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Flavonoid complex is protective against sodium arsenite-induced hepatotoxicity and nephrotoxicity in rats

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Abstract

Introduction: Flavonoids are a class of polyphenols that are widely available in plant being part of the four main classes of polyphenols. They possess strong antioxidant, anti-inflammatory and anti-cancer properties. The effects of flavonoid on sodium arsenite-induced hepatorenal toxicity was therefore investigated.

Methods: Twenty-four adult (160 ± 20g) male Wistar rats were randomly divided into four groups (A-D), with six rats per group. Group A (negative control) received distilled water; Group B received sodium arsenite (SA) only; Group C received flavonoid complex (FL) only and Group D initially received flavonoid complex and later received sodium arsenite every other day. SA was administered at 5mg/kg and FL at 10mg/kg, all treatments were for fourteen days.

Results: Arsenic exposure resulted in significant reduction ($P<0.05$) in in total protein, percentage body and relative liver weights. Biochemical alterations in ALT, AST, ALP activities, urea and creatinine levels in the blood were significantly decreased. Furthermore, a reduction in GSH level, GST and CAT activities in the arsenic exposed group were also significant. The LPO and NO levels were also increased significantly. FL significantly restored the hepatorenal parameters and promoted hepatocytes and renal cells regeneration.

Conclusion: The present study indicates that flavonoid complex is protective against hepatorenal toxicities induced by sodium arsenite in Wistar rats.

Keywords: *Sodium arsenite, Hepatotoxicity, Nephrotoxicity, Flavonoid complex, Antioxidant, Oxidative/Nitrosative stress*

Abstrait

Introduction: Les flavonoïdes sont une classe de polyphénols largement disponibles dans les plantes faisant partie des quatre principales classes de polyphénols. Ils possèdent de fortes propriétés antioxydantes, anti-inflammatoires et anticancéreuses. Les effets des flavonoïdes sur la toxicité hépato-rénale induite par l'arsénite de sodium ont donc été étudiés.

Méthodes: Vingt-quatre rats Wistar mâles adultes (160±20 g) ont été répartis au hasard en quatre groupes (A-D), avec six rats par groupe. Le groupe A (témoin négatif) a reçu de l'eau distillée ; Le groupe B a reçu uniquement de l'arsénite de sodium (SA); Le groupe C a reçu uniquement un complexe flavonoïde (FL) et le groupe D a initialement reçu un complexe flavonoïde, puis de l'arsénite de sodium tous les deux jours. SA a été administré à 5 mg/kg et FL à 10 mg/kg, tous les traitements ont duré quatorze jours.

Résultats: L'exposition à l'arsenic a entraîné une réduction significative ($P < 0,05$) du poids total des protéines, du pourcentage corporel et du poids relatif du foie. Les altérations biochimiques des activités ALT, AST, ALP, ainsi que des taux d'urée et de créatinine dans le sang ont été significativement diminuées. En outre, une réduction du niveau de GSH, des activités de GST et de CAT dans le groupe exposé à l'arsenic était également significative. Les niveaux de LPO et de NO ont également augmenté de manière significative. FL a restauré de manière significative les paramètres hépato-rénaux et favorisé la régénération des hépatocytes et des cellules rénales.

Conclusion: La présente étude indique que le complexe flavonoïde protège contre les toxicités hépato-rénales induites par l'arsénite de sodium chez les rats Wistar.

Mots clés: Arsénite de sodium, Hépatotoxicité, Néphrotoxicité, Complexe flavonoïde, Antioxydant, Stress oxydatif/nitrosatif

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Introduction

Arsenic is now a severely toxic metallic contaminant in groundwater, natural processes and agricultural spills, which can have a negative impact on health[1].

Sodium arsenite is an organic sodium salt with a chemical formula of $NaAsO_2$. Sodium arsenite is a clastogen which causes chromosomal breakage[2] and they interact with other metals to make their effects potent [3]. Arsenic is mostly ingested by humans through contaminated drinking waters such as subterranean water, agricultural waste, and industrial effluents. Skin cancer, conjunctivitis, melanosis, hyperkeratosis, renal dysfunction, liver and respiratory disorders, and hematological changes are considered to be some of the most common health problems caused by arsenic poisoning[4,5]. The USEPA [6] and the International Agency for Research on Cancer [7]classify them as Group A and Category 1 human carcinogens, respectively. High intakes of inorganic arsenic via contaminated water or food are thought to increase the risk of cancers, especially skin cancer, lung cancer, liver cancer, and lymphoma [8]. After chronic long-term human exposure to arsenic, it has been shown to be carcinogenic [9].

Genetic and epigenetic modifications, DNA repair inhibition, oxidative stress, changes in cell death and proliferation, and abnormal activation of signal transduction pathways have all been hypothesized as mechanisms for arsenic-induced carcinogenesis [9].Keratosis, hyperpigmentation, hypopigmentation, cardiovascular illness, diabetes mellitus, central nervous system problems, etc. are other health issues that can be brought on by arsenical exposure [10]. It was previously hypothesized that arsenic causes oxidative stress by cycling between its metallic oxidation states or by an imbalance of antioxidants and a high rate of inflammation, leading to the formation of free radicals within cells [11].

