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PREVENTIVE ROLE OF *Ficus exasperata* N-HEXANE: ETHYL ACETATE (85:15 v/v) FRACTION AGAINST POTASSIUM DICHROMATE INDUCED OXIDATIVE STRESS IN FEMALE WISTAR RATS

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ABSTRACT

Humans and animals exposed to potassium dichromate develop oxidative stress-associated diseases. Given *Ficus exasperata*'s medicinal properties, this study investigated how *Ficus exasperata*'s n-hexane: ethyl acetate fraction (FR2) can prevent potassium dichromate-induced oxidative stress in female Wistar rats. The study assessed oxidative stress indicators (malondialdehyde (MDA), total antioxidant capacity (TAC) aconitase (ACON) and non-enzymatic antioxidants (total thiols (TSH), vitamin C (Vit C), and vitamin E (Vit E). Thirty female Wistar rats, divided into six groups (n=5), were used for this study. Group A (control) and B were administered olive oil (2 ml/kg b.wt (body weight)) for fourteen days. For an exact number of days, group C (Standard) was administered vitamin E (100 mg/kg b.wt) and omega 3 (72 mg/kg b.wt eicosapentaenoic acid+ 48 mg/kg b.wt docosahexaenoic acid). In comparison, groups D (30 mg/kg b.wt), E (60 mg/kg b.wt), and F (60 mg/kg b.wt) were administered *Ficus exasperata* n-hexane: ethyl acetate fraction (FR2). The experiment lasted for fourteen days, and potassium dichromate (100 mg/kg b.wt) was administered on the fourteenth day to rats in groups B, C, D, and E. Groups D and E pre-treated with FR2 showed the protective role of *Ficus exasperata* n-hexane: ethyl acetate fraction against high levels of serum ACON and MDA with concomitant low levels of T.SH, Vit C, Vit E, TAC and tissues (heart, liver, and kidney) ACON when compared to group B (Potassium dichromate intoxicated). This research revealed that the n-hexane: ethyl acetate fraction of *Ficus exasperata* leaf extract protects female Wistar rats against potassium dichromate-induced oxidative stress.

Keywords: Potassium dichromate, Oxidative stress, *Ficus exasperata*, Intoxication.

INTRODUCTION

Various studies have shown that exposure to specific heavy metals through environmental pollution can lead to toxic and carcinogenic effects in humans and animals. Chromium, for instance, has been found to generate an excess of reactive species (RS) that can cause lipid peroxidation, protein depletion, and other harmful effects (Patlolla *et al.*, 2009; Ejoh *et al.*, 2021; Murthy *et al.*, 2023). Chromium (Cr) is a naturally found heavy metal that is widely pre-

sent in the environment in two valence states: trivalent Cr (III) and hexavalent Cr (VI) (Patlolla *et al.*, 2009; Murthy *et al.*, 2023). Commercial chromium compounds are utilized in industrial processes such as welding, metal finishing, leather tanning and wood preservation. They constitute a significant environmental pollutant on a global scale (Murthy *et al.*, 2023). Potassium dichromate ($K_2Cr_2O_7$), a type of hexavalent chromium, has been shown to cause toxicity related to oxidative stress in humans and animals. It enters cells rapidly by utilizing the sulfate anion transport system in the cell membrane, where it is then reduced to lower oxidation states. This results in its accumulation in different organs, leading to the generation of reactive species (RS) and organ damage (Ejoh *et al.*, 2021; Murthy *et al.*, 2023).

Ficus exasperata (Vahl) is a terrestrial shrub or tree native to Africa, particularly Nigeria, valued for its medicinal properties. This plant can reach a height of 20 meters and thrives in settings like evergreen and secondary woods. The plant is often known as sandpaper leaf and is called "Epin," "Anwerenwa," and "Kawusa" by the Yorubas, Igbos, and Hausas in Nigeria, respectively (Olaoluwa *et al.*, 2022; Akinloye and Ugbaja, 2022). Previous research has shown that the leaf extract of *F. exasperata* has various beneficial properties, including anti-inflammatory, antipyretic, analgesic, anti-ulcer, antioxidant, anticonvulsant, antimicrobial, anti-arthritic, and antinociceptive effects (Oyetayo *et al.*, 2018; Agunloye and Oboh, 2018; Adekeye *et al.*, 2020; Akinloye and Ugbaja, 2022;Akinloye *et al.*, 2023).

Interest in medicinal plants as antioxidants has increased due to concerns about the side effects of synthetic antioxidants used to treat or prevent xenobiotic intoxication. These interest researches aim to explore the therapeutic potential of different medicinal plants in reducing or preventing adverse effects from different xenobiotic intoxication (Akinloye and Ugbaja, 2022; Akinloye *et al.*, 2023; Abd El Rahman *et al.*, 2023). Akinloye and Ugbaja (2022) and Akinloye *et al.* (2023) have documented the nutritional benefits of *Ficus exasperata* leaves and their role in combating oxidative stress and inflammation. Additional information is required regarding the advantageous impact of *Ficus exasperata* leaves in mitigating and preventing the negative consequences of environmental pollution. This study evaluated the preventative effects of the *F. exasperata* n-hexane: ethyl acetate fraction on potassium dichromate-induced oxidative stress in female Wistar rats. Indices such as vitamin C (Vit C), vitamin E (Vit E), total thiols (TSH), total antioxidant capacity (TAC), malondialdehyde (MDA), and aconitase (ACON) activity were measured.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents were obtained from Sigma-Aldrich Chemical (Missouri, USA) in analytical and pure grades.

