

Pumpkin (*Cucurbita pepo*) Fruit Extract Attenuates Paraquat-induced Toxicity in Wister Rats

Abstract

Introduction: Paraquat is a bipyridil herbicide commonly used in modern agriculture for weed and pest control. Paraquat poisoning usually occurs through direct ingestion for suicidal intent, causing mortality as a consequence to oxidative damage, inflammation and multi-organ failure.

Objectives: In this study, we investigated the ameliorative potential of *Cucurbita pepo* fruit on paraquat-induced oxidative damage, inflammation, hyperlipidemia, hepatotoxicity and nephrotoxicity in rats.

Methods: Rats were orally administered paraquat (2 mg/kg b.w) with or without the fruit extract (500 mg/kg b.w) or resveratrol (3.57 mg/kg b.w) co-administration for 2 weeks.

Results: We found *C. pepo* fruit extract significantly restored paraquat-induced oxidative damage, acute inflammation and hyperlipidemia. Reduced glutathione (GSH) concentration was significantly abridged in paraquat treated group, while the concentration of GSH increases substantially across groups administered fruit extract and resveratrol. Furthermore, paraquat induced concomitant reduction in the activity of glutathione peroxidase (GPx) and superoxide dismutase (SOD), which was restored by the fruit extract and resveratrol. However, nitric oxide level demonstrated a substantial elevation in paraquat-treated group, but restored by both the fruit extract and resveratrol. Moreover, pumpkin fruit extract and resveratrol supplementation suppressed overproduction of total cholesterol, triglycerides, low density lipoproteins, high density lipoproteins and thus, alleviated paraquat-induced hyperlipidemia. In addition, administration of *C. pepo* fruit extract and resveratrol ameliorated the hepatotoxic and nephrotoxic effect caused by paraquat.

Conclusion: We conclude that *C. pepo* fruit extract and resveratrol administration ameliorated oxidative damage, acute inflammation, hyperlipidemia, hepatotoxicity and nephrotoxicity caused by paraquat ingestion.

Keywords: Oxidative stress; Paraquat toxicity; Antioxidant; Pumpkin fruit; Resveratrol

Introduction

Paraquat is a bipyridil herbicide commonly used in modern agriculture for weed and pest control.

Paraquat poisoning usually occur through ingestion, in most cases, for suicidal intent; causing

death from hypoxemia, cardiovascular collapse and multi-organ failure in moderate to severe case with high ingestion volume (1). Intentional poisoning with rodenticides and pesticides is a major problem of public health in developing countries, with paraquat being the prime target for suicide attempts in these regions. The menace of paraquat intoxication is not limited to developing countries alone, but also span through developed nations like the UK and America (2–4). For this reason, the use of paraquat has been abolished in many countries (5,6).

The mechanism of paraquat toxicity begins when paraquat concentrate in lung alveolar type I and II cells via an active transport system and interferes with mitochondrial electron transfer, leading to production of large amount of reactive oxygen species (ROS) and eventually induce lipid peroxidation injury and consequent respiratory failure due to fibrosis (7).

Exposure to paraquat has been linked to the pathogenesis of neurodevelopmental and neurodegenerative disorders such as attention deficit/hyperactivity, autism spectrum disorders and Parkinsonism due to permanent toxicity in the nigrostriatal dopamine system (8). Exposure to paraquat during development at nontoxic concentration for the adult brain, can lead to neuronal loss and functional deficits (8).

In spite of the fact that paraquat poisoning has mortality rate of 60-80 %, there is no specific, clearly defined and effective treatment or antidote for paraquat poisoning (6,9). Current treatment of paraquat poisoning focuses on prevention of absorption, elimination from the body by hemodialysis or hemoperfusion and reduction of ROS generation; in addition to ROS scavenging, ROS-induced lesion repair, as well as reduction of inflammation (1,5).

Development in modern sciences has demonstrated that a wide range of plants-derived constituents called phytochemicals has the potential to detoxify or reverse the deleterious effects

of toxins and protect biological system from the adverse effect of xenobiotics and other toxicants. The utilization of medicinal plants and plant-derived products for therapeutic purpose is rapidly increasing and gaining attention globally because they exhibit little or no safety concern and their biofriendliness compared to synthetic medications. *Cucurbita pepo* (Pumpkin), an economically and nutritionally important fruit-bearing vegetable belonging to the family *Cucurbitaceae*, is a reservoir of variety of nutrients and bioactive compounds such as unsaturated fatty acids, micro and macro minerals, α and γ tocopherols, β -carotene, proteins (albumin and globulins), vitamins and phytosterols (10–14).