Flavonoids are the most frequently and extensively dispersed group of plant phenolics, and they are abundant in diets [12]. Flavonoids are major bioactive compounds and they have been used in the treatment of many chronic diseases, including cancer, viral infections, inflammation, cardiovascular and neurodegenerative diseases. It is widely believed that an effective dietary ingredient is an antioxidant nutrient found in fruits and vegetables [13].

The flavonoid complex used in this study encompass diverse phytonutrients which contains: catechins, anthocyanins, proanthocyanins, flavones, flavanones, flavonols and ellagic acid. Whole cranberries, kale, green tea (without caffeine), beets, red and black grapes, oranges, lemons and grapefruits can be used to create an exclusive blend of flavonoid-rich extracts and concentrates. Absorption is aided by the addition of natural vitamin C [14]. They have great benefits due to their abilities to reduce free radical (antioxidant activity) [15]. Other biological functions of flavonoids include skin protection from harmful UV light, DNA protection, capillary strengthening, anti-inflammatory impact, radiation protection, moistening, softening, calming, antimicrobial, and others. Flavonoids can be useful as component of cosmetics and medicinal goods because of their biological activities [16]. Flavonoids antioxidant capacity is due to the combination of iron chelating and free radical capture properties and the inhibition of oxidase enzymes such as lipoxygenase, cyclooxygenase, myeloperoxidase, NADPH oxidase and xanthine oxidase, which prevent the formation of reactive oxygen species and organic matter[17]. In addition, flavonoids possess the capacity to inhibit enzymes that are indirectly involved in the oxidative process. B. Phospholipase A2, while also stimulating antioxidant enzymes including catalase and superoxide dismutase. Flavonoids are used in conjunction with other antioxidants, vitamins, and minerals to reduce cancer development, cardiovascular mortality, and ischemic vascular disease [18].

Nevertheless, limited studies have been done on the role of flavonoid complex in the protection or restoration of arsenic-induced liver and kidney dysfunction.This study was therefore designed to evaluate the protective role of flavonoid complex in arsenic-induced hepatotoxicity and nephrotoxicity in male Wistar rats.

Materials and Methods

Chemicals, Reagents and Kits

Sodium arsenite waspurchased from Sigma Aldrich Co. United Kingdom andflavonoid complex was obtained from Danax pharmacy, at Ibadan, Oyo State, Nigeria. The flavonoid Complex is a very concentrated source of water-soluble flavonoids from green tea, kale, cranberries, elderberries, red and black grapes, beetroot root, lemons, oranges and grapefruit. Vitamin C is also present all encapsulated in one.A commercially available kit was used to measure serum liver enzyme activity according to the manufacturer's procedures. Serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT) activities, urea, and creatinine levels were determined using the

RANDOX® Laboratory Reagent Kit from RANDOX Laboratories Ltd., Ardmore, UK.

Experimental Animals

Mature male Wistar rats (n = 24) weighing 160 ± 20g (8 weeks old) were purchased for this study from the Preclinical animal house facilities of the Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria. Prior to the commencement of treatment, the animals were allowed to acclimatize for 1 week, they were housed in well ventilated cages in the animal house of our department. All animals were allowed free access to rat food and water that was delivered fresh every day. Experimental animals were given a photoperiod of 12 hours of light/dark cycles, which corresponded to the natural light cycle, and were handled in adherence to the guide for the care and use of experimental animals, as specified by the National Institute of Health (NIH publications number 85–93 revised in 1985). Hence the weight of the animals was taken weekly.

Experimental Design

The classification of animals used for this study is indicated below with their grouping.

Group A (Control): Given distilled water, 2 ml/kg of distilled water.

Group B: Administered Sodium arsenite at 5 mg/kg for 14 days at every other day.

Group C: Administered flavonoid (10 mg/kg) for 14 days

Group D:Pretreated with flavonoid(10mg/kg) daily for 14 days and then administered SA (5 mg/kg) for 14 days at every other day.

All treatments were done by oral gavage. Sodium arsenite was administered in line with published report [19]. Flavonoid complex was administered at 10mg/kg since it was pretreated as this was chosen based on the recommended dose of the flavonoid complex which is 18mg/kg.

Preparation of tissues

Rats were sacrificed by cervical decapitation, 24 hours after the last treatment administration and fasted overnight, blood samples were collected. The liver and kidney were carefully removed, washed in rinsing buffer solution and weighed. A section of each of the liver and kidney samples was fixed in 10% formalin solution for histological investigations. The original weights of the harvested liver and kidney organs were calculated after they had been rinsed in an icy cold solution of 1.15% KCl, blotted with filter paper, and weighed. The liver samples were then divided into sections for histological analysis and put in 10% Formalin. Utilising a Teflon homogenizer, the remaining pieces of the extracted liver were homogenised with 0.1M Phosphate buffer (pH 7.4). To acquire the post mitochondrial fraction, the homogenates were spun at 10,000 revolutions per minute for 15 minutes at 4 degrees in a cool centrifuge. The supernatants were collected after centrifugation and kept at -20 degrees until the biochemical analyses.