Preparation of *Ficus exasperata* N-hexane: ethylacetate fraction (FR2)

Fresh leaves of *Ficus exasperata* were collected from the botanical garden of the Department of Pure and Applied Botany, Federal University of Agriculture Abeokuta, Nigeria, with identification and authentication herbarium number FUNAABH0039. The leaves were washed, air-dried to constant weight, and milled using a mechanical grinder. To 100 g of milled sample, 500 ml of 85:15 (v/v) n-hexane: ethylacetate was used for the extraction with the aid of a Soxhlet extractor

for 24 hours. The filtrate was therafter concentrated using a Rotary evaporator at 60°C to get the crude extract. The crude extract was fractionated into fractions on a silica gel -packed column. A non-polar solvent (n-hexane) was used to elute the first fraction of the extract. Then, semi-polar (85% n-hexane + 15 % ethylacetate) solvent was added in drops to elute the second fraction (FR2), which was further concentrated and used for the study.

Experimental Animals

Before starting the experiment, ethical clearance FUNAAB - BCH- DI 023 was obtained from the Departmental Animal Ethical Committee (FUNAAB- BCH). The committee's authorized protocols and standards were followed when running the studies. Fifty (50) female Wistar rats weighing 60 g - 100 g were purchased from animal farms. The animals were acclimatized for four weeks.

Pilot study and an acute toxicity test (LD^{50} (lethal dose)

Following the report of Arivarasu *et al.* (2012), a pilot study was carried out to ascertain the dose of potassium dichromate that would induce toxicity and not cause mortality in female rats (a dose that will provide toxicity without lethality). This study was divided into three stages, each determining the next step (whether to terminate or proceed to the next stage). Three rats were used per stage and received 60, 75, and 100 mg/kg of body weight of potassium dichromate. All animals were observed continuously for four hours and thereafter for twenty-four and forty-eight hours for any behavioral changes; and the number of survivors noted. At the end of the study, when no mortality occurred, 100 mg/kg of body weight of potassium dichromate was concluded to be used for induction of oxidative stress.

An acute toxicity test for *Ficus exasperata* n-hexane: ethylacetate fraction (FR2) was carried out according to the method described and modified by Chinedu *et al.* (2013). Nine rats divided into three groups (n = 3) were subjected to three stages of experimental protocol, one after the other. The outcome from each stage determined whether to terminate or proceed to the next stage. In the first stage, 500 mg/kg body weight of FR2 was administered orally, and when no sign of toxicity or death was seen, 1000 mg/kg body weight was given orally to another 3 rats. Then, the dose was increased to 2000 mg/kg body weight extract when no death or sign of toxicity was observed in the second stage. At the end of the final stage, when no sign of toxicity or death was observed, it was concluded that the LD^{50} (lethal dose) of FR2 was more than 2000 mg/kg body weight.

Experimental Animals and Design

After acclimatization, pilot study (100 mg/kilogram body weight was chosen) and LD^{50} (lethal dose) test (more than 2000 mg/kg body weight), the remaining rats were divided into six groups of five rats each (n=5) for the experiment (Table 1).

Table 1: Experimental design

Groups (n = 5)	Pre-treatment for 14 days	Potassium dichromate Single dose intoxication
Olive oil	2mL/kg b.wt olive oil	2mL/kg b.wt distilled water
$K_2Cr_2O_7$	2mL/kg b.wt olive oil	2mL/kg b.wt $K_2Cr_2O_7$
Standard + $K_2Cr_2O_7$	2mL/kg b.wt standard	2mL/kg b.wt $K_2Cr_2O_7$
FR2 30mg/kg b.wt + $K_2Cr_2O_7$	2mL/kg b.wt FR2	2mL/kg b.wt $K_2Cr_2O_7$
FR2 60mg/kg b.wt + $K_2Cr_2O_7$	2mL/kg b.wt FR2	2mLl/kg b.wt $K_2Cr_2O_7$
FR2 60mg/kg b.wt	2mL/kg b.wt FR2	2mLl/kg b.wt distilled water

kg b.wt = kilogram body weight, standard = standard drug consisting of 100mg Vitamin E and Omega-3 essential fatty acid (72 mg Eicosapentaenoic acid (EPA) + 48 mg Docosahexaenoic acid (DHA).

All treatments were given by oral gavage for 14 days while the potassium dichromate was administered an hour after the last day of treatment. The animals were fasted overnight prior to the day of sacrifice. Blood samples were collected via retro-orbital puncture using a capillary tube into clean plain tubes to get the serum. Then, the liver, kidney, and heart harvested were homogenized (10 % homogenate) and used for biochemical analysis.

