leaves (FRESML) against D-galactose-induced oxidative stress in rats.

MATERIALS AND METHODS

Materials

Basic laboratory materials such as UV-Visible Spectrophotometer, Centrifuge Machine, Thermostatic Water Bath, Mortar and Pestle, Stop Watch, weighing balance, pH meter, micropipette and glasswares were used for this work. Reagents includes: methanol, D-galactose, Tris HCl, KCl, Distilled water, Corn Oil, Magnesium ribbon, Adrenaline, H_2O_2, Potassium dichromate, Glacial acetic acid, Thiobarbituric acid, Trichloroacetic acid and Ellman's reagent. All other reagents were of the best pure analytical grade available and were obtained from Sigma Aldrich Chemical Company, St. Louis, Missouri, USA.

Sample Collection and Preparation

Solanum macrocarpon plants were collected from the vegetable garden in Ote Village, via Ilorin, Kwara State, Nigeria and were identified and authenticated with voucher herbarium number LHO 481 issued by Prof. Ogunkunle, a taxonomist at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. Fresh leaves of the

plant were air-dried at room temperature and further powdered. About 625 grams of the powdered leaves was extracted in 6.25 Litres of methanol and kept in the dark for 72 hours. The solvent was concentrated at temperature below 40^0C and the resulting methanol extract was subjected to liquid-liquid chromatographic separation technique using n-hexane, chloroform and ethyl acetate as the solvent to obtain their corresponding fractions.[34] The three fractions were screened for total flavonoids, and the ethyl acetate fraction was found to be the most potent, thereafter it was used for the biological study.

Experimental Animals

Twenty eight male albino rats of Wistar strain, weighing averagely 120g were used for this study. Animals were randomly selected into four groups of seven per group and treated for 28 days. Group A (control) were given distilled water, Group B (extract only) were administered 100mg/kg/day of FRESML, Group C (D-galactose only) were administered 500mg/kg/day of D-galactose and Group D (D-galactose + extract) received combined administration of 500mg/kg/day of D-galactose and 100mg/kg/day of FRESML. The animals were sacrificed 24 hours after the last administration using a mild anaesthetizing solvent. Established international guiding principles for using animals in biomedical research was adopted for the experiments.

Phytochemical Evaluation

Total Flavonoids content was determined by magnesium hydrochloride reduction test and sodium hydroxide test described by Trease and Evans.[35]

Biochemical Evaluation

Tissues and serum Total Protein concentration was determined by the Biuret method.[36] ALT and AST activities were determined by the method of Reitman and Frankel.[37] GGT activity was determined by the method of Szasz.[38] ALP activity was determined by optimized standard method according to the recommendation of the Deutsche Gesellschaft fur Klinische Chemie.[39] Urea concentration was determined by Urease-Berthelot method. Creatinine concentration was determined by modified method of Jaffe.[40,41] SOD activity was determined by the method of Misra and Fridovich.[42] Catalase activity was determined by the method of Sinha.[43] GSH concentration was determined by the modified method of Ellman.[44,45] MDA concentration was determined by the method reported by Varshney and Kale.[46] PCV was estimated using micro haematocrit.[47] WBC was counted using improved Neubaur haemocytometer. Fibrinogen concentration was measured by the Clauss method.[48]

Statistical Analysis

Results are presented as Mean ± SEM. Paired Student's t-test was used to compare variations amongst groups. The minimum level of significance was considered at $p<0.05$. Statistical analysis was carried out using a software program (GraphPad Prism Ver. 5; GraphPad Software, San Diego, CA).

RESULTS AND DISCUSSION

Oxidative stress is associated with decline in synthesis rate of muscle proteins, such as myosin heavy chain[49] and mitochondrial protein.[50] The statistically significant decreased in serum (Fig. 1), liver (Fig. 2) and kidney (Fig. 3) total protein concentrations in the D-galactose treated group further emphasized the fact that total protein concentration decreases in oxidative stress condition. These results (Fig. 1, Fig. 2 and Fig. 3) are in agreement with the results of others which reported evidences of generalized decline of whole body protein turnover and mixed muscle proteins respectively.[49,51-53] Therefore, generalized decline in the remodelling process of tissues with oxidative stress may be responsible for the significant decrease in total protein reported in this study. In support of this, a previous study explained that the significant decrease in total protein with oxidative stress may indicate an impaired liver synthesis and excretory functions[54]. Interestingly, combined administration of D-galactose and FRESML brought about increased serum (Fig. 1), hepatic (Fig. 2) and renal (Fig. 3) total protein concentrations. This may be due to the ability of the flavonoid contents of the extract to improve the synthesis and excretory functions of the tissues.[54]

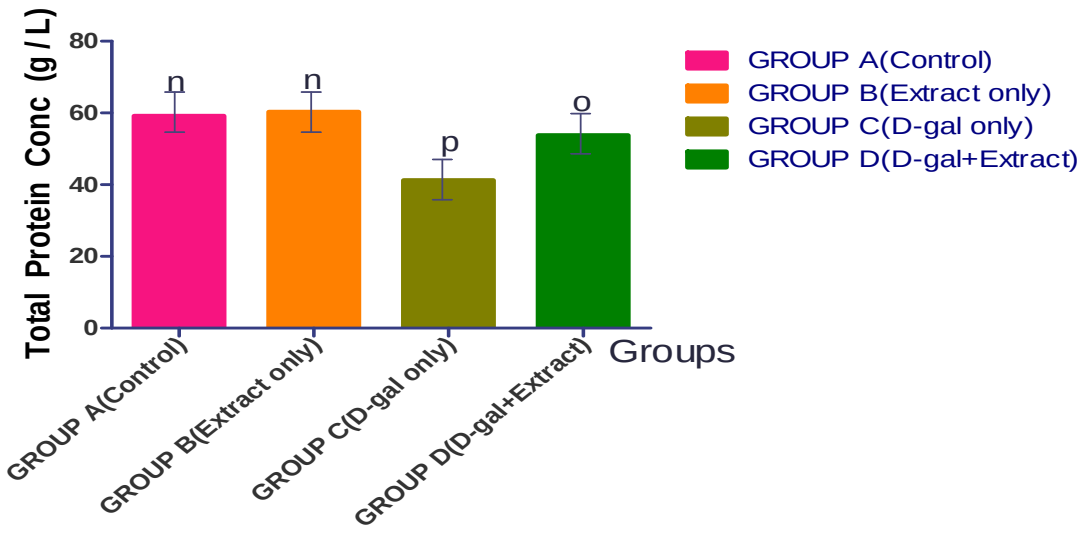

Fig. 1: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on serum total protein concentration in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

n = significantly different from **C&D** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

o = significantly different from **A, B&C** ($p<0.05$)