Preparation of Serum and Blood Analysis

Blood was drawn from the periorbital sinus and placed in plain tube to coagulate at room temperature. The coagulated blood sample was centrifuged at 3,000 g for 10 minutes at 4 °C to prepare serum. Prior to the analyses, the serum was decanted and kept at -20 °C.Blood was drawn from the periorbital sinus and placed in lithium heparinized flask. Packed cell volume (PCV) was calculated using the microhematocrit method and hemoglobin (Hb) concentration was calculated using cyanmethemoglobinmethod. The new enhanced Neubauer hemocytometer was used to count Red Blood cells (RBCs) and white blood cells (WBCs). A conventional approach was used to assess differential leukocyte counts [20].

Biochemical assays

Total Protein level assay

Total Protein level was determined following the protocol outlined in Randox Laboratories Limited kits. Absorbance was read at 546 nm. This assay was done using biuret method to evaluate the total protein which was used as a reference for other assays carried out.

Kidney function assay

Urea and creatinine levels were determined in the blood using the method of Fawett and Scott [21].

Liver function assay

Alanine and aspartate aminotransferases (ALT and AST) activities were determined based on the method of Reitman and Frankel [22]. Serum alkaline phosphatase (ALP) activitywas determined according to the method previously described by Rec [23].

Assay for antioxidant markers

Claiborne's approach for determining Catalase (CAT) activity was followed[24]. At 240nm, the absorbance

was measured. Misra and Fridovich's approach [25] were used to determine Superoxide dismutase (SOD) activity, at 480nm, the absorbance was measured. Glutathione-S- transferase (GST) activitywas measured according to the method published by Habig [26] with absorbance read at 340nm.

The Rotruck [27] method was used to measure Glutathione peroxidase (GPx) activity. The activity of Gluatathione (GSH) was measured using Beutler's technique [28]. Rice-Evans method was used to assess Lipid peroxidation (LPO) activity, which involves a reaction between 2-thiobarbituric acid (TBA) and malonaldehyde (MDA). At 532nm, the absorbance was measured. Adam-Vizi and Seregi's approach was used to calculate MDA levels [29].

Assay for markers of inflammation

The Myeloperoxidase (MPO) activity was determined using a modified version of Trush et al. technique [30]. Green et al. described a method for determining nitric oxide (NO) activity [31].

Histological evaluation of the liver and kidney tissues

Tissue sections from the liver and kidney werefixed in 10% neutral buffered formalin. A standard paraffin-wax embedding procedure was used to prepare these tissues for histological analysis. Hematoxylin and eosin-stained sections of 5 micrometer thickness were prepared for light microscopy at a magnification of X400.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) to compare experimental groups.Using GraphPad prism6 software, a post hoc test (Bonferroni) was used to confirm the level of significance between groups (version 4; GraphPad Software, La Jolla). P values of less than 0.05 were considered significant.

Results

Administration of sodium arseniteled to a reduction in body and relative liver weight of the rats

The results in (Figure I) show that the rate of change in body weight in the SA-only group was significantly reduced compared to the control group, and that the FL + SA group was also significantly increased compared to the SA-only group. The relative liver weight changes in the SA group are also reduced compared to the control group, and the FL + SA group is also increased compared to the SA group. There is no significant change in relative kidney weight of the various experimental groups.

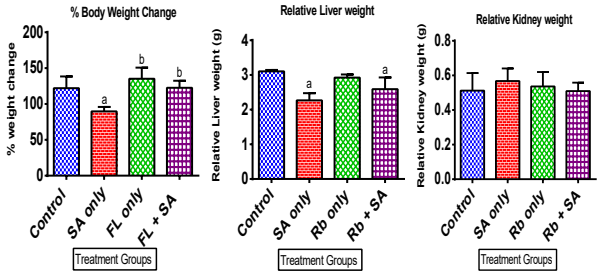


Figure I: Effect of sodium arsenite and flavonoid complex on percentage body weight, and relative liver and kidney weights respectively. Values are expressed as mean ± SD (n=6). a= significant difference ($p< 0.05$) when compared with the positive and negative control-(which is SA only and Control group).

Sodium arsenite caused a decrease in serum and liver total protein

There was a significant reduction ($p <0.05$) in total serum protein in animals treated with SA alone compared to the control group. Significant reduction in total protein in the liver of SA-treated animals only when compared to the control group. There are no major changes in the kidney total protein of the various experimental groups (Figure II).

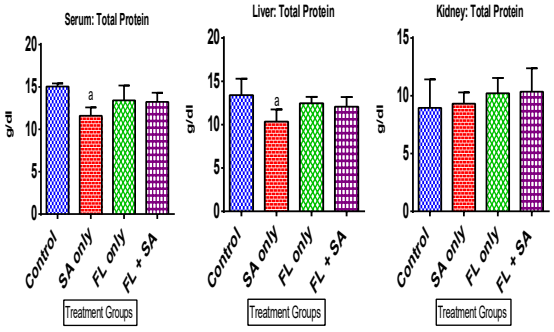


Figure II: Effect ofsodium arsenite and flavonoid complex on serum, liver and kidney protein levels respectively. Values are expressed as mean ± SD (n=6). a= significant difference ($p< 0.05$) when compared with the positive and negative control-(which is SA only and Control group)

Flavonoid complex offered protection against hepatoxicity induced by sodium arsenite

The result in (Figure III) shows Serum ALT, AST and ALP were significantly increased in the SA (5mg/kg) only treated group compared to the control. Also, a significant decrease in the FL + SA treated group compared to SA only. The Liver ALT, AST and ALP were significantly ($p<0.05$) increase in SA alone (5 mg/kg) treatment group compared to controls (Figure III). Similar protection is seen in the histological section of the liver (Figure IV).

