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Research Article

Ficus Racemosa Extract and Vitamin C Ameliorate Paraquat-Induced Oxidative Stress in Wistar Rats

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Abstract

Ficus racemosa is an important medicinal plant found ubiquitously. The immunomodulatory potential of this plant has not been delved much. In the present study, we evaluated the *in vivo* antioxidant potential of *Ficus racemosa* in paraquat-induced oxidative stress in Wistar rats. The ethanolic extract of the leaves of *F. racemosa* was used for the study. For the *in vivo* study, 5 groups of 6 rats per group, including paraquat-induced oxidative stress were evaluated for responses in a 30-day experimental trial using doses of 250 mg/kg b wt. for *F. racemosa* extract. Oxidative stress induced by Paraquat caused an increase in ROS, caused severe liver damage, increased lipid peroxidation but not many hematological alterations. Group using *F. racemosa* extract and combination of Ficus extract and vitamin C showed significantly proficient results by decreasing the LPO production and helped in recovering the liver alterations.

However, further research is needed to identify the different molecular mechanisms involved in mediating the antioxidant response.

Keywords: Ficus Extract; Antioxidant; Lipid Peroxidation; Superoxide Dismutase

Introduction

Oxidative injury and inflammation have been regarded as the primary cause of the majority of chronic diseases in animals and humans. The state of oxidative stress that occurs in various disorders is a result of an imbalance in the pro-oxidants and antioxidants inside the body [1]. Herbal antioxidants are attracting present-day researchers and clinicians due to their remarkable ability to provide ancillary therapy in various infectious and metabolic disorders. Moreover, they are drawing the attention of consumers due to better safety concerns [2].

Ficus racemosa, commonly described as the cluster fig tree has been used around the globe as a medicinal agent in traditional practices like Ayurveda and Unani medicine for a long time [3]. Most of the parts of this plant are known to exhibit therapeutic effectiveness in various disease conditions, including diabetes mellitus, diarrhoea, inflammation, pyrexia, fungal infections, bacterial diseases, hyperlipidemia, filariasis, and hepatic disorders [4]. The leaves of this plant possess significant amounts of flavonoids, triterpenoids, alkaloids, and tannins that are known to exhibit potent antioxidant properties.

Vitamin C or ascorbic acid is a naturally occurring water-soluble vitamin, having exceptional antioxidant properties. It is actively involved as a cofactor in the essential enzymatic reactions that synthesize important metabolites [5]. Various studies have already proved the protective effect of Vitamin C against oxidative stress in both in-vitro and in-vivo experiments [6,7]. Besides, it has demonstrated to be a potential agent in alleviating paraquat-induced toxicity both in animal and human studies [8].

Paraquat is a non-selective and toxic bipyridyl compound used as a contact herbicide in agriculture practice [9]. Accidental poisoning with paraquat is common both in humans and farm animals. It exhibits a toxic effect on non-target mammalian species by inducing oxidative injury in the susceptible organs [10]. The lung seems to be the primary organ target for paraquat toxicity, while secondary effects are seen in the kidneys, liver, and brain [11]. One of the principal aims in the treatment and management of paraquat toxicity are to lessen the impact of free radical injury by the use of antioxidants that scavenge these reactive intermediates. In this direction, many compounds with significant antioxidant potential have been tested in animal models of paraquat toxicity [12]. Also, paraquat-induced oxidative stress is routinely used in animal studies for screening the antioxidant potential of natural compounds [13]. In this respect, our work aims at investigating the antioxidant potential of leaf extract of *Ficus. racemosa* in paraquat-induced oxidative stress in Wistar rats. Further, a standard antioxidant Vitamin C is used alone as well as in combination with the leaf extract to access the comparative free radical scavenging ability in this study.

Materials and Methods

Animals and ethical approval

A total of 54 male Wistar rats (100- 150g) of 6-8 weeks were obtained from the Laboratory Animal Resource Section of the Indian Veterinary Research Institute and housed under standard laboratory conditions with *ad libitum* access to standard rodent chow and water. The experiments performed were in full compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Government of India).