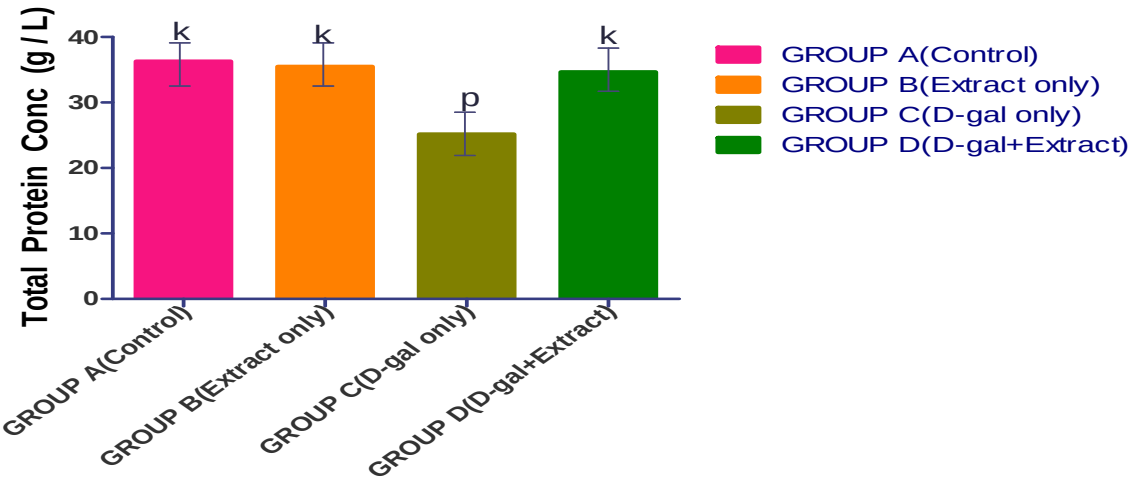

Fig. 2: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on liver total protein concentration in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

k = significantly different from **C** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

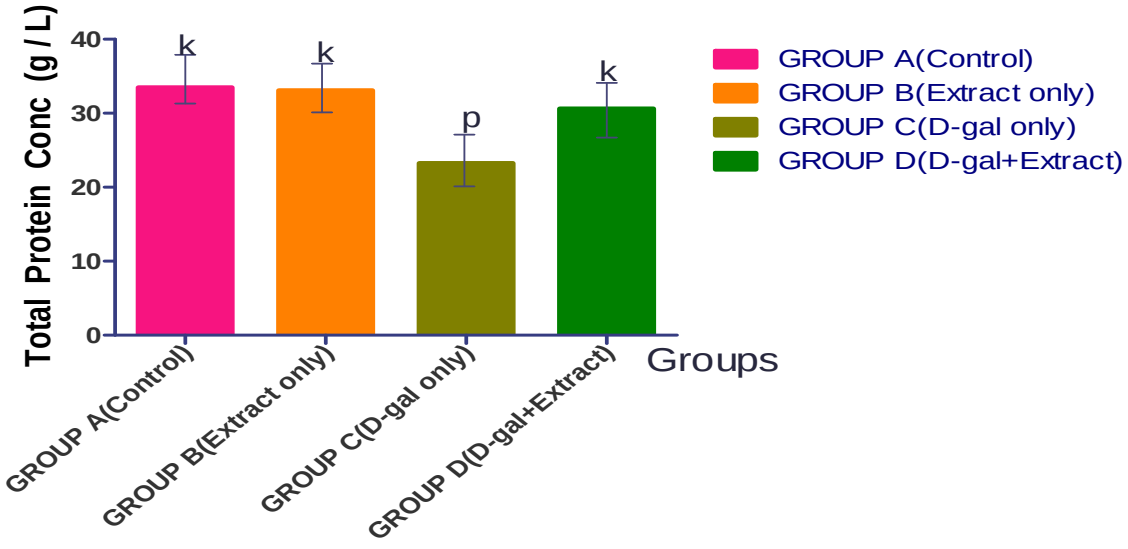

Fig. 3: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on kidney total protein concentration in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

k = significantly different from **C** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

The study of different enzyme activities such as ALT, AST and GGT have been found to be of great value in the assessment of clinical and experimental liver damage.[55,56] In the present investigation, it was observed that the animals treated with D-galactose resulted in elevated levels of serum ALT (Fig. 4), AST (Fig. 5) and GGT (Fig. 6). These changes in the marker levels may affect the hepatic structural integrity. The rise in the AST (Fig. 5) is usually accompanied by an elevation in the level of ALT (Fig. 4), which play a vital role in the conversion of amino acids to keto acids.[57] The combined administration of D-galactose and FRESML significantly attenuated the elevated levels of these serum markers (Fig. 4, Fig. 5 and Fig. 6). The normalization of these serum markers by FRESML suggests that the extract was able to condition the hepatocytes so as to protect the membrane integrity against oxidative stress that facilitated leakage of these marker enzymes into the blood circulation from the liver.[58,59]