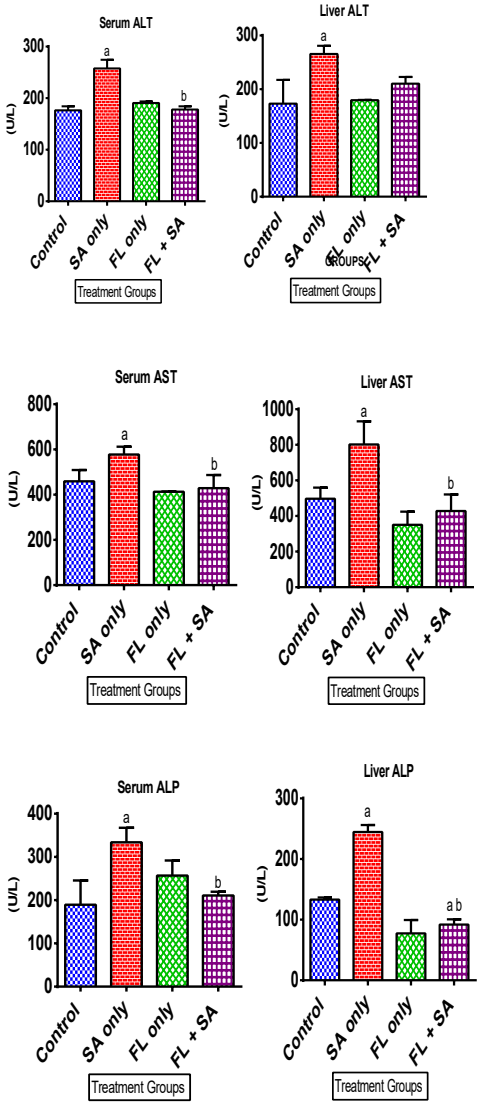


Figure III: Flavonoid complex protects against sodium arsenite-induced hepatotoxicity. The data is presented as a mean standard deviation (n=6), a indicates a significant difference ($p < 0.05$) when juxtaposed the negative control, whereas b indicates a significant difference ($p< 0.05$) when compared to the group that received only SA.

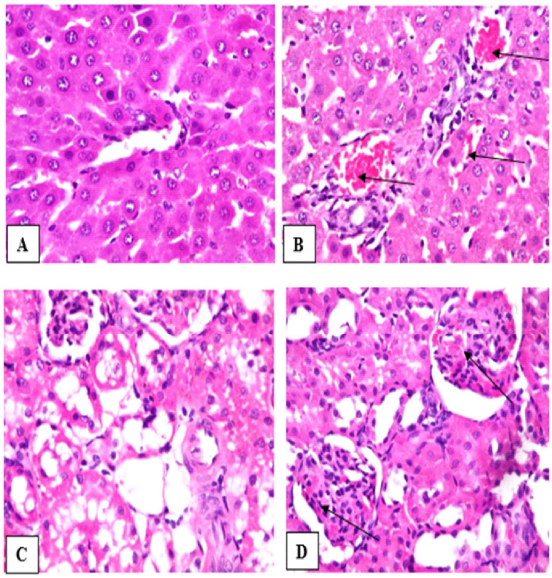


Figure IV: Photomicrographs showing liver sections from rats that had been treated for 14 days with flavonoid complex and sodium arsenite (Mag. X400). (A) Hepatocytes in normal condition**.** (B) SA only (5mg/kg body weight) showing mild vascular congestion and mild periportal inflammation. (C) Flavonoid (10mg/kg body weight) showing moderate tubular necrosis and replacement by inflammatory cells. (D) FL + SA (10mg/kg and 5mg/kg body weight) showing mild periportal inflammation and hepatocytes regeneration.

Protection was offered against renal-toxicity induced by sodium arsenite

The results in (Figure V) show that serum urea was significantly increased and decreased in the SA (5mg/kg) only and FL + SA treated groups respectively compared to the control. Also, a significant decrease in the FL + SA treated group compared to SA only. Also, renal urea was significantly ($p <0.05$) increased in the SA group (5 mg/kg) compared to the control. There was also a significant decrease in the FL + SA treatment group compared to SA alone. The results show that serum creatinine was significantly increased in the SA group (5mg/kg) compared to the control group. There was significant reduction of creatinine in the FL + SA treatment group compared to SA alone. The results show that renal creatinine was significantly ($p <0.05$) increased in the SA group (5 mg/kg) compared to the control (Figure V). The protection offered by flavonoid complex in the renal tissues is shown in Figure VI