Biochemical assay

The levels of vitamin C (Jagota and Dani, 1982), vitamin E (Prieto *et al.*, 1999), total thiols (Ellman, 1959), total antioxidant capacity (Prieto *et al.*, 1999), malondialdehyde (Fernandez *et al.*, 1997) and aconitase activities (Racker, 1950) were assayed under standard protocols.

Statistical analysis

Data generated were expressed as mean ± standard error of the mean (SEM). One-way Analysis of Variance (ANOVA) was used to analyze the means followed by Duncan's test, where homogeneity occurred. Statistical Package for Social Science (SPSS 20.0) for Windows was used for the analysis; values with $p<0.05$ were regarded as being statistically significant.

RESULTS

All animals remained stable during the pilot research, and there was no mortality within 24 hours after the tests. No signs of toxicity or changes in rats were observed and there was no mortality at any of the tested doses (500 mg, 1000 mg, and 2000 mg/kg body weight of FR2) after 24 h of observation. Thus, the LD^{50} (lethal dose) of FR2 was more than 2000 mg/kg body weight.

Biochemical effects of *F. exasperata* FR2 on serum, heart, liver, and kidney vitamin C (Vit C) and E (Vit E)

Pretreatment with *Ficus exasperata* n-hexane: ethyl acetate fraction (FR2) significantly prevented depletion of vitamin C in the serum, liver, heart, and kidney when compared respectively with control (olive oil) and dichromate ($K_2Cr_2O_7$) intoxicated group (Figure 1). The protective effects of *F. exasperata* (FR2) against depletion of vitamin C in the liver, heart, and kidney by dichromate-induced oxidative stress depend on the fraction doses. F. exasperata's (FR2) protective role against depletion of vitamin E by dichro-

mate administration showed the same effect despite the dose difference (Figure 2). The levels of respective liver, heart, and kidney vitamin C and E in the group given 60 mg/ kg body weight without dichromate (FR2) showed no significant difference when compared with control, with olive oil (Figures 1 and 2).

Biochemical effects of *F. exasperata* FR2 on serum, heart, liver, and kidney total thiols (TSH) and aconitase (ACON)

Administration of *F. exasperata* (FR2) before dichromate ($K_2Cr_2O_7$) induction of oxidative stress was able to prevent the depletion of serum, liver, heart, and kidney total thiols (TSH) levels in a dose-dependent manner when compared with group B treated with $K_2Cr_2O_7$ (Figure 3). Despite the dose difference, *F. exasperata* (FR2) protection against perturbation of aconitase activities by dichromate administration was observed to be non-dose dependent (Figure 4). The levels of respective liver, heart, and kidney TSH of the group given 60mg/ kg body weight without dichromate (FR2) showed no significant difference when compared with control (olive oil). No significant difference was observed in the liver and kidney ACON of the group given 60mg/ kg body weight (FR2) without dichromate compared with the control group.