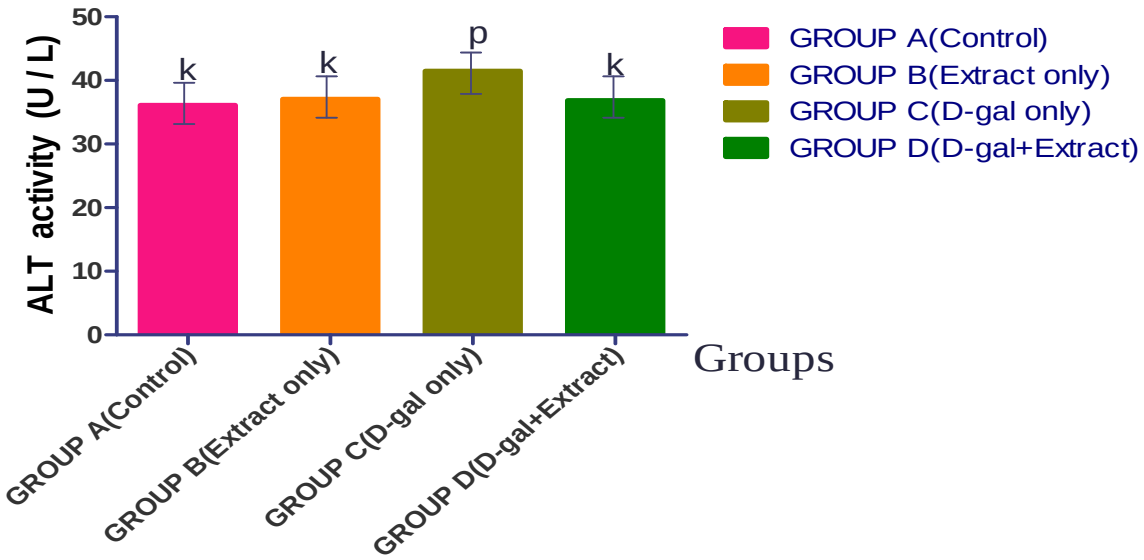

Fig. 4: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on serum ALT activities in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

k = significantly different from **C** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

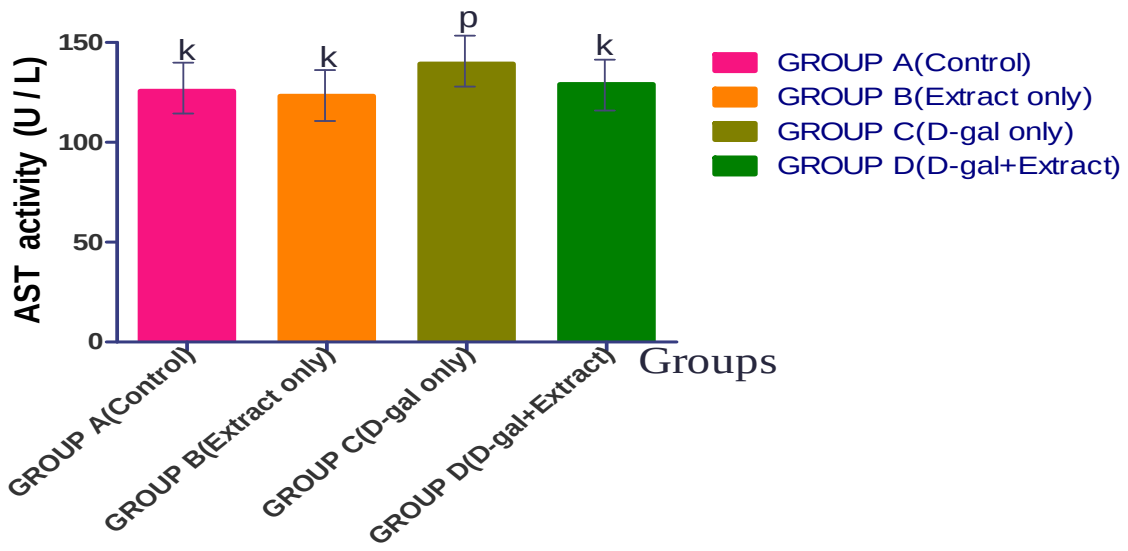

Fig. 5: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on serum AST activities in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

k = significantly different from **C** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

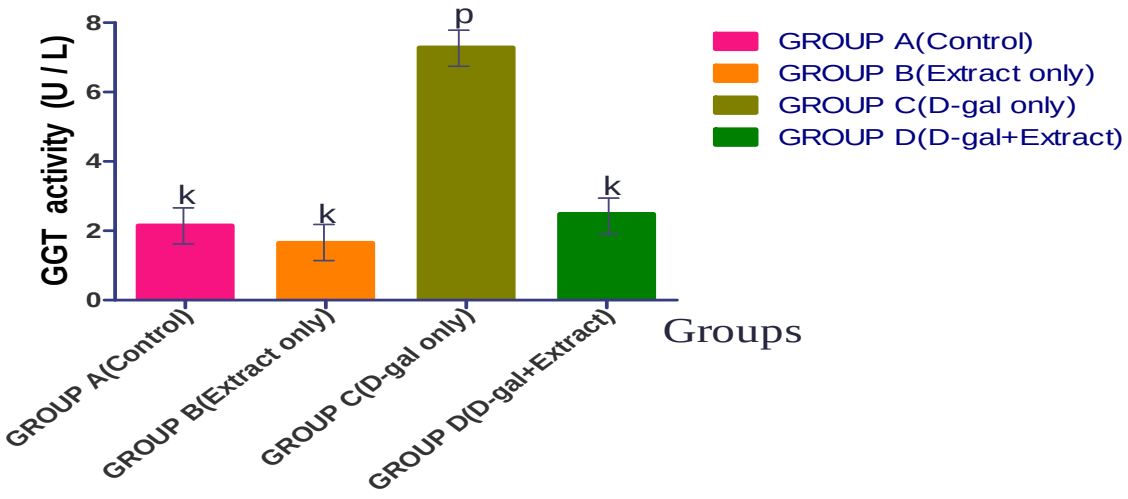

Fig. 6: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on serum GGT activities in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

k = significantly different from **C** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

The elevated levels of serum alkaline phosphatase (Fig. 7), urea (Fig. 8) and creatinine (Fig. 9) obtained in the D-galactose treated group in this study are suggestive of abnormal renal function caused by oxidative stress induced by D-galactose administration, especially as it relate to glomerular functions. Bishop *et al.*[60] observed an increase in blood urea, creatinine and ALP levels which was closely correlated with histopathological degenerative changes in the kidney and these changes caused disturbance in the transport system of biochemical constituents. Urea is the principal end product of protein catabolism.[60] Therefore its elevation in the serum (Fig. 8) may be due to enhanced protein catabolism and accelerated amino acid deamination for gluconeogenesis as most conditions that increases serum urea brings about decreases in serum total protein.[61] Creatinine is the last variable of non-protein nitrogenous blood constituents.[62] Low clearance rates of urea and creatinine (Fig. 8 and Fig 9) may indicate diminished ability of the kidneys to filter these waste products from the blood and excrete them in the urine.[63] However, the ability of FRESML to restore the levels of these parameters to nearly control level (Fig. 7, Fig. 8 and Fig. 9) in the combined treatment group suggests the ability of the extract to prevent amino acid deamination (nephroprotective) and could be credited to its antioxidant activities.[64]