Experimental design

To study the antioxidant effect of *F. racemosa* extract, the rats were randomly divided into 5 groups containing 6 animals each. Group I received saline through oral gavages at 10 ml/kg body weight. The rats in groups II, III, IV, and V were dosed weekly twice with paraquat at 5mg/kg body weight intraperitoneally on the first and third week of the experiment [14]. The *F racemosa* extract was given to groups III and IV through oral gavages at 250 mg/kg body weight for 30 days. Besides, groups IV and V also received a standard antioxidant Vitamin C orally at 100 mg/kg body weight daily for 30 days [15].

Blood samples were collected on day 0, 15, and 30 for determining oxidative stress, haematological and biochemical parameters. After the last blood collection, the animals were humanely sacrificed by using volatile inhalational anaesthetic (halothane) in a glass chamber. The gross lesions in the visceral organs were observed and representative samples from the lung, liver and kidney were collected for histopathology.

Hematological parameters

The total erythrocyte count (TEC) and total leukocyte count (TLC) were done manually on Neubauer's chamber as per the standard protocol. Hemoglobin concentration (g/dl) in the whole blood was estimated by the cyanomethemoglobin method [16]**.** Packed Cell Volume (PCV%) was determined by the capillary microhematocrit method.

Serum biochemistry

Serum glucose concentration, total cholesterol, and triglycerides were estimated with commercially available kits (Span Diagnostics, India) employing a UV-VIS spectrophotometer (Thermo Fisher Scientific).

Assessment of oxidative stress

The oxidative stress parameters were assessed in rats of all groups using the hemolysate. The lipid peroxidation and malondialdehyde (MDA) levels were measured spectrophotometrically as per the method described by [17]. The reduced glutathione (GSH) content was determined by the standard method using DTNB reagent [18] and catalase activity was assayed by using H_2O_2 a substrate as per the method of [19]. Superoxide dismutase activity was measured as per the method suggested by [20].

Histopathology

Liver, lung, and kidney tissue samples collected at the time of sacrifice were fixed in 10% buffered formal saline overnight. Small

pieces of the tissue (2-3mm) were cut and tissues were processed by standard protocol to obtain 4-5 µm thick paraffin-embedded sections which were subsequently stained with routine hematoxylin and eosin stain for histopathological examination.

Statistical analysis

The results obtained were compared between the groups using the ANOVA. The level of statistical significance for all the comparisons made was established at P = 0.05. All data were analyzed through the statistical package SPSS 15 (SPSS Sciences, Chicago, USA).

Results and Discussion

In our study, weekly twice intraperitoneal injection of paraquat given during the first and the third week of experimental protocol, induced oxidative changes in the Wistar rats characterized by alteration in oxidant-antioxidant balance, haemato-biochemical profile, and histopathological changes in the lung, liver, and kidney. We evaluated the potential beneficial effects of *Ficus racemosa* extract and a standard antioxidant Vitamin C, alone and in combination, against paraquat-induced oxidative stress. Several studies have demonstrated the efficacy of Vitamin C in mitigating pesticide-induced toxicity [21,22]. *Ficus racemosa* has also proven to be an effective antioxidant in both in-vitro and in-vivo studies [23,24].

The hematological parameters like hemoglobin (a), PCV (b), total erythrocyte counts TEC (c), and total leukocyte counts TLC (d) are shown in figure 1 in the control and treatment groups at day 0, 15, and 30. No significant differences were observed during the study period in the hematological values and no relevant studies were found attempted specifically to assess the significance or changes in the hematology.