Another important plant derivative with therapeutic properties is the polyphenolic phytoalexin called resveratrol (3,5,4'-trihydroxystilbene) that is found in various plant parts and products, including peanut skins, berries, red wine and grapes (15).

The aim of this research is to investigate the ameliorative potentials of *Cucurbita pepo* (pumpkin) fruit and resveratrol on paraquat-induced toxicity.

Materials and Methods

Chemicals and Kits

50 mM sodium phosphate buffer with 0.40 mM ethylenediaminetetraacetic acid (EDTA), pH 7.0; 1.0 mM sodium azide solution; β -nicotinamide-adenine dinucleotide phosphate reduced (NADPH); reduced glutathione (GSH); glutathione enzyme solution; glutathione peroxidase enzyme solution, hydrogen peroxide; nitric oxide assay kit purchased from ThermoFisher Scientific (catalog number EMSNO).

Animals

Twenty-Four (24) adults Wistar Albino male rats (250-265g body weight) were obtained from National Veterinary Research Institute, Vom, Jos, Plateau State, Nigeria. Ethical approval was obtained from Ethical Committee for Animal Studies, Nasarawa State University, Keffi. The animals were handled in accordance with principles of Planning Research and Experimental Procedures on Animals: Recommendation for Excellence. The rats were housed in the Animal House of the Department of Biochemistry and Molecular Biology, Nasarawa State University Keffi, where they were maintained on standard rat vital feed and water which were made available *ad libitum* for 14 days. The rats were maintained at an ambient temperature between 28-30 °C, humidity of 55 ±5%, and standard (natural) photoperiod of approximately 12 hours light (06:30 h – 18:30 h) alternating with approximately 12 hours of darkness (18:30 h - 06:30 h) to acclimatized the laboratory condition prior to the commencement of the research.

Collection and Extraction of Pumpkin Fruit

Pumpkin fruits were obtained from a farmland in Nasarawa Local Government Area, Nasarawa State, Nigeria. The Pumpkin fruits were then washed, pilled, cut into pieces and allowed to air-dry for five (5) days. Thereafter, converted into powder and preserved in a refrigerator at -4 °C in plastic container. The preserved powder was used when required by suspending it in a distilled water to prepare fresh stock solutions of 0.1 mg/mL and 0.5 mg/mL. The volume of the solution that corresponds with the dose to be administered to each animal based on animal weight was obtained using the formula; $\text{volume in mL} = \text{dosage (mg/kg)} \times \text{body weight in kg} / \text{concentration (mg/mL)}$.

Experimental design

After the acclimatization, the animals were weighed and divided into four (4) groups with 6 animals each per group.

Group 1: Control – received feed and water throughout the period of 2 weeks.

Group 2: Received paraquat only at 2 mg/kg body weight once a day and haphazardly for the period of 2 weeks.

Group 3: Received paraquat at 2 mg/kg and *C. pepo* extract 500 mg/kg body weight twice a day for the period of 2 weeks.

Group 4: Received paraquat at 2 mg/kg and Resveratrol supplement extract 3.57 mg/kg body weight twice a day for the period of 2 weeks.

The treatments were administered orally and with the aid of a feeding tube and all the animals were allowed access to feed and water *ad libitum* throughout the treatment period.

Collection of Blood Sample

After the administration for two (2) weeks, the rats were anaesthetized with diethyl ether and blood sample was collected with the aid of capillary tube via an ocular vein puncture into plain bottle for biochemical analysis.

Biochemical Assay

Determination of Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) was assayed using method described by Xin *et al.* (1991) (16).

Liver and Kidney Function Parameters

Determination of liver and kidney function parameters were performed using Standardized diagnostic kits (Randox® by Randox laboratories ltd. United Kingdom) according to modified conventional methods as described by the kit's manufacturer (17).

Determination of Reduced Glutathione (GSH)

Glutathione (GSH) reacts with 5,5'-dithiobis-2-nitrobenoic acid (DTNB) to give a yellow product that absorbs maximum ally at 412nm. The amount of GSH was determined as GSH mg/dL of blood (18).