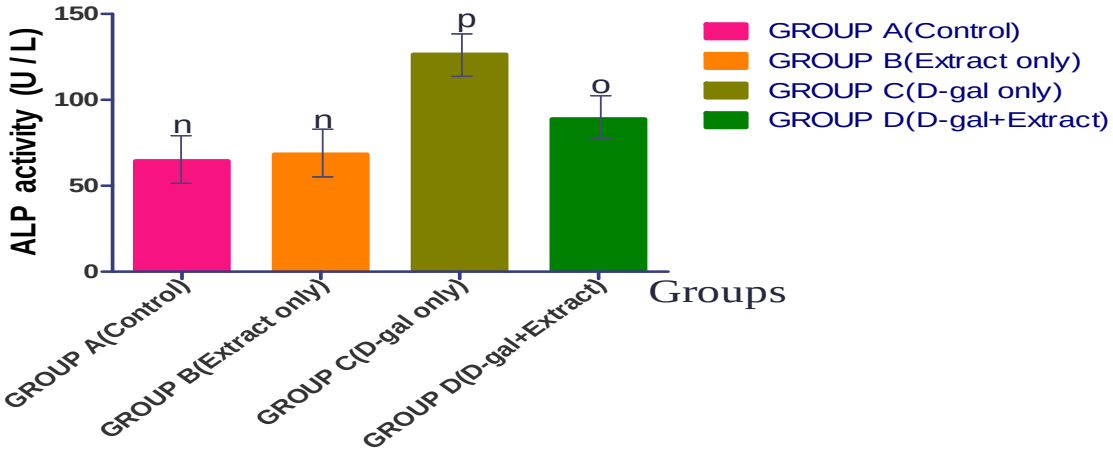

Fig 7: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on serum ALP activities in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

n = significantly different from **C&D** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

o = significantly different from **A, B&C** ($p<0.05$)

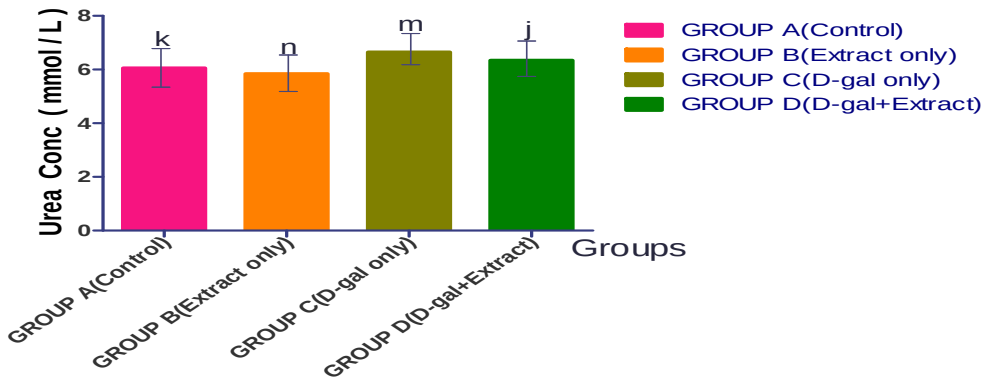

Fig. 8: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on serum urea concentration in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

k = significantly different from **C** ($p<0.05$)

n = significantly different from **C&D** ($p<0.05$)

m = significantly different from **A&B** ($p<0.05$)

j = significantly different from **B** ($p<0.05$)

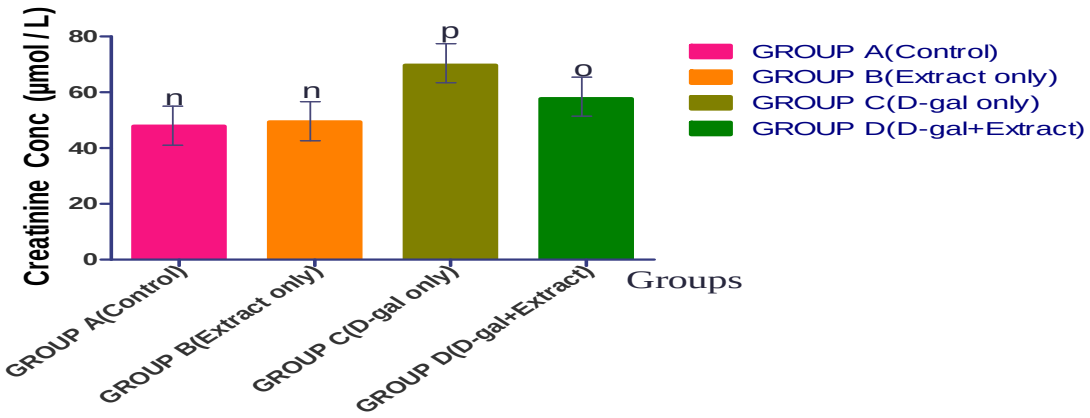

Fig. 9: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on serum creatinine concentration in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

n = significantly different from **C&D** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

o = significantly different from **A, B&C** ($p<0.05$)

It has been reported that animal body had an effective mechanism to prevent the free radical induced tissue damage; this is accomplished by a set of endogenous antioxidant enzymes and proteins such as SOD, Catalase and GSH.[65] In the present study, SOD and catalase activities

as well as GSH concentration were measured in liver (Fig. 10, Fig 12 and Fig. 14) and kidney (Fig. 11, Fig. 13 and Fig. 15). The depletion of SOD activity to nearly half of the control level in liver and kidney as seen in D-galactose treated group is another indication of oxidative stress induced by D-galactose. In the same manner, the depletion of Catalase activity to nearly quarter of the control level and half of the control level in liver and kidney respectively in D-galactose treated group also confirmed hepatotoxicity and nephrotoxicity of D-galactose. The reduction in GSH levels in the liver and kidney of the animals administered D-galactose alone by three-quarter of its concentration compared to the control group, further established the hepatotoxicity and nephrotoxicity of D-galactose. The decreases in these antioxidant parameters may be due to rapid consumption and exhaustion of storage of the enzymes and protein in fighting free radicals generated during development of oxidative stress.[65]

Significant increases ($p<0.05$) in the hepatic and renal levels of SOD, Catalase and GSH observed in the combined treatment group and extract only group showed that FRESML may reduce reactive free radicals and boost the antioxidant status. Similar claims were made by Iweala and Ogidigo,[66] where they attributed the increase of these antioxidant indices by *Solanum macrocarpon* leaves extract to the abundant antioxidant polyphenolic compounds in the extract. However, they could not ascertain the specific class of polyphenolic compound responsible for this action. Having used Flavonoids-Rich Extract of *Solanum macrocarpon* Leaves in this study, it may be suggested that the flavonoids content of the extract was responsible for the ability of the extract to increase the levels of SOD, Catalase and GSH *in-vivo*.