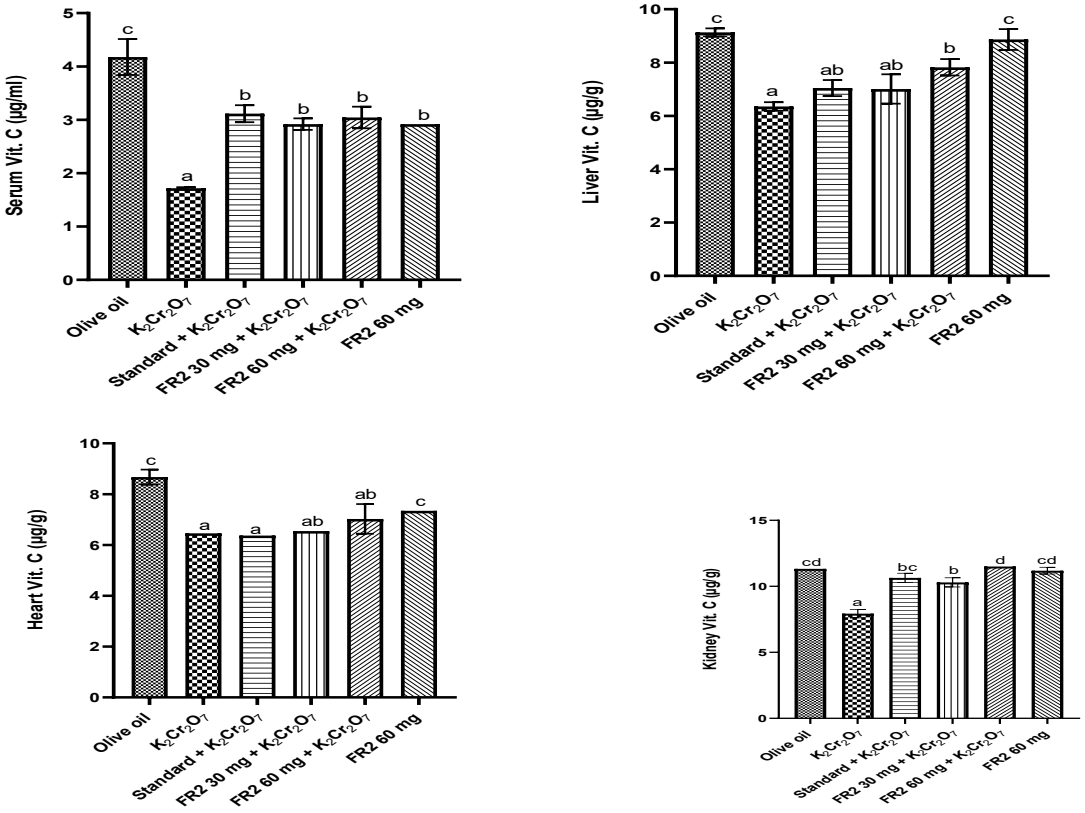


Figure 1: Effects of *F. exasperata* n-hexane: ethyl acetate fraction (FR2) on serum, liver, heart and kidney vitamin C.

Bars represent mean ± SEM (n=5). Bars with different letters are significantly different at $p < 0.05$.

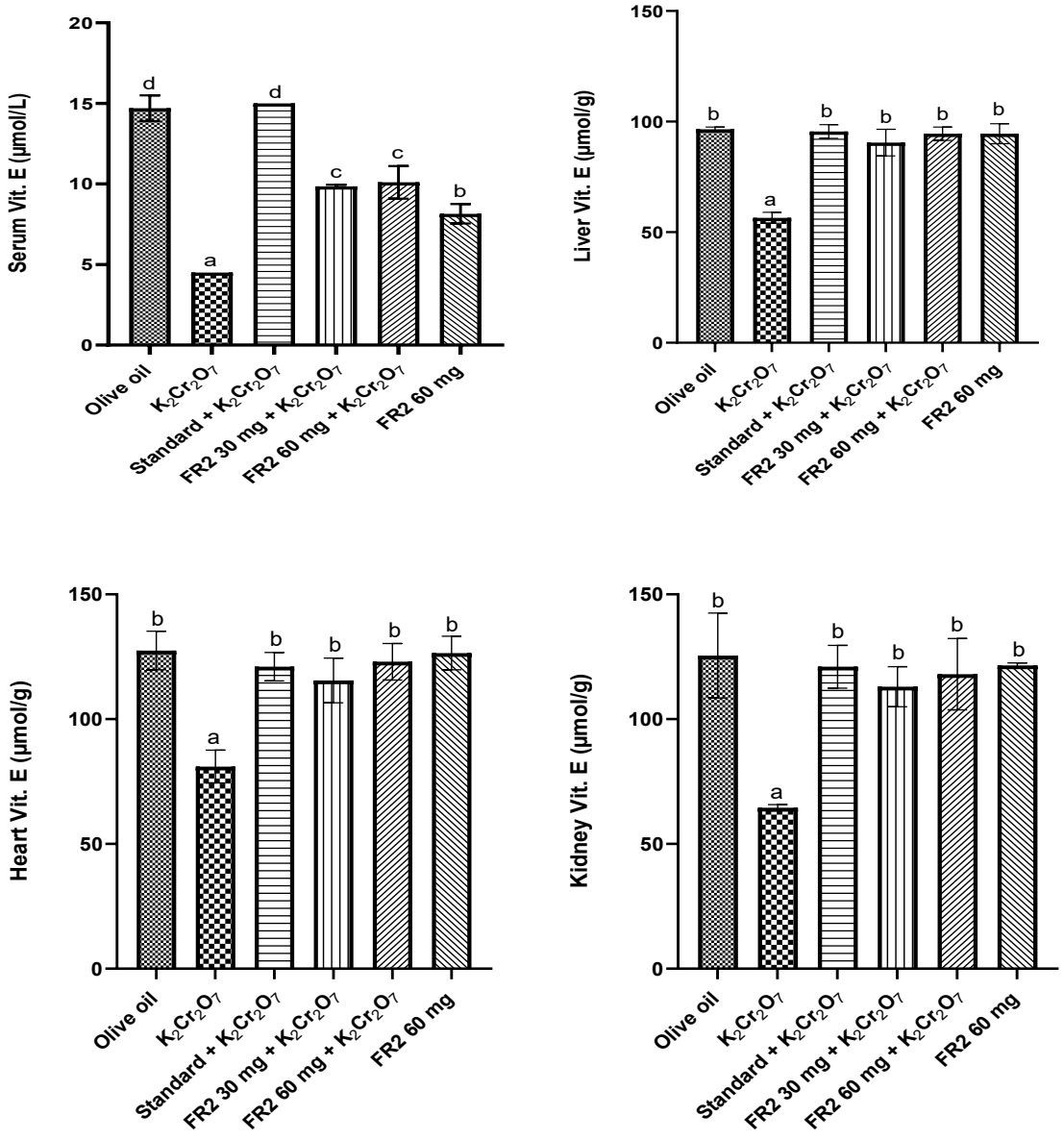


Figure 2: Effects of *F. exasperata* n-hexane: ethyl acetate fraction (FR2) **on serum, liver, heart and kidney vitamin E.**

Bars represent mean ± SEM (n=5). Bars with different letters are significantly different at $p < 0.05$.