Serum glucose levels were found to be significantly high in the paraquat exposed group (109.3 ± 2.90) as compared to healthy control (97.83 ± 2.1) on day 30. Significant differences were also observed in the triglyceride levels between the paraquat exposed (107.71 ± 7.76) and healthy group (99.68 ± 10.88) on day 30, but other groups were not showing any significant changes among them. There was a significant (P < 0.05) reduction in the cholesterol values in the *Ficus racemosa* treated group (130.41 ± 11.28) as compared to the standard antioxidant treated group (135.26 ± 8.06) and PQ exposed group (177.98 ± 10.42) with values relatively

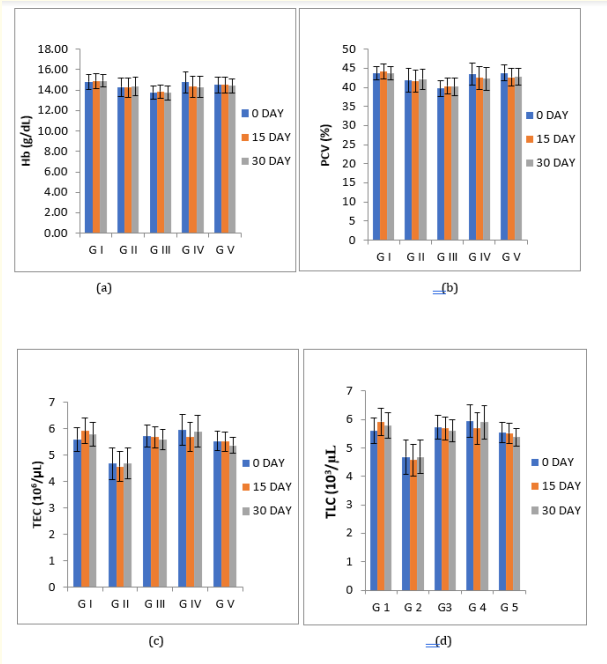

Figure 1: Hematological parameters in different groups at 0, 15 and 30 days (Mean ± S.E.). (a) Hemoglobin levels expressed in g/dL; (b) Packed cell volume in percentage; (c) Total erythrocyte count in10^6/µL; (d) Total leukocyte count in 10^3/ µL.

close to healthy control (121.21 ± 7.69) on day 30, which was in compliance with the study of [25], using *Ficus racemosa* bark extract where inhibition of endogenous lipid synthesis was reported.

The results are compiled in table 1. Serum blood glucose (mg/dl) levels (Mean ± SE) were significantly high in Group II, III and IV (106.6 ± 2.90, 109.36 ± 3.21 and 106.21 ± 3.08), respectively on day 15, as compared to the healthy control (98.37 ± 1.45) and the standard antioxidant treated group (99.32 ± 6.1) which may be due to the elevated need to counteract the stress and impaired glucose metabolism. In contrast to the present study, there was a lowering of blood glucose demonstrated by the tannin fraction of bark of *Ficus racemosa*, due to its anti-diabetic activity; however the effects of tannin fraction on triglycerides were insignificant [26]. Also in our study there were no significant differences in the triglyceride levels between all the groups, except the paraquat exposed group.

Parameter		Day 0	Day 15	Day 30
Glucose (mg/dl)	G I	99.35 ± 2.9^{xA}	97.95 ± 2.92^{xA}	97.83 ± 2.1^{xA}
	G II	103.5 ± 2.9^{xB}	106.6 ± 2.90^{yB}	109.3 ± 2.90^{zB}
	G III	101.21 ± 3.33^{xA}	109.36 ± 3.21^{yB}	101.9 ± 4.62^{xA}
	G IV	100.36 ± 2.79^{xA}	106.21 ± 3.08^{yB}	99.98 ± 3.1^{xA}
	G V	97.93 ± 20.4^{xA}	99.41 ± 11.4^{xA}	100.68 ± 10.88^{xyA}
Triglycerides (mg/dl)	G I	97.93 ± 20.4^{xA}	95.63 ± 6.3^{xA}	99.68 ± 10.88^{xA}
	G II	101.25 ± 9.92^{xA}	105.93 ± 7.77^{yB}	107.71 ± 7.76^{yB}
	G III	100.13 ± 6.33^{xA}	101.21 ± 5.55^{xA}	95.1 ± 5.49^{yB}
	G IV	89.88 ± 1.93^{xA}	91.68 ± 8.61^{xA}	83.15 ± 0.71^{yC}
	G V	88.31 ± 4.32^{xA}	91.75 ± 4.24^{xA}	92.35 ± 3.20^{xA}
Cholesterol (mg/dl)	G I	119.56 ± 11.4^{xA}	116.96 ± 8.56^{xA}	121.21 ± 7.69^{xA}
	G II	122.25 ± 11.68^{xA}	173.91 ± 10.7^{yB}	177.98 ± 10.42^{yB}
	G III	118.5 ± 12.63^{xA}	157.2 ± 12.62^{yC}	130.41 ± 11.28^{zC}
	G IV	122.5 ± 9.98^{xA}	145.11 ± 9.72^{yC}	124.33 ± 9.84^{xA}
	G V	120.41 ± 6.87^{xA}	154.11 ± 7.96^{yC}	135.26 ± 8.06^{zC}