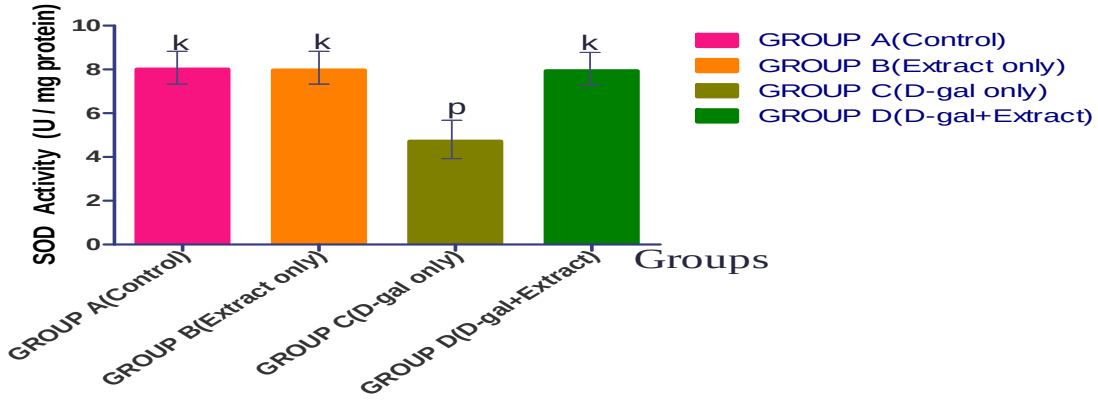

Fig. 10: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on liver SOD activities in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

k = significantly different from **C** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

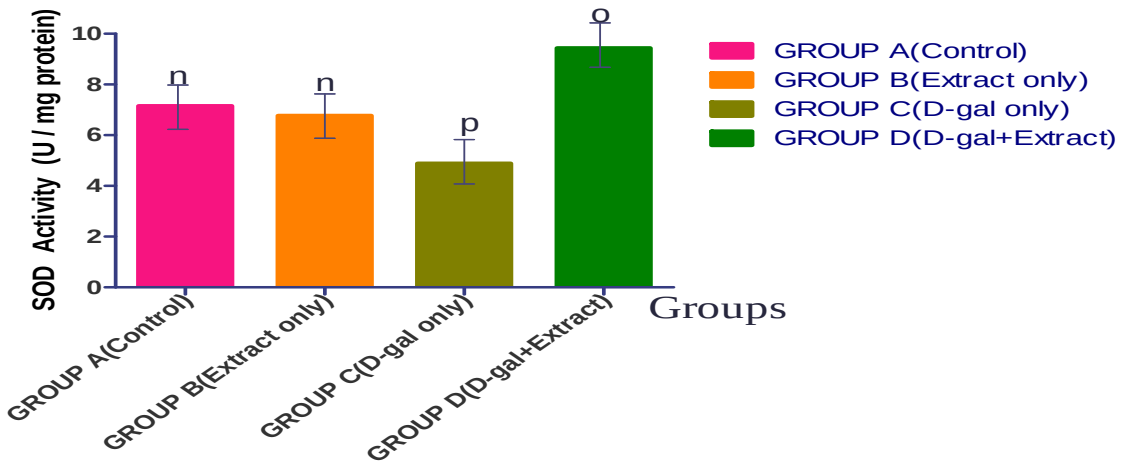

Fig. 11: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on kidney SOD activities in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

n = significantly different from **C&D** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

o = significantly different from **A, B&C** ($p<0.05$).

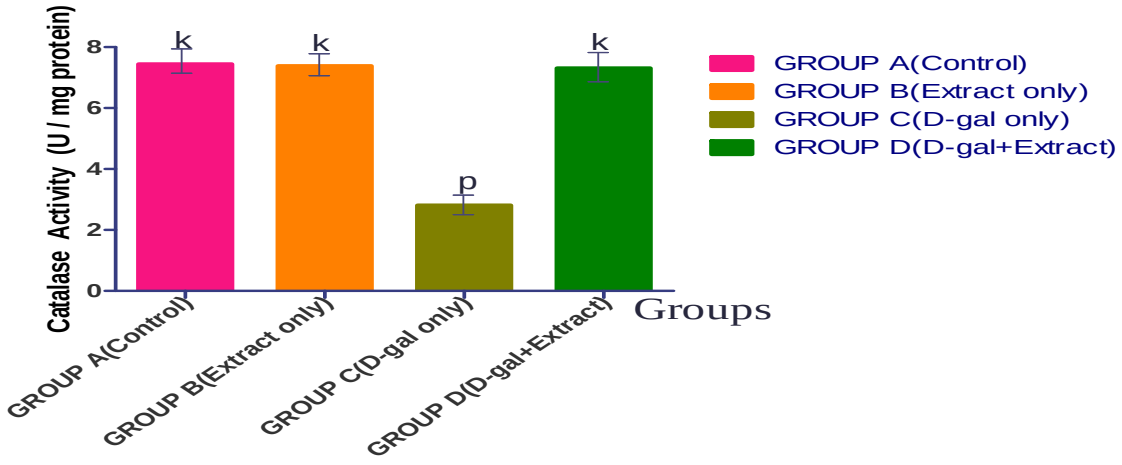

Fig 12: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on liver catalase activities in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

k = significantly different from **C** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