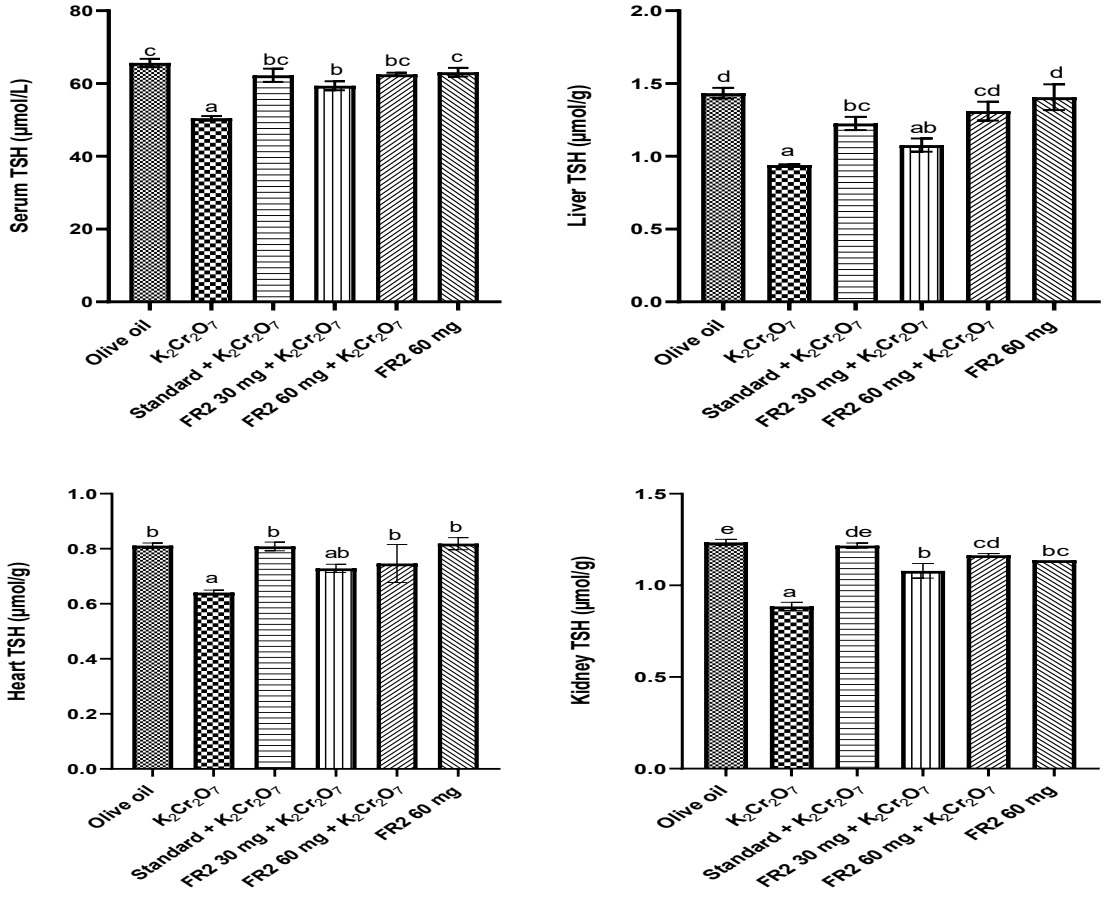
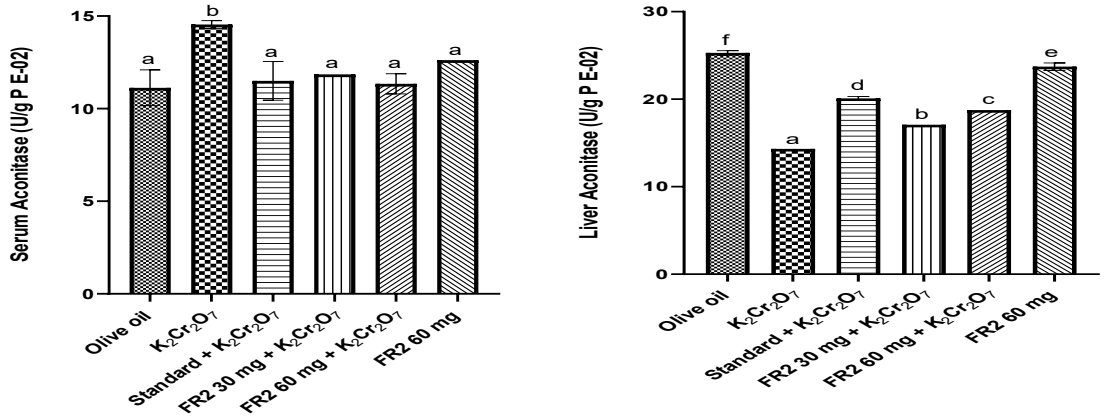


Figure 3: Effects of *F. exasperata* n-hexane: ethyl acetate fraction (FR2) **on serum, liver, heart and kidney TSH.**

Bars represent mean ± SEM (n=5). Bars with different letters are significantly different at $p < 0.05$.