Glutathione Peroxidase Assay

Glutathione peroxidase (GPx) protects the cell from the damaging effect of free radicals using GSH as reducing equivalent via catalytic reduction of H_2O_2 to water and organic peroxides to corresponding alcohols. GPx activity was measured according to manufacturer's (Biodiagnostic Diagnostic and Research Reagents) instruction. Briefly, GPx the assay involves indirect measurement of c-GPx activity spectrophotometrically by measuring the decrease in absorbance of NADPH upon oxidation to $NADP^+$ at 340 nm.

Nitric Oxide Assay

Nitric oxide (NO) was assayed as described in the manual by the kit's manufacturer. The kit uses the enzyme nitrate reductase to convert nitrate to nitrite; thereafter, nitrite is detected as colored azo dye product of the Griess reaction which absorb visible light at 540 nm.

Lipid Profile

Triglycerides (TGs), total cholesterol (TC), high density lipoprotein (HDL), and low density lipoprotein (LDL) were analyzed as described by the manufacturer Randox lipid profile kits based on the protocol of (19), (20), (21) and (22), respectively.

Results

Table 1 below shows the findings of antioxidant status, endogenous antioxidant enzymes activity and NO concentration. GSH concentration was observed to decrease significantly (7.28 ± 0.756 and 17.18 ± 0.43 mg/dL, respectively; $p < 0.05$) in paraquat treated group compared to the control. However, the level of GSH was significantly increased in groups administered paraquat and pumpkin fruit extract (9.16 ± 1.06 mg/dL), and paraquat with resveratrol (10.52 ± 1.18 mg/dL) in comparison with paraquat only treated group.

Table 1: Effects of Pumpkin Fruit Extract and Resveratrol on Antioxidant Status and Antioxidant Enzymes Activity

| Group | GSH (mg/dL) | GPx (IU/L) | SOD (IU/L) | NO (μ mol/mg) |
|-------------------------|--------------------|--------------------|-------------------|--------------------|
| 1 Control | 17.18 ± 0.43 | 25.30 ± 1.32 | 7.00 ± 1.59 | $0.60 \pm .49$ |
| 2 PQ only | 7.28 ± 0.76^a | 16.06 ± 1.49^a | 1.15 ± 0.17^a | $0.82 \pm .39^a$ |
| 3 PQ+Pumpkin | 9.16 ± 1.06^b | 17.67 ± 1.44^b | 4.68 ± 0.99^b | $0.55 \pm .45^b$ |
| 3 PQ+Resveratrol | 10.52 ± 1.18^b | 17.91 ± 1.33^b | 5.78 ± 0.65^b | $0.68 \pm .31^b$ |

The data represent mean \pm SD (n=6). a: indicate a significant difference ($P < 0.05$) compared to the control group, values with b are significantly different compared to group 2. PQ: paraquat, GSH: Reduced Glutathione, GPx: glutathione peroxidase, SOD: superoxide dismutase, NO: Nitric Oxide.

In a similar way, there was significant reduction in the activity of GPx (16.06 ± 1.49 IU/L) and SOD (1.15 ± 0.17 IU/L) in paraquat treated group compared to control. In contrast, significant increase in the activity of GPx and SOD was observed in group co-administered paraquat with pumpkin extract and paraquat with resveratrol compared to paraquat treated group.

Furthermore, the result of NO analysis revealed a substantial elevation ($P < 0.05$) in NO level of group 2 ($0.82 \pm 0.39 \mu\text{mol/mg}$) compared to the control ($0.60 \pm 0.49 \mu\text{mol/mg}$). However, the trend was reversed in pumpkin fruit extract and resveratrol treated groups.

The concentration of total cholesterol, triglyceride, low density lipoprotein (LDL) and high density lipoprotein (HDL) was observed to increase markedly ($P < 0.05$) in group treated with paraquat as compared to control (table 2).