Table 1: Serum biochemical parameters of rats from various groups (Mean ± SE).

Superscript x, y, z indicates significant ($P < 0.05$) difference within same row.

Superscript A, B, C indicate significant ($P < 0.05$)) difference within same column.

In our study, the paraquat challenge significantly suppressed the activity of SOD (.59 ± 0.09,.87 ± 0.22,.77 ± 0.19,.65 ± 0.10) in GII, GIII, GIV and GV, GSH (0.112 ± 0.023, 0.099 ± 0.002, 0.09 ± 0.003) in GII, GIV,GV and CAT(.591 ± 0.24,.703 ± 0.25,.84 ± 0.12,.67 ± 0.16) in GII, GIII, GIV and GV on day 15 to produce oxidative injury, while there was significant ($p < 0.05$) elevation in the levels of MDA (1.92 ± 0.21, 1.03 ± 0.09, 0.649 ± 0.12, 0.753 ± 0.10), in group II, III, IV and V respectively, a product of lipid peroxidation in the blood, compared with the control group (0.45 ± 0.01) on day 15 of treatment. *Ficus racemosa* extract and Vitamin C supplementation significantly ($P < 0.05$) alleviated the oxidative stress induced by paraquat in rats. The comparison between all the groups and standard antioxidant is shown in table 2. Our results showed that *Ficus racemosa* and Vitamin C either alone or in combination reversed the oxidative stress induced by paraquat.

The levels of MDA, GSH, Catalase and SOD were normalized with the greatest alleviation seen within the combination group of Vitamin C and *Ficus racemosa* extract indicating a protective role in membrane stabilization. Also Group V supplemented by standard antioxidant i.e. Vitamin C showed highest improvement in the SOD levels in affected animals, supporting the fact that Vitamin C was able to reverse the oxidative stress parameters and hepatotoxicity in common carps exposed to paraquat by reducing MDA, increasing the total antioxidant capacity, and catalase activities. In another study, high doses of Vitamin C treatment augmented the total antioxidant capacity of human serum and acted as a free radical scavenger in human patients poisoned with paraquat [27]. Vitamin C also reduced reactive oxygen species production in mouse embryonic stem cells treated with paraquat [28]. *Ficus racemosa* stem bark and fruit extracts exhibited potential in-vitro antioxidant capacity [29,30]. Similarly, ethanolic extract of the leaves from the