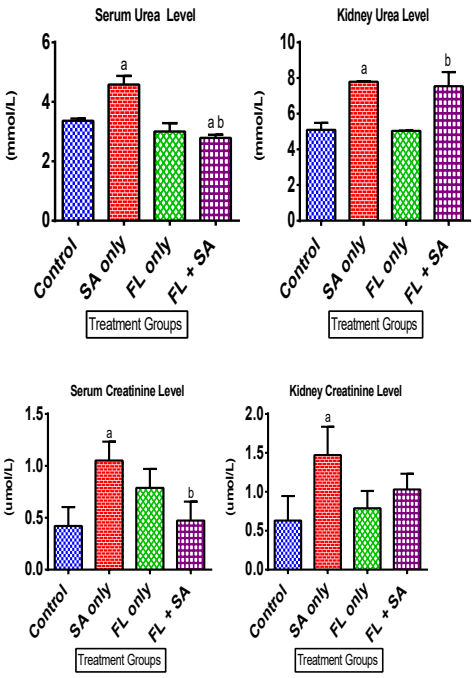


Figure V: Protective effects of flavonoid complex (Fl) on sodium arsenite-induced renal toxicity. Each bar represents mean ± SD (n=6). *a* = significant difference ($p< 0.05$) in comparison to the negative control; b = significant difference ($p< 0.05$) relative to the group treated with sodium arsenite (SA) alone.

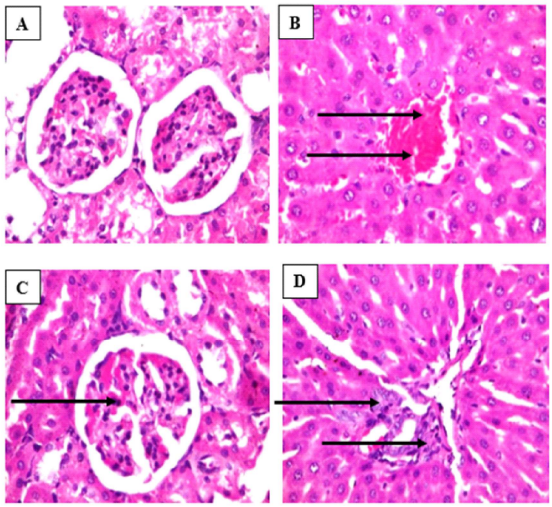
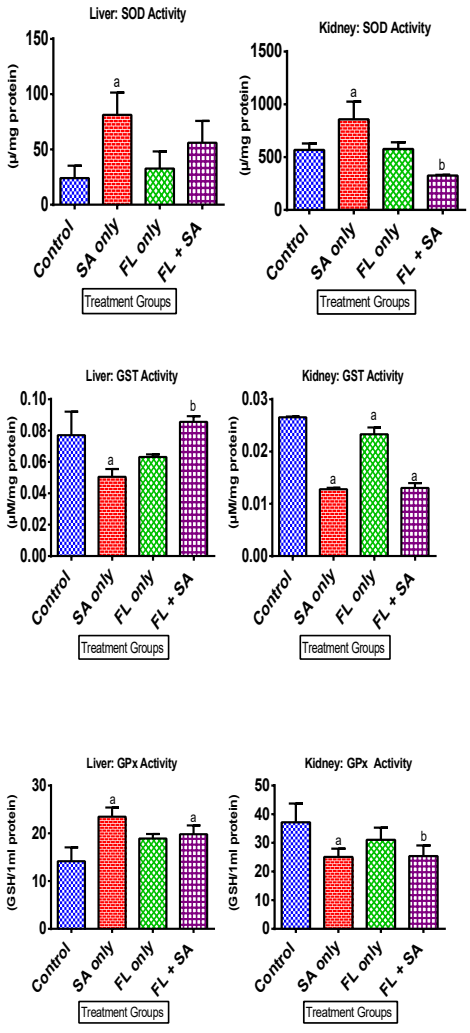
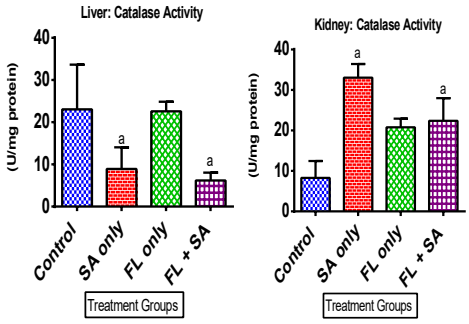


Figure VI: Photomicrographs of kidney sections from rats treated for 14 days with flavonoid complex and sodium arsenite (Mag X400). (A) Hepatocytes in normal condition. (B) SA alone (5 mg/kg body weight) demonstrates minor vascular congestion and periportal irritation. (C) Flavonoid (10mg/kg body weight) showing show moderate periportal inflammation (slender arrow). (D) FL + SA (10mg/kg and 5mg/kg body weight) prevented such degeneration in the renal cells.

Amelioration of the antioxidant biomarkers distorted by sodium arsenite via flavonoid complex treatment

The results in (Figure VII) show that there is a significant($p <0.05$) increase in liver SOD in the group treated with SA alone, compared to the control group. In addition, the SOD for the FL + SA group showed a decrease compared to the SA treatment group. The results show that the group treated with SA alone had a significant ($p <0.05$) increase in renal SOD compared to the control group. There was also a significant decrease when comparing only the FL + SA group with the SA group. Comparing the SA group with the control group, the results show that the liver GST activity in the SA group was significantly lower ($p <0.05$). In addition, the FL + SA group showed a significant increase compared to the SA group. Renal GST activity in the SA alone and FL + SA groups was significantly lower than in the control group ($p <0.05$), whereas renal GST activity in the FL group up was significantly higher than in the SA only group ($p <0. 05$) group (Figure VII).