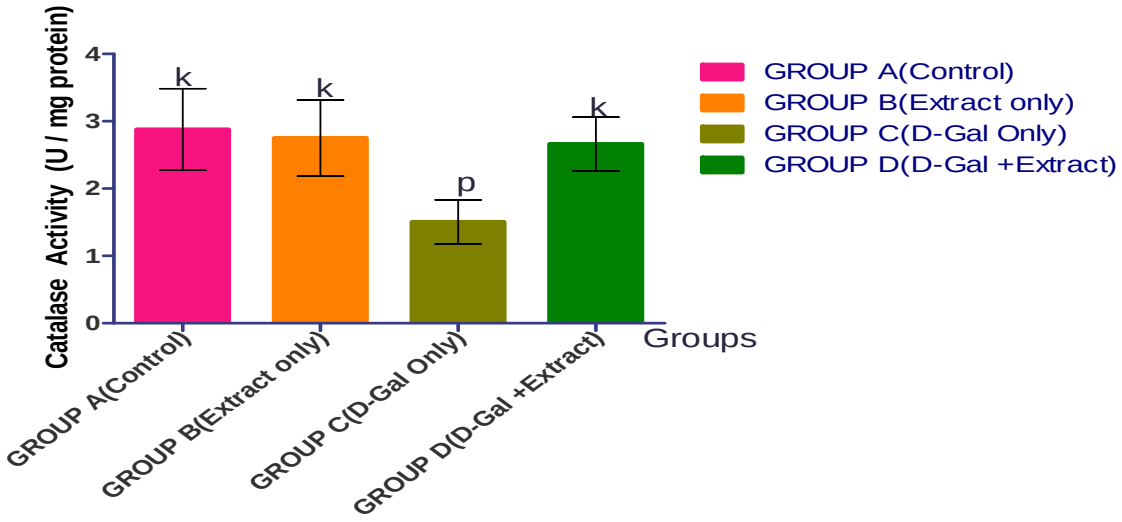

Fig. 13: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on kidney catalase activities in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

k = significantly different from **C** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

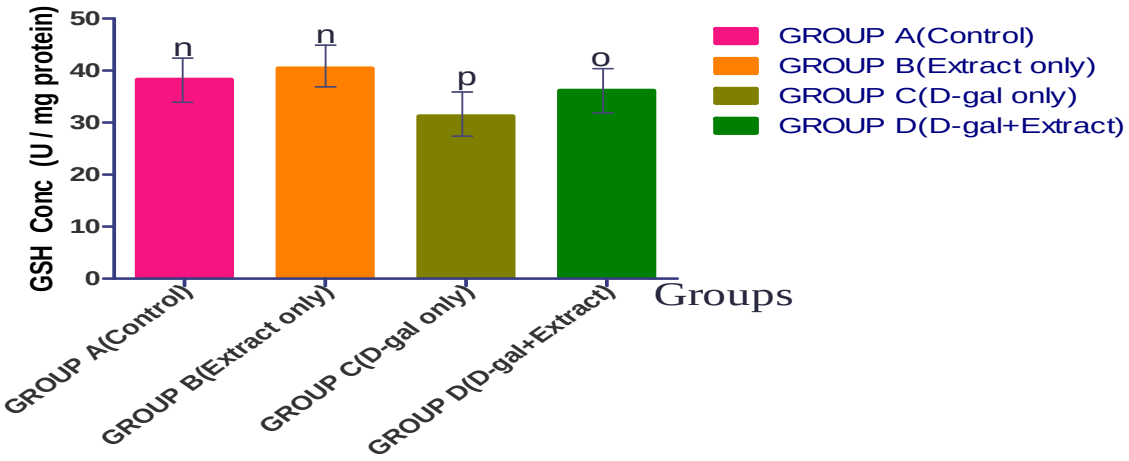

Fig. 14: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on liver GSH concentration in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

n = significantly different from **C&D** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

o = significantly different from **A, B&C** ($p<0.05$)

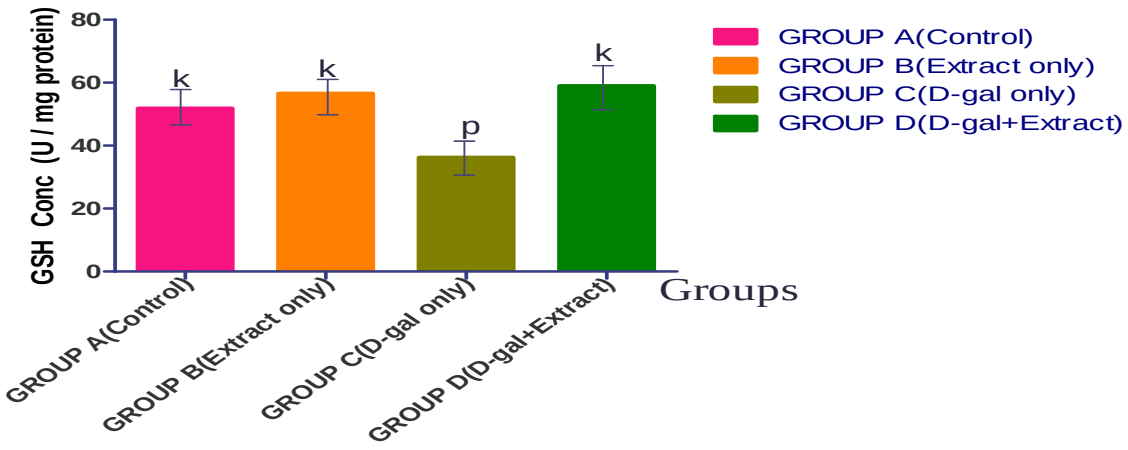

Fig. 15: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on kidney GSH concentration in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

k = significantly different from **C** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

Lipid peroxidation has been implicated in processes such as carcinogenesis, inflammation and aging.[67] In the present study elevation in the levels of end products of lipid peroxidation, Malondialdehyde (MDA) in the liver (Fig. 16) and kidney (Fig. 17) of rats treated with D-galactose was observed. The increase in Malondialdehyde (MDA) levels in the liver and kidney suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence system to prevent formation of excessive free radicals. Combined treatment of D-galactose and FRESML significantly reversed these changes. Hence it may be possible that the mechanism of liver protection and renal protection by FRESML may be due to its antioxidant effects.[26,68-70]