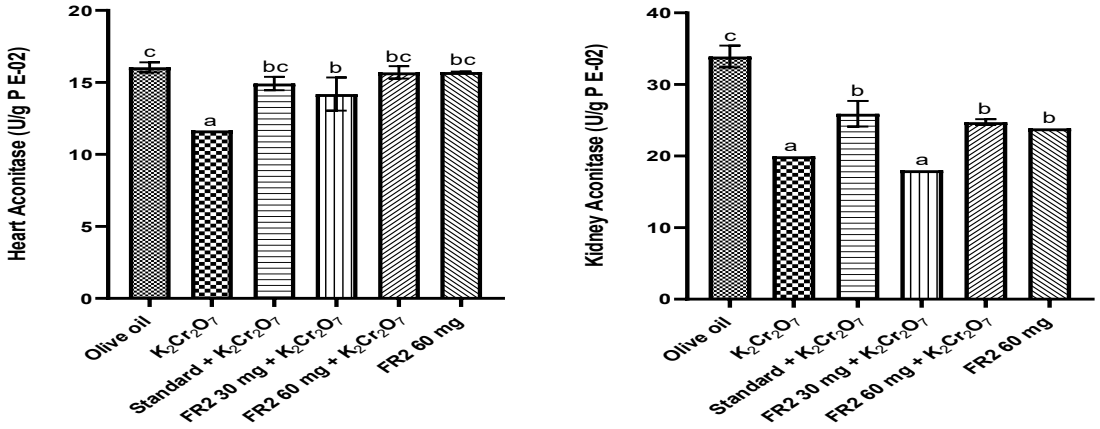


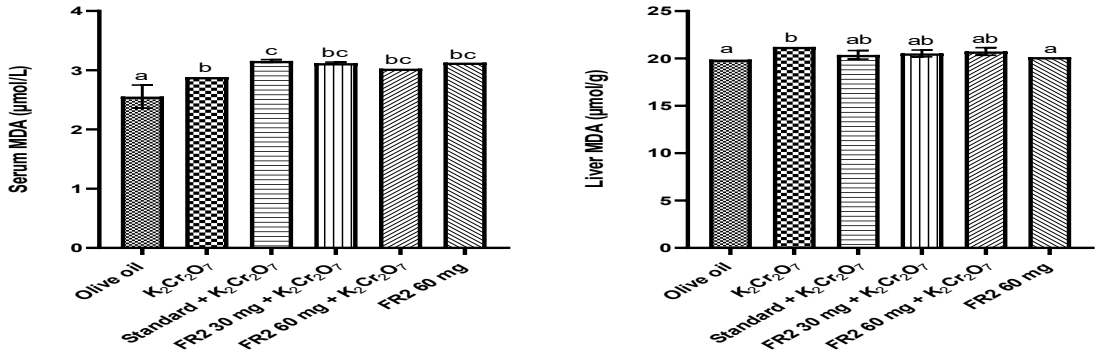
Figure 4: Effects of *F. exasperata* FR2 on serum, liver, heart and kidney ACON. Bars represent mean ± SEM (n=5). Bars with different letters are significantly different at $p < 0.05$.

Biochemical effects of *F. exasperata* FR2 on serum, heart, liver, and kidney malondialdehyde (MDA)

Pre-treatment with the n-hexane: ethyl acetate fraction (FR2) of *Ficus exasperata* considerably showed reduced levels of elevated MDA in the liver and heart, with no impact on MDA levels in the serum and kidneys, compared to the control (olive oil) and dichromate ($K_2Cr_2O_7$) intoxicated group (Figure 5). The group treated with FR2 at a dose of 60 mg/kg body weight (F) did not exhibit a statistically significant difference in liver and heart MDA levels compared to the control group treated with olive oil.

Biochemical effects of *F. exasperata* FR2 on serum, heart, liver, and kidney total antioxidant capacity (TAC):

A single dose of potassium dichromate ($K_2Cr_2O_7$) reduced total antioxidant capacity compared to the control (olive oil), suggesting the development of dichromate-induced oxidative stress (Figure 6). Previous administrations of varying dosages of *F. exasperata* FR2 successfully prevented oxidative damage produced by dichromate. The blood, liver, heart, and kidney TAC levels in the group administered *F. exasperata* FR2 at a dose of 60mg/kg body weight did not exhibit a statistically significant difference compared to the control group treated with olive oil (Figure 6).