Parameter		Day 0	Day 15	Day 30
LPO (nmol MDA/mg Hb)	G I	0.42 ± 0.01^{xA}	0.45 ± 0.01^{xA}	0.38 ± 0.03^{xA}
	G II	0.45 ± 0.01^{xA}	1.92 ± 0.21^{yB}	2.155 ± 0.20^{yB}
	G III	0.388 ± 0.03^{xA}	1.03 ± 0.09^{yC}	0.68 ± 0.19^{zC}
	G IV	0.353 ± 0.17^{xA}	$0.649 \pm 0.^{12yD}$	0.505 ± 0.12^{zC}
	G V	0.388 ± 0.034^{xA}	0.753 ± 0.10^{yD}	0.47 ± 0.04^{xD}
GSH (µmol/ mg Hb)	G I	0.19 ± 0.005^{xA}	0.184 ± 0.025^{xA}	0.156 ± 0.03^{xA}
	G II	$0.173 \pm 0.00x^{xA}$	0.112 ± 0.023^{yB}	0.065 ± 0.06^{zB}
	G III	0.16 ± 0.011^{xA}	0.187 ± 0.034^{xA}	0.509 ± 0.094^{yC}
	G IV	0.194 ± 0.005^{xA}	0.099 ± 0.002^{yC}	0.32 ± 0.009^{zc}
	G V	0.18 ± 0.12^{xA}	0.09 ± 0.003^{yC}	0.43 ± 0.09^{zC}
SOD (U/mg Hb)	G I	1.23 ± 0.050^{xA}	1.25 ± 0.23^{xA}	1.156 ± 0.03^{xA}
	G II	1.29 ± 0.06^{xA}	1.59 ± 0.09^{yB}	0.15 ± 0.02^{zB}
	G III	1.27 ± 0.10^{xA}	1.87 ± 0.22^{yaC}	1.90 ± 0.03^{zAC}
	G IV	1.32 ± 0.06^{xA}	1.47 ± 0.19^{yB}	2.15 ± 0.09^{zC}
	G V	1.35 ± 0.15^{xA}	1.45 ± 0.10^{yB}	2.23 ± 0.06^{zC}
CAT (U/mg Hb)	G I	0.984 ± 0.08^{xA}	1.10 ± 0.22^{xA}	0.981 ± 0.34^{xA}
	G II	1.30 ± 0.15^{xA}	$.591 \pm 0.24^{yB}$	$.42 \pm 0.123^{yB}$
	G III	1.17 ± 0.16^{xA}	$.703 \pm 0.25^{yB}$	2.591 ± 3.40^{zC}
	G IV	1.10 ± 0.06^{xA}	$.84 \pm 0.12^{yAB}$	1.093 ± 4.16^{xA}
	G V	1.21 ± 0.7^{xA}	$.67 \pm 0.16^{yB}$	1.526 ± 3.2^{zC}

Table 2: Markers of oxidative stress in blood of rats in various groups (Mean ± S.E.).

Superscript x, y, z indicates significant ($P < 0.05$) difference within same row.

Superscript A, B, C indicate significant ($P < 0.05$)) difference within same column.

same plant showed both nitric oxide and superoxide scavenging activity in in-vitro studies which are in agreement with or in-vivo results [31].

Paraquat treatment to rats induced significant structural alterations in the liver, lungs, and kidneys. These histopathological changes were ameliorated by the supplementation of *F. racemosa* extract and Vitamin C.

Microscopically the sections of hepatocytes in samples of the control group group I (a) were normal and did not reveal any specific pathological changes. In paraquat treated group II (b) rats, there was degeneration and necrosis of hepatocytes with loss of architecture. The cords of hepatocytes were ruptured having dark stained basophilic nuclei. Group III (c) also showed fatty and degenerative changes in the liver with mild lesions in hepatocytes. Group IV (d) and V (e) liver sections exhibited seemingly normal hepatocytes with an intact central vein as seen in figure 2.

figure 3 demonstrates the histopathological findings of the lungs in different study groups. The lung histopathology of rats in group I (a) revealed normal architecture with intact alveoli. Group