Table 2: Effects of Pumpkin Fruit Extract and Resveratrol on Lipid Profile

| Group | T. cholesterol (mmol/L) | Triglyceride (mmol/L) | LDL (mmol/L) | HDL (mmol/L) |
|-------------------------|----------------------------|--------------------------|-------------------|-------------------|
| 1 Control | 8.48 ± 0.82 | 5.40 ± 1.29 | 4.71 ± 0.56 | 0.48 ± 0.43 |
| 2 PQ only | 12.57 ± 1.14^a | 9.46 ± 0.98^a | 5.95 ± 0.79^a | 1.71 ± 1.43^a |
| 3 PQ+Pumpkin | 10.34 ± 0.72^b | 7.39 ± 0.95^b | 6.20 ± 0.83^a | 0.97 ± 0.48 |
| 3 PQ+Resveratrol | 8.39 ± 0.94^b | 6.25 ± 1.03^b | 3.50 ± 0.61^b | 0.38 ± 0.31^b |

The data represent mean \pm SD (n=6). a: indicate a significant difference ($P < 0.05$) compared to the control group, values with b are significantly different compared to group 2. PQ: paraquat, T.Cholesterol: total cholesterol, LDL: low density lipoprotein, HDL: high density lipoprotein

However, the concentration of total cholesterol, triglyceride, LDL and HDL were significantly decreased ($P < 0.05$) across the groups administered pumpkin fruit extract and resveratrol compared to group treated with paraquat only.

Result of the effect of pumpkin fruit and resveratrol on paraquat hepatotoxicity in wister rats is presented in table 3 below. The results showed that AST activity ($16.01 \pm 2.15 \text{ IU/L}$) of the group administered paraquat increased significantly ($p < 0.05$), compared to the control group

(10.11±1.98 IU/L), while the AST level of the group administered paraquat and pumpkin as well as paraquat and resveratrol, (12.63±2.53 IU/L) and (11.73±1.46 IU/L); respectively, were non-significantly ($p < 0.05$) lowered compared to the group treated with paraquat only (16.01±2.15 IU/L).

The result of ALT activity, total and direct bilirubin were found to be significantly ($p < 0.05$) higher in group treated with paraquat in comparison to the control, whereas a substantial decrease in ALT activity and total bilirubin level were observed in group administered paraquat with pumpkin fruit and paraquat with resveratrol when compared to group 2, except for direct bilirubin level which showed a non-significant decrease ($p > 0.05$) in the group administered paraquat and pumpkin (15.58±1.58 IU/L) compared to group 2 (15.58±1.58 IU/L) (table 3).

Table 4: Effect of Pumpkin Fruit Extract and Resveratrol on Liver Function Indices

| Group | AST (IU/L) | ALT (IU/L) | Total Bilirubin (mg/dL) | Direct Bilirubin (mg/dL) | T. Protein (mg/dL) |
|-----------------------------|-------------------------|-------------------------|------------------------------------|-------------------------------------|-------------------------------|
| 1 Control | 10.11±1.98 | 4.71±1.3 | 11.89±1.17 | 15.58±1.58 | 32.70±3.97 |
| 2 PQ only | 16.01±2.15 ^a | 14.69±1.09 ^a | 20.35±1.97 ^a | 31.03±1.88 ^a | 24.70±3.73 ^a |
| 3 PQ+Pumpkin | 12.63±2.53 | 8.07±0.75 ^b | 18.29±2.38 ^b | 17.38±1.65 | 27.20±2.47 ^b |
| 3 PQ+Resveratrol | 11.73±1.46 | 9.49±1.94 ^b | 17.28±1.97 ^b | 19.45±1.74 ^b | 25.71±4.38 ^b |

The data represent mean ± SD (n=6). a: indicate a significant difference ($P < 0.05$) compared to the control group, values with b are significantly different compared to group 2. PQ: paraquat, ALT: Alanine amino transferase, AST: Aspartate amino transferase.

The level of total protein in group treated with paraquat exhibit a significant decrease compared to the control, while the group treated with pumpkin fruit extract and resveratrol indicate significant elevation in total protein concentration in comparison to group 2 (table 4).