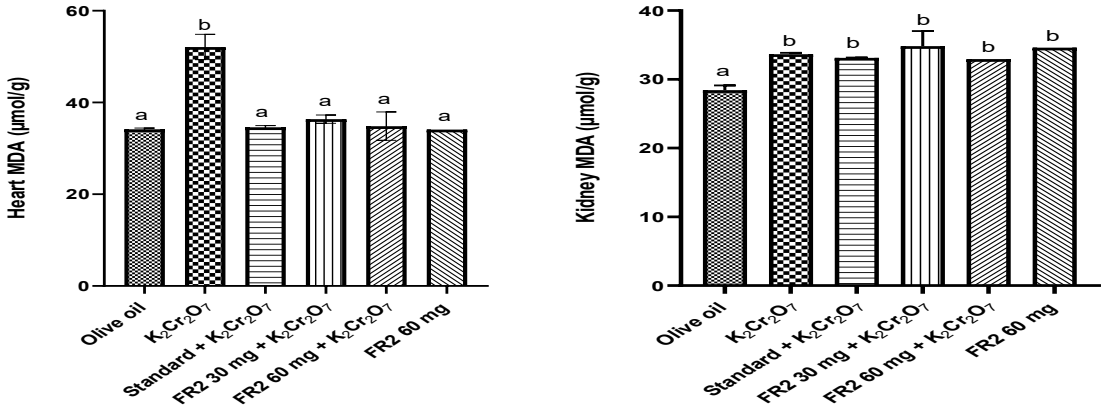


Figure 5: Effects of *F. exasperata* FR2 on serum, liver, heart and kidney MDA.
Bars represent mean ± SEM (n=5). Bars with different letters are significantly different at $p < 0.05$.

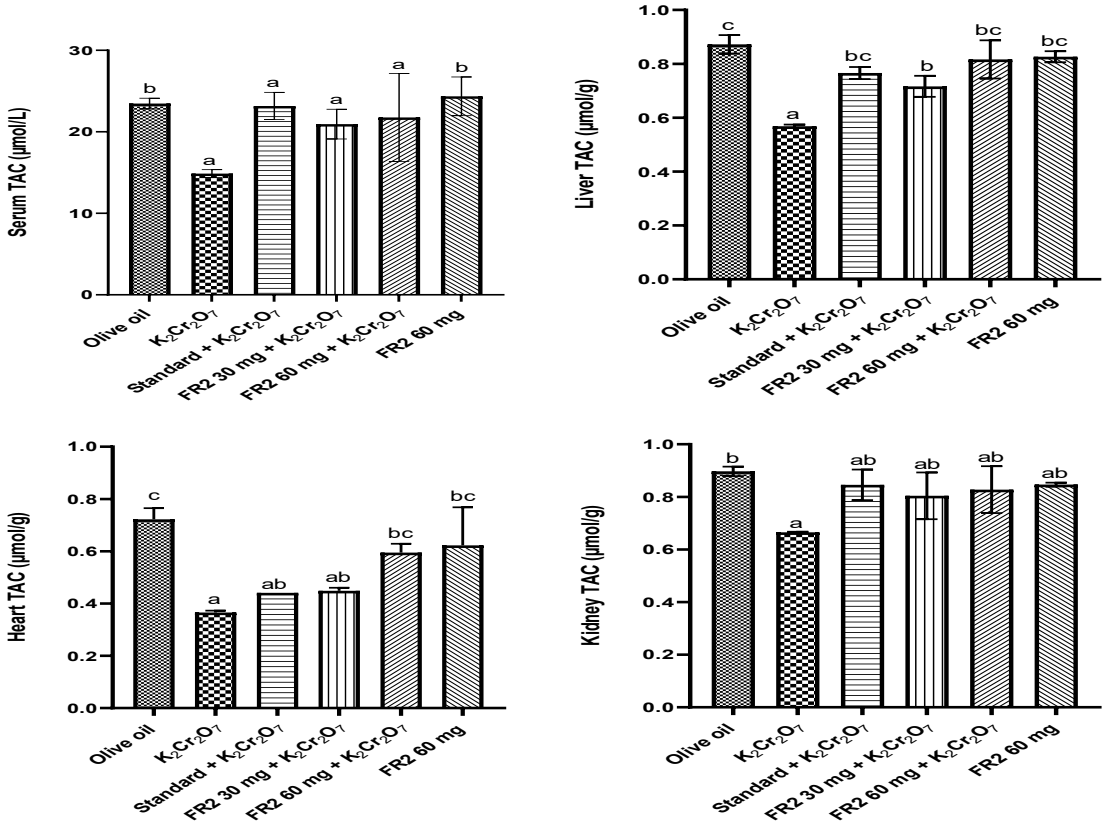


Figure 6: Effects of *F. exasperata* FR2 on serum, liver, heart and kidney TAC.
Bars represent mean ± SEM (n=5). Bars with different letters are significantly different at $p < 0.05$.