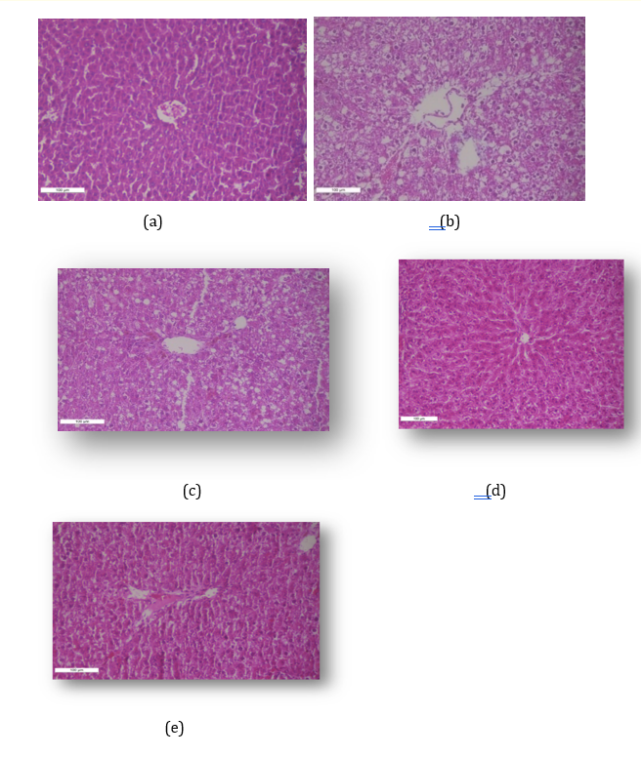

Figure 2: Histopathology of liver (HEx20). (a) Group I (Healthy control) Normal architecture with central vein (arrow); (b) Group II (Positive control) show detached central vein with loss of architecture and necrotized hepatocytes; (c) Group III (EXT) Fatty changes and central vein is intact; (d) Group IV (EXT + VIT C). Apparently normal hepatocytes surrounding central vein, hepatocytes in paracentral area shows degenerative changes; (e) Group V (VIT C) normal hepatocytes, central vein is intact.

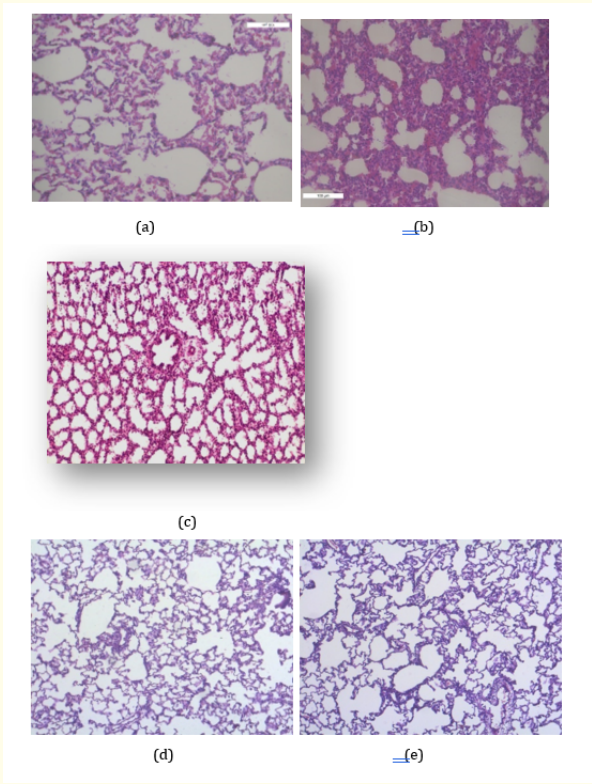

Figure 3: Histopathology of lungs (HE x20). (a) Group I (Healthy control) Normal architecture; (b) Group II (Positive control) Infiltrative cells (arrow), interstitial thickening;(c) Group III (EXT)Mild emphysema; (d) Group IV (EXT+VIT C) Apparently normal architecture; (e) Group V (VIT C).

II (b) animals showed interstitial thickening with congested blood vessels, oedema, and mononuclear infiltration of lung lymphocytes. Group III (c) presented mild emphysema with the regular architecture of cells. The animals in group IV (d) and V (e) exhibited normal lung histopathology.

In the kidney sections of the group I (a) normal morphology with intact glomeruli and renal tubules were noticed. Group II (b) exhibited mild degenerative changes in renal tubules with linear haemorrhages in the peri-tubular area. There was a mild degeneration in tubules of group III (c) and an increased peri-glomerular space in Group IV (d). The renal tubules of group V (e) were having a normal architecture similar to Group I (Figure 4). We found paraquat action to alter the normal histopathological architecture of the liver, lung, and kidney in our work which is of the same opinion as previous studies in rats, mice, and fish [32-34]. Free radical injury to alveolar epithelium leading to respiratory failure is attributed as a major reason for death in paraquat poisoning [35]. *Ficus racemosa* extract and Vitamin C supplementation to paraquat treated rats alleviated the pathological changes in our investigation suggesting a potential anti-oxidant activity of these agents in the major organs.