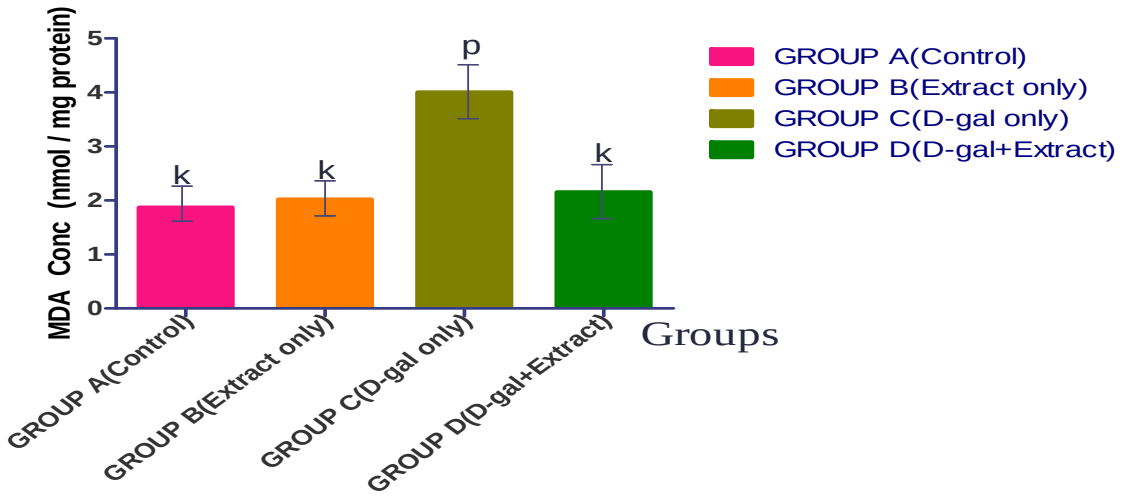

Fig. 16: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on liver MDA concentration in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

k = significantly different from **C** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

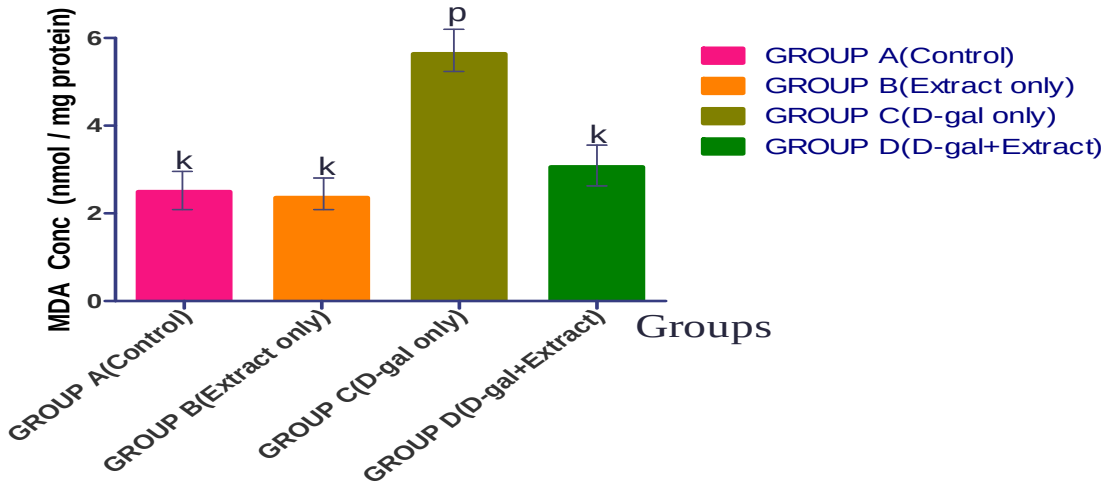

Fig. 17: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on kidney MDA concentration in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

k = significantly different from **C** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

Both haematological and inflammatory indices are needed to characterize tissues' response to injurious stimuli.[71] The relative increase in percentage PCV of the animals treated with D-

galactose compared to the control group in this study (Fig. 18) signified acute systemic inflammation typical of oxidative stress in animal model.[72] Evidence of increased PCV in oxidative stressed animals has been documented in literature.[72] However, increased PCV has been strongly correlated with increased fibrinogen in the plasma.[73,74] Therefore the increased percentage Packed Cell Volume witnessed in the D-galactose treated group in this study may be attributed to the increase in fibrinogen concentration in the rats. Interestingly, the increased PCV was adequately attenuated in the combined treatment group as proved by significant decrease in the percentage PCV of the animals in this group to nearly control level (Fig. 18). This correction may be as a result of FRESML ability to regulate haematopoiesis in bone marrow with a subsequent modulation of PCV as shown in this study.[75]

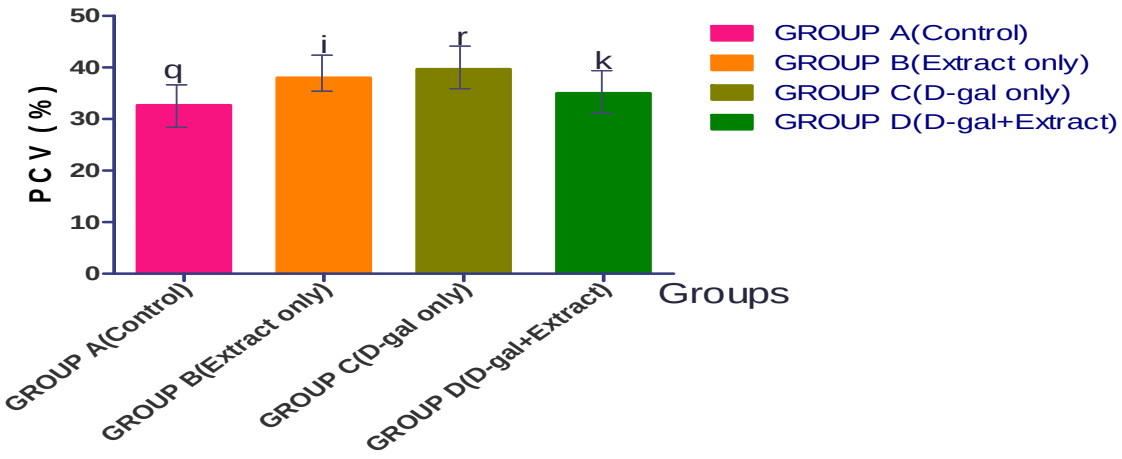

Fig. 18: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on blood PCV levels in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

q = significantly different from **B&C** ($p<0.05$)

i = significantly different from **A** ($p<0.05$)

r = significantly different from **A&D** ($p<0.05$)

k = significantly different from **C** ($p<0.05$)