DISCUSSION

Excessive production of reactive species (RS) can negatively impact biological components, including proteins, lipids, and DNA, leading to oxidative stress and potential bodily harm. Reactive species (RS) at low to moderate concentrations are crucial for some physiological processes in animals (Akinloye *et al*., 2022). According to Murthy *et al.* (2023), the rise in the use of dichromate in industrial settings has resulted in the substance becoming a widespread environmental threat. Several studies have demonstrated that dichromate-induced oxidative stress leads to the generation of reactive species (RS) that are not under control, which in turn leads to a reduction in mitochondrial function and an irreversible suppression of aconitase activity (Myers *et al*., 2010; Abd El-Rahman *et al*., 2023). According to Myers *et al.* (2010), aconitase is considered an adequate measure of mitochondrial and cellular oxidative stress. Because they are highly reactive, these excessive RS creations further affect the physiological and biochemical features, ultimately damaging a variety of cell membranes and structures (Myers *et al*., 2010; Akinloye *et al*., 2022; 2023; Abd El-Rahman *et al*., 2023). Akinloye and Ugbaja (2022), Akinloye *et al.* (2023), and Abd El-Rahman *et al.* (2023) have demonstrated that the use of natural antioxidant supplements can facilitate the protection of body cells against the detrimental effects that are caused by the excessive formation of reactive substances (RS). The only alternative to prevent illnesses related to excessive RS generation is to use antioxidant molecules that come from natural sources. Therefore, any medicinal plant that exhibits antioxidant activity through RS scavenging property has the potential to prevent or block cellular disruption or damage that is associated with excessive RS formation (Agunloye and Oboh, 2018; Akinloye and Ugbaja, 2022). It has been discovered that the leaves of *F. exasperata* contain phenolic compounds. These compounds, which include phenolic hydroxyl groups, are highly efficient in eliminating reactive species (Agunloye and Oboh, 2018; Akinloye and Ugbaja, 2022; Akinloye *et al*., 2023).

The in-*vitro* antioxidant potential of *F. exasperata* aqueous leaf extracts was reported by Akinloye and Ugbaja (2022). They found that the extracts were able to scavenge cation radicals such as DPPH (1, 1-diphenyl-2-picrylhydrazyl) and ABTS (2, 2'-azino-bis-3-ethyl benzothiazole-6-sulfonic acid). Additionally, the extract showed an increase in its total antioxidant capacity (TAC) in a concentration-dependent manner. According to Akinloye and Ugbaja (2022), it has been determined that the leaf extract of *F. exasperata* is reasonably safe and, as a result, has the potential to serve as a nutritional source that can contribute to improving human health. It was also suggested by Akinloye *et al.* (2023) that the biochemical changes that were associated with the alcohol-induced cellular disturbance of the endogenous antioxidant defense system could be reversed by this extract. The authors suggested that the natural phytochemical constituents in the *F. exasperata* aqueous leaves extract played both direct and synergistic protective roles in maintaining the cellular redox state by scavenging reactive species. This study was conducted to provide evidence supporting the idea that the n-hexane: ethyl acetate fraction (FR2) of *Ficus exasperata* might protect against potassium dichromate-indued oxidative stress in female Wistar rats.

Malondialdehyde, often known as MDA, is a crucial lipid peroxidation indicator because it is the principal oxidation product of perox-

idized polyunsaturated fatty acids. When MDA levels are increased, it causes damage to physiologically significant molecules and tissues (Abd El-Rahman *et al*., 2023). Previous reports have demonstrated that the measurement of MDA levels using different methods of thiobarbituric acid-reactive substances (TBARS) lacks selectivity, which in turn limits its usefulness in clinical studies (Altomare *et al*., 2021; Akinloye *et al*., 2022). The inconsistent results observed in serum and kidney MDA levels support these reports. According to Ellman (1959) and Akinloye *et al*. (2023), total thiols (T.SH) are made up of both intracellular and extracellular thiols. These thiols can be found in the free form as reduced glutathione, or they can be those attached to proteins. It was believed that their position within the bodily system was that of antioxidant radical scavengers that provided protection (Akinloye *et al* 2023). According to Akinloye *et al*. (2023), an imbalance between thiol and disulfide might indicate oxidative damage in the entire body system. As an effective radical scavenger, vitamin E is crucial in shielding membranes from excessive RS assault. Vitamin C, on the other hand, acts in conjunction with vitamin E to combat several forms of RS-induced damage (Akinloye *et al*., 2023). This investigation showed that exposure to a single dosage of potassium dichromate poisoning resulted in a substantial decrease in aconitase activities and total antioxidant capacity, vitamins (E and C), and total thiol levels. Additionally, there was an increase in MDA levels and serum aconitase activity. These findings imply that the membrane peroxidation rate is a consequence of the activities of reactive species that are not under control. Despite this, a pre-treatment with *Ficus exasperata* leaves n-hexane: ethyl acetate fraction (FR2) was able to protect against the changes brought about by potassium dichromate intoxication. These protective benefits might result from synergistic activities of the bioactive substance in *F. exasperata* leaves n-hexane: ethyl acetate fraction (FR2) in scavenging excess reactive species created by potassium dichromate intoxications.

CONCLUSION

This study was conducted to provide evidence supporting the idea that the n-hexane: ethyl acetate fraction (FR2) of *Ficus exasperata* might protect against potassium dichromate-indued oxidative stress in female Wistar rats. According to this study, oxidative stress (ACON and MDA) and antioxidant (vit C, vit E, TSH, and TAC) indices are perturbed due to the excessive RS production by dichromate intoxication. However, the adverse effects were prevented by the two doses of *F. exasperata* FR2. Each dose could scavenge excess RS by demonstrating protective roles against dichromate-induced oxidative stress through its antioxidant properties. Thus, the antioxidant characteristics of *F. exasperata* leaves are responsible for their beneficial benefits, including protecting rats from oxidative stress ensued potassium dichromate intoxication.

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