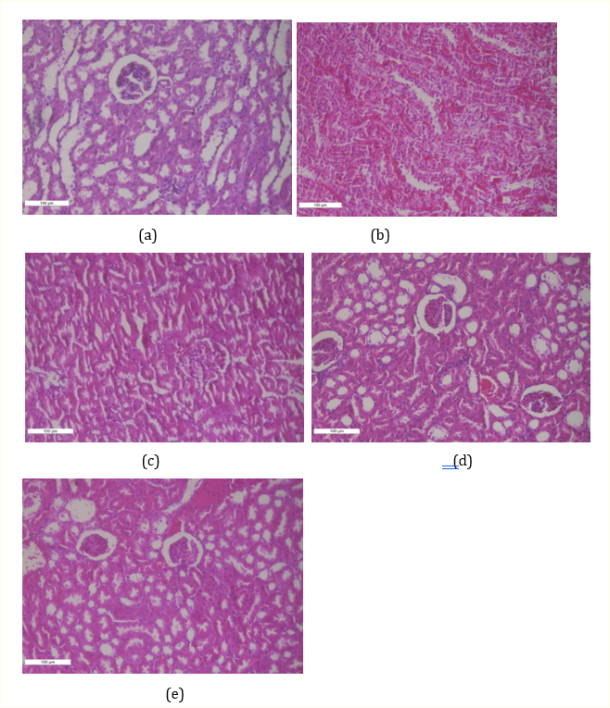

Figure 4: Histopathology of renal tubules (HE x20). (a) Group I (Healthy control) Normal architecture with intact glomeruli and tubules; (b) Group II (Positive control) a: Linear hemorrhages, b: Tubules show degenerative changes; (c) Group III (EXT) Apparently normal architecture with mild Degenerative changes in tubules; (d) Group IV (EXT+VIT C) Normal architecture with hypercellular glomeruli; (e) Group V (VIT C) Normal architecture.

However, a combination of both gave better protection than either agent alone which may be due to additive interaction with respect to the antioxidant property. Our results are in accordance with previous studies with Vitamin C against paraquat-induced morphological changes in the kidney and liver of rats [33]. Similarly, Vitamin C increased the anti-oxidant capacity in the lungs by moderating SOD and catalase levels further reversing the lung fibrosis induced by paraquat [36]. Intraperitoneal injection of paraquat to rats decreased the Vitamin C level in the lungs indicating a potential involvement of Vitamin C mediated pathway in lung chemical toxigenesis of paraquat [37]. In one of the studies, *Ficus racemosa* leaves extract ameliorated the chronic hepatic damage in rats induced by carbon tetrachloride [38]. Also, *Ficus racemosa* plant extract protected the rats against Ferric nitrilotriacetate-induced renal damage and oxidative stress [24]. The tannins from the stem bark of the same plant improved antioxidant status in the heart, liver, and kidney of high-fat-fed diabetic rats [26].

Conclusion

Paraquat is used in agriculture and is known to cause toxicity in non-target species by inducing major histopathological alteration in the lungs, liver, and kidneys. These modifications are likely to be caused by an overall impaired oxidative status. We conclude that *Ficus racemosa* extract and Vitamin C ameliorate the toxic changes in rats exposed to paraquat. A combination of these antioxidants exhibits synergy in their protective effects when compared to the use of either agent alone. However, more studies are required to standardize the dose requirements for their use in clinical conditions.

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Declaration

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Disclosure/Conflict of Interest

No potential conflict of interest was reported by the authors.

Availability of Data and Material

All the relevant data is provided in the manuscript.

Authors' Contributions

S.K. D. and U.K. D conceived the work. S.Y. participated in data acquisition. S.Y., U.K. D., S.K.S., and P.K.P. participated in data analysis and interpretation and in manuscript drafting. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Ethics Approval

Our study is in compliance and approved with the ethical guidelines proposed by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals, Govt. of India).

Consent to Participate

NA.

Consent for Publication

NA.

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