The above results show that there is a significant ($p < 0.05$) increase in liver GPx activity in the SA alone and slightly increased the FL + SA groups though lower compared to the control group. There is a significant ($p < 0.05$) reduction in renal GPx activity in the SA alone and FL + SA groups compared to the control group. The above results show that there is a significant ($p < 0.05$) reduction in liver catalase activity in the SA and FL + SA groups compared to the control group. There is a significant increase in renal catalase activity in the SA and FL + SA groups compared to the control group.

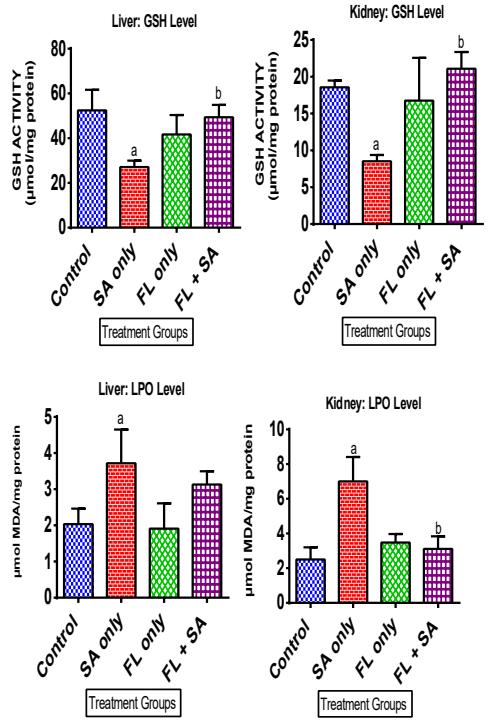


Figure VII: Flavonoid complex ameliorated sodium arsenite induction of distortion of enzymatic and nonenzymatic antioxidant in liver and kidney of treated rats. Each bar represents mean ± SD (n=6). *a* = significant difference ($p < 0.05$) relative to the negative control; b = significant difference ($p < 0.05$) in comparison to the group treated with sodium arsenite (SA) alone.

Comparing the SA-only and FL + SA-treated groups with the control, the results show that liver GSH in the SA-only was significantly lower but was increased in the FL +SA group. The results show a significant ($p < 0.05$) decrease in renal GSH in the SA treated group and an increase in the FL + SA treatment group compared to the control group. The results show that the SA group showed a significant increase in liver MDA activity compared to the control group, while the FL + SA group showed a significant decrease in liver MDA activity compared to the SA group. Compared to the control group, the SA group alone showed a significant ($p < 0.05$) increase in renal MDA activity. There was also a significant decrease in the FL + SA group compared to the SA group. In addition, a significant decrease was seen when comparing the FL + SA group to the SA-only group.

Flavonoid complex ameliorates the effects of sodium arsenite on inflammatory biomarkers

The results in (Figure VIII) show that liver NO in the SA group significant ($p < 0.05$) increased compared to the control group, whereas the FL + SA group showed a significant decrease compared to the SA only group. Renal NO activity in the SA-only group is significantly increased compared to the control group, while the FL + SA group is significantly

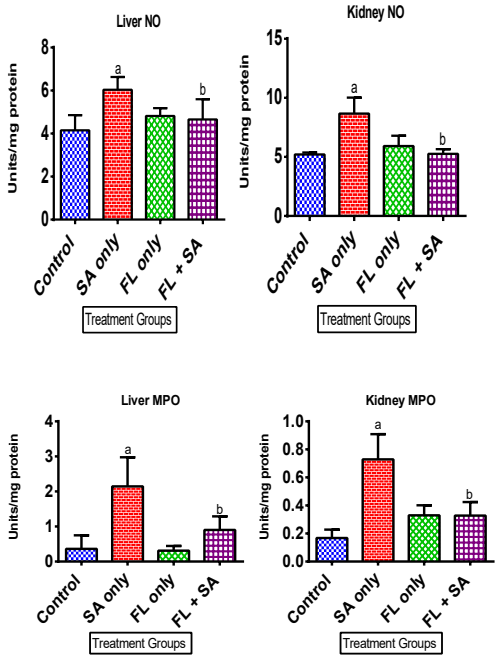
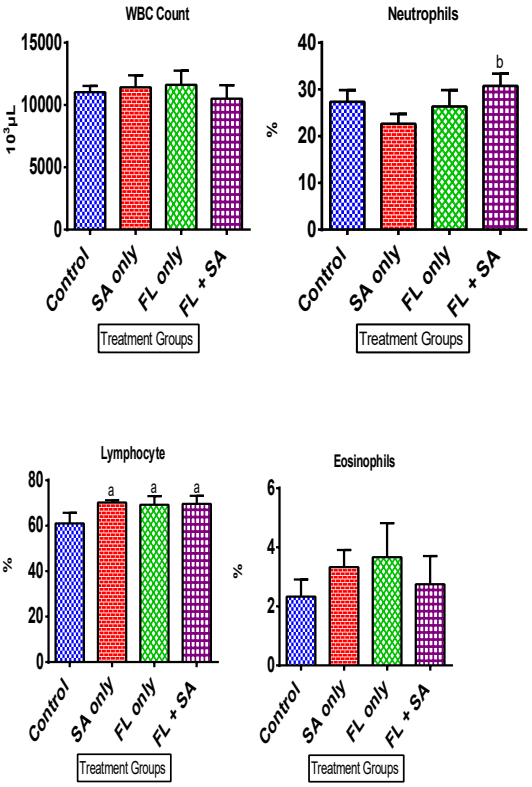