In the present study, there was a significant increase in white blood cell count in D-galactose treated group compared to the control group and the extract only group (Fig 19). Mansour *et al.*[76] revealed that increased WBC counts can occur as a result of immune response to an infection, cancer, or toxic chemical. Significant reduction of WBC counts in groups B (extract only group) and D (D-galactose + extract group) nearly to the control level (Fig 19) showed that the immune system may not be challenged in animals fed with FRESML. This

may be due to its content of immune-boosting flavonoids as flavonoids have been reported to have immune-boosting ability as a result of its antioxidant activities.[77,78] This result (Fig 19) agreed with previous studies that *Solanum macrocarpon* may help preserve the body's adaptive response to stress.[79]

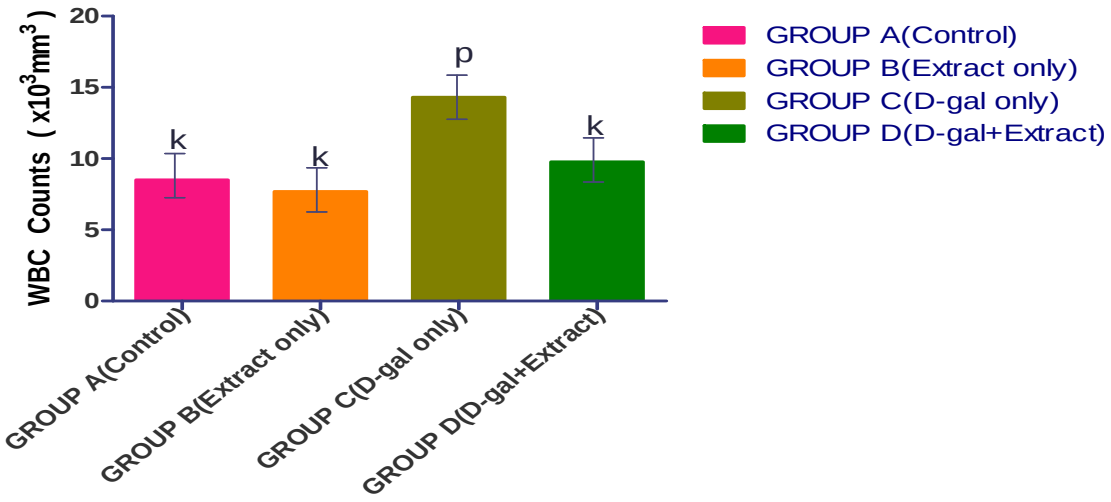

Fig. 19: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on plasma WBC counts in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

k = significantly different from **C** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$)

Inflammation is a protective attempt by the organism to remove the injurious stimuli and initiate the healing process.[71] Fibrinogen concentration has previously been found to be particularly useful in detecting inflammatory diseases.[80] Fibrinogen concentration rises with systemic inflammation.[81,82] The significant increase in the plasma concentration of fibrinogen observed in D-galactose treated group in this study (Fig. 20) may be because its concentration increases gradually in response to tissue injury.[83,84] However, the feature of inflammation disappeared in the combined treatment group of D-galactose and FRESML as the plasma fibrinogen concentration was restored nearly to the control levels (Fig. 20). This improvement noticed after FRESML administration might be attributed to the anti-inflammatory properties of the extract and strong antioxidant effect of flavonoids in *Solanum macrocarpon* extract on hematopoietic cells.[75]

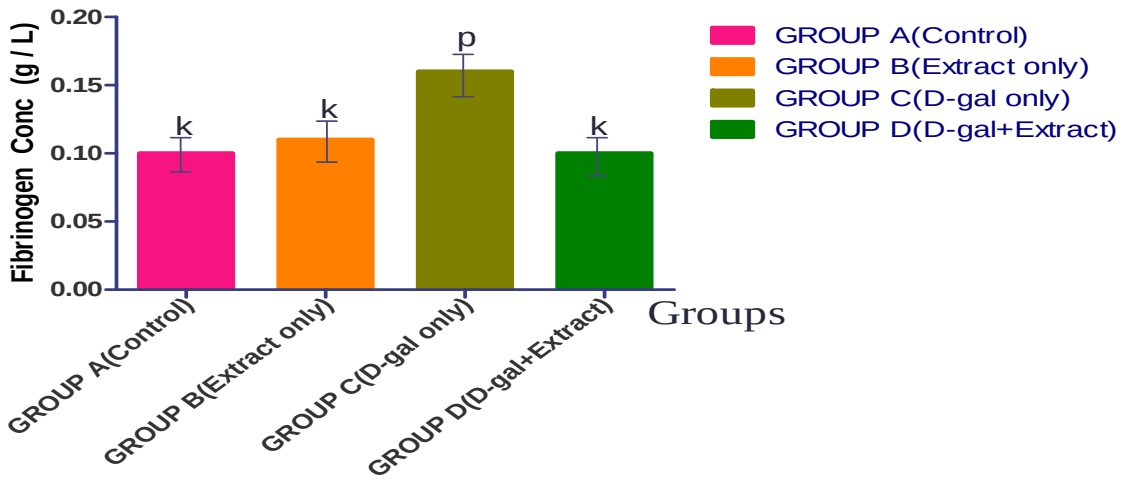

Fig. 20: Effects of flavonoid-rich extract of *Solanum macrocarpon* leaves on plasma fibrinogen concentration in D-galactose-treated rats.

Each value is Mean ± S.E.M of 7 rats in each group.

k = significantly different from **C** ($p<0.05$)

p = significantly different from **A, B&D** ($p<0.05$).

CONCLUSION

The results obtained in this study demonstrated that combined treatment of D-galactose and Flavonoid-Rich Extract of *Solanum macrocarpon* Leaves showed significant improvement in protein synthesis, hepatic integrity, renal integrity and antioxidant status compared with D-galactose exposed rats. Therefore, Flavonoid-Rich Extract of *Solanum macrocarpon* Leaves could be seen as a good source of useful chemoprotective agents or food supplements and a possible template for discovery of drugs for ameliorating oxidative stress and other diseases associated with it.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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