Table 5: Effect of Pumpkin Fruit Extract and Resveratrol on Renal Function Parameters

| Group | Urea (mg/dL) | Creatinine (mg/dL) | Na ⁺ (mmol/L) | K ⁺ (mmol/L) | HCO ₃ ⁻ (mmol/L) |
|-------------------------|-------------------------|------------------------|-----------------------------|----------------------------|---|
| 1 Control | 4.85±1.21 | 0.74±0.05 | 182.17±6.18 | 10.02±4.35 | 1.30±0.49 |
| 2 PQ only | 11.26±0.93 ^a | 0.77±0.24 | 160.58±5.99 ^a | 4.88±1.19 ^a | 0.82±0.42 ^a |
| 3 PQ+Pumpkin | 8.41±1.95 ^b | 0.61±0.19 ^b | 173.98±3.52 ^b | 6.72±1.28 ^b | 0.59±0.09 ^b |
| 3 PQ+Resveratrol | 4.07±1.36 ^b | 0.46±0.41 ^b | 169.92±3.26 ^b | 6.25±0.79 ^b | 0.86±0.17 ^b |

The data represent mean ± SD (n=6). a: indicate a significant difference (P<0.05) compared to the control group, values with b are significantly different compared to group 2. PQ: paraquat

The concentration of serum urea was remarkably higher in paraquat treated group (11.26±0.93mg/dL) compared to the control (4.85±1.21 mg/dL). However, the trend was reversed in groups administered pumpkin fruit and resveratrol in contrast to group treated with paraquat alone (table 5). Although the increase was non-significant (p>0.05), the level of serum creatinine was observed to increase in group treated with paraquat (0.77±0.24 mg/dL) as compared to the control (0.74±0.05 mg/dL), while a significant decrease in level of serum creatinine were observed in group administered paraquat with pumpkin fruit extract (0.61±0.19 mg/dL) and paraquat with resveratrol (0.46±0.41 mg/dL) when compared to group 2 (0.77±0.24 mg/dL).

Moreover, the concentration of serum electrolytes (Na⁺, K⁺ and HCO₃⁻) were significantly lowered compared to the control, while a significant elevation in level of serum electrolytes were

observed in groups co-administered paraquat and pumpkin fruit extract, as well as paraquat and resveratrol in contrast to paraquat treated group.

Discussion

In this study, a significant reduction in the level of GSH was observed in group administered only paraquat compared to the control. It has been reported that paraquat induces toxicity through a reduction and reoxidation steps (called redox cycling) involving series of enzymes and NADPH, which serve as electron donor, subsequently resulting in the production of superoxide radical in the presence of molecular oxygen, and ultimately stimulate the activation of a cascade, leading to generation of hydrogen peroxide and other reactive oxygen species (23). Reduced glutathione (GSH) is a tripeptide that utilize the sulfhydryl (-SH) group in its cysteine residue to neutralize the deleterious effects of hydrogen peroxide and hydroxyl free radicals in the cell, thereafter, leading to the formation of oxidized form of glutathione (GSSG). NADPH is an essential cofactor required by glutathione reductase to maintain optimum physiologic concentration and regenerate GSH from GSSG formed during detoxification of hydrogen peroxide into water by glutathione peroxidase caused by paraquat toxicity. A decrease in the ration of NADPH/NADP⁺ on lung tissues exposed to paraquat has been documented in both in vivo (24) and in vitro (25) experiments. Hence, the decrease level of GSH observed in our experiment is likely in part, a reflection of excessive utilization NADPH, which is a prerequisite for initiation of paraquat redox cycling. However, our result revealed a significant elevation in GSH level on groups co-administered paraquat with pumpkin fruit extract and paraquat with resveratrol. Several authors (26,27) have reported the antioxidant activity of *Cucurbita pepo*. The observed improvement in GSH level by pumpkin fruit extract and resveratrol could be through

potentiation of body's antioxidant status by compounds of *C. pepo* fruit, perhaps by scavenging of ROS or interfering with their production and propagation of oxidative stress.

Our investigation revealed a significant reduction in glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities following paraquat exposure; which may be the reflection of the enzyme response to free radicals generated during paraquat metabolism. This finding conforms with the report of (28,29) which showed a significant impairment in GPx activity 24 hour after 1 mM paraquat exposure and 48 hour following exposure to 0.5 mM paraquat respectively in pulmonary microvascular endothelial cells (PMECs). Similarly, an *in vitro* study by (29) revealed reduced activities of GPx on exposure to 0.1 and 0.5 mM paraquat when cumene hydroperoxide or phospholipid hydroperoxide was used as substrate. (29) attributed the observed decrease in GPx activity to the impairment of glutathione redox cycle and inactivation of GPx. Redox state of the cell is maintained by glutathione system (glutathione/glutathione peroxidase or glutathione/glutaredoxin) and thioredoxin system; whose roles are sometimes complementary and overlapping in cytoprotection (28).