Figure VIII: Effects of sodium arsenite (SA) and/or flavonoid complex (Fl) on inflammatory biomarkers in rats' liver and kidney. Each bar indicates the mean standard deviation (n=6). a = significant change ($p < 0.05$) from the negative control; b = significant difference ($p < 0.05$) from the group given sodium arsenite (SA) alone.

decreased compared to the SA-only group. Patterns similar to the observation made with NO concentrations were observed with effect of flavonoid complex on SA treatment on liver MPO.

Flavonoid complex restores to normal levels of PCV, Hb and RBC

The results show that the PCV in the SA group was significantly ($p < 0.05$) lower than the PCV in the control group ($p < 0.05$), and the PCV in the FL only and FL + SA groups were significantly higher ($p < 0.05$). 0.05). The number of RBCs in the SA group is much lower than in the control group, and the number of RBCs in the FL + SA group is significantly higher than in the SA group. Comparing the SA-only, FL-only, and FL + SA groups with the control group, the results show a significant increase in lymphocyte counts in the SA-only, FL-only, and FL + SA groups ($p < 0.05$) when compared with the control. There was no significant ($p < 0.05$) difference in the number of eosinophils when comparing the experimental group with the control and SA groups (Figure IX).



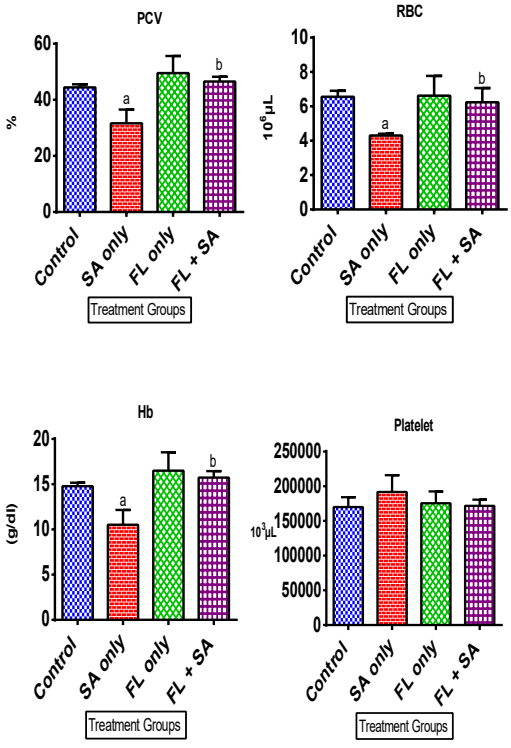


Figure IX: Effects of sodium arsenite (SA) and/or Flavonoid complex on hematological parameters in the treated rats. Data are presented as mean ± SD (n=6). *a* = significant difference ($p < 0.05$) relative to the negative control; b = significant difference ($p < 0.05$) in comparison to the group treated with SA alone.

Discussion

Increased exposure to environmental pollutants such as arsenicals, constitute a major threat to the survival of animals and humans in industrialized countries. Studies have shown that secondary phenolic metabolites such as flavonoids possess variety of pharmacological effects[32,33]. Exploration of functional foods and bioactive compounds have been considered to curtail multiple organ toxicity and liver cancers due to their potent therapeutic potential [34].

The results of this investigation revealed a substantial decrease in body weight in rats treated with sodium arsenite alone compared to the control group, as well as a significant rise in body weight change of rats pretreated with FL and then treated with SA compared to those treated with SA only.

Treatment with Flavonoid complex restored total protein levels in rats with sodium arsenite poisoning. Decreased serum protein levels may be the result of damage caused by sodium arsenate, especially protein oxidation by reactive oxygen species produced by arsenic poisoning [35].Protein

catabolism (breakdown) may increase as a result of probable oxidative stress, resulting in a decrease in protein levels.

The AST, ALP and ALT activities from the data indicates that there was an increase in AST, ALP and ALT activity in serum and liver of rats treated with SA alone (5 mg/kg) compared to controls. This effect may have been caused by oxidative stress induced by sodium arsenite, with hepatocyte membrane breakdown and hepatic transaminases leaking into extracellular spaces finally reaching the bloodstream from the liver [36].

However, when comparing the FL + SA pretreatment group to the SA only group (5mg/kg), the serum and liver activity of AST, ALP, and ALT were significantly lower in the FL + SA pretreated group. This suggests that flavonoid complex therapy protects hepatocytes from SA-induced cytotoxic damage. The antioxidant action of the flavonoid complex can be credited for these effects. This is consistent with the findings of Das and Sengupta [37], who recommend the use of antioxidant-rich foods and fruits, herbs and medicinal plants to treat arsenic poisoning.

The nephrotoxicity effects of drugs in animals and humans are studied using raised serum urea and creatinine concentrations [38]. Serum urea and creatinine levels in rats treated with SA (5 mg/kg) were significantly increased compared to those obtained in controls and decreased in FL+SA pretreated groups. There was also a significant decrease in FL+SA pretreated groups when compared to the values obtained in the SA only group. In addition, renal urea and creatinine levels were significantly higher in SA (5 mg/kg) treated rats compared to controls and significantly reduced in FL + SA pretreated groups compared to SA treated groups. A low clearance between creatinine and urea nitrogen indicates a diminished ability of the kidneys to filter waste products from the blood and excrete them into the urine. In other words, as the clearance value decreases, the blood levels of creatinine and urea and nitrogen rise.

Arsenic-induced lipid peroxidation in the liver is thought to be the outcome of arsenic-induced increased microsomal oxidation capacity. SA group only compared to control group has a substantial drop in liver and kidney GSH levels however, flavonoid complex substantially improves the level of GSH following exposure to sodium arsenite. This is as a result of the antioxidant and free radical scavenging properties of the flavonoids and similar trends was seen in our study on riboceine antioxidant property on sodium arsenite induced hepatorenal toxicity [39].

Furthermore, although GST and CAT activity in a single group were lower than the control group, GST and CAT activity in the FL + SA group were significantly higher than SA group. In the liver of SA-only treatment group, compared with the control group, the decrease in FL + SA group was reduced compared to the SA group, and there was no significant increase in kidney SOD activity compared to the control group. The FL + SA group showed a significant decrease compared to the SA group. Ramanathan et al. [40] Study showed that changes in the characteristics of oxidative stress are caused by overwork disorders of the antioxidant defense system secondary to the formation of reactive oxygen species. When it comes to the defense system against oxidative damage or stress, the employment of endogenous antioxidants has become more crucial.

The chain of reactions that mediate free radical and once it is initiated it results in an oxidative deterioration of polyunsaturated lipids which also causes oxidative degradation of lipids called lipid peroxidation. It involve the process by which free radicals take electrons from the lipids in cell membranes which leads to cell damage [9]. This process is proceeded by a free radical chain reaction mechanism of which malondialdehyde the end products of the LPO is mutagenic and carcinogenic[41]. In the liver and kidney cells, the protective potential of flavonoid complex was found, with a substantial rise in the MDA activity. Significant reduction in MDA activity was seen in the FL + SA group compared to the SA group.

NO is a signal transduction molecule that plays an important role in the pathophysiology of inflammation and other diseases. It also has an anti-inflammatory impact under normal physiological conditions, but it is termed a pro-inflammatory mediator when it causes inflammation owing to overproduction in abnormal situations [42]. When compared to the control group, the SA only group's liver and kidney NO levels increased, but the FL + SA group's NO activity decreased significantly.The significant increase in NO levels in the liver and kidney tissues of rats treated with SA in this study could lead to nitrosative stress and tissue damage via peroxynitrite production [43]. In the production of hepatic and renal damage by the toxicants which might be linked to the deleterious effects heavy metals like arsenicals have on the organ biological activities.

Pretreatment of rats with flavonoid complexes significantly increased the number of neutrophils in the FL + SA group compared to the SA group and lymph in the SA, FL and FL + SA groups compared to the rats in the control group. There were no significant changes in the WBC count, eosinophil and platelet counts in the experimental group compared to the control and SA groups, but the PCV and RBC concentrations after exposure to sodium arsenite were compared to the control group, decreased significantly and increased in FL + SA group compared to SA group. The suppression of porphyrin or heme production causes a decrease in the amount of PCV and RBC counts. The activity of aminolevulinic acid dehydratase is known to be inhibited by arsenic, changing the heme production route [44].

Recent studies have shown protective effect of specific flavonoid or flavonoid complexes. Abbasi *et al*. [45] showed the neuroprotective effects of Vitexin, on seizure induced by pentylenetetrazole in rats. Krylova *et al*. [46] also showed the gastroprotective effects of flavonoid complex from *Lychnischalcedonica.* Also, Omer *et al.*[47] showed that Quercetin protects against streptozocin-induced oxidative stress in rat's pancreas. Hence, to the best of our knowledge we are reporting the protective effects of flavonoid complex on hepatorenal toxicities induced by Sodium arsenite in Wistar rats.

Conclusion

The findings from this study revealed that sodium arsenite altered the activities of antioxidant enzymes in plasma and was also capable of inducing significant changes in some biochemical parameters, with the protective effect of flavonoid complex seen on hematological, serum biochemical, and histological parameters in Wistar rats. The regulation of natural antioxidant enzymes, as well as the suppression of oxido-inflammatory mediators like MDA and NO, may have a protective effect. On the basis of this study, the protective effect of flavonoid complex may be useful in the prevention of sodium arsenite toxicity which induces liver and kidney diseases.

Summary

- The results of this study showed that sodium arsenite may significantly impact various biochemical parameters and changed the plasma's antioxidant enzyme activity.
- A protective effect could be produced by controlling naturally occurring antioxidant enzymes and suppressing oxido-inflammatory mediators like MDA and NO.
- The current work shows that flavonoid complex protects Wistar rats from hepatorenal toxicities brought on by sodium arsenite.
- The preventive impact of flavonoid complex may be helpful in preventing sodium arsenite poisoning, which causes liver and renal problems, based on the findings of this study.

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