In order to achieve cellular homeostasis, many antioxidants and antioxidant enzymes which include α -tocopherol (vitamin E), ascorbic acid, GPx, SOD, catalase, glutathione *S*-transferase (GST) are involved in cell protection (29). However, imbalance between these defense systems and oxidative stress induced by free radicals can interfere with cellular homeostasis. It was reported that treatment with SOD mimetics and overexpression of SOD has proven to protect against toxicity induced by paraquat (30–32).

This study showed the effect of *C. pepo* fruit extract and resveratrol in attenuation of paraquat-induced impairment in activities of GPx and SOD. The antioxidant potential of resveratrol has been established (33,34). This improvement in activities of the antioxidant enzymes GPx and

SOD caused by *C. pepo* fruit extract could be likely due to its ascorbic acid (35), α -tocopherol (36) and selenium (37) composition. In the work of Sharifinasab and coauthors, combined supplementation with ascorbic acid and chitosan enhanced the detoxification system in fish muscles and protect against paraquat toxicity (38). Vitamin C is effective in scavenging free radicals; such as hydroxyl radicals, aqueous peroxy radicals and superoxide radicals and confers protection to the cell by donating electron to reduce these radicals, thus neutralizing their effects (23). Furthermore, ascorbic acid also exhibits its antioxidant potential through its ability to regenerate small antioxidant molecules, including GSH, β -carotene and α -tocopherol (39). Vitamin E is a potent antioxidant which exert its effect through scavenging of free radicals and stabilization of membranes containing polyunsaturated fatty acids (40). In another study, the use of α -tocopherol has proven success in ameliorating lung injury in many survival cases of paraquat poisoning (41). GPx is a selenoenzyme that contain selenolate at the active site. The oxidation of this selenolate into selenonic derivative, resulting in inactivation of GPx, probably, is caused by hydrogen peroxide and other free radical generated by paraquat (42–44). Supplementation of selenium to culture medium was reported to increase the activities GPx and alleviate paraquat-induced cytotoxicity (28).

Our experiment demonstrated a remarkable increase in level of nitric oxide (NO) in group exposed to paraquat. This effect was ameliorated by *C. pepo* fruit extract and resveratrol following administration. NO has been reported to play a crucial role in regulation of acute and chronic inflammation and its generation is demonstrated to increase substantially in certain types of inflammatory conditions (45,46). Scientific investigation revealed that inflammation is another mechanism employed by paraquat in inducing its toxicity. In addition, evidences indicated that paraquat induces the expression nitric oxide synthase (NOS) in various tissues

(47). Human lung epithelial cells and rodents' liver tissue treated with paraquat show an upregulation of inducible nitric oxide synthase (iNOS) mRNA (48,49). The ameliorative effect demonstrated by pumpkin fruit extract and resveratrol could be attributed to inhibition of NOS activity by compounds of pumpkin fruit and resveratrol as evidenced in the work of Muhammad and colleagues.

The result from this study demonstrates that paraquat administration caused a significant increase in total cholesterol, triglycerides, LDL and HDL levels compared to the control. However, the trend was reversed following administration of *C. pepo* fruit extract and resveratrol. The paraquat-induced elevation in atherogenic indices is an indication that paraquat exposure could predispose animals to coronary heart disease (50). The increased level of triglycerides observed may be attributed to impairment in production of very low density lipoproteins (VLDL). As reported by Havinga and Beisiegel, triglyceride is not transported when VLDL is not synthesized, leading to accumulation of triglycerides in the tissues (51). The increase in atherogenic indices induced by paraquat reported from this investigation is in consonant with the findings of (52–54). The suppression of increased atherogenic indices due to paraquat by pumpkin fruit extract and resveratrol may be suggestive of their potential in preventing the development of coronary heart disease.

In this study, the level of serum AST, ALT, total bilirubin and direct bilirubin in paraquat treated group were substantially higher when compared to the control. In addition, the concentration of serum total protein in paraquat treated group is significantly reduced when compared to the control. This could be indicative of severe hepatic injury probably promoted by free radical generation. However, administration of *C. pepo* fruit extract and resveratrol restored the level of liver biomarkers, which is a suggestive of protective effect against paraquat-induced

hepatotoxicity and amelioration of liver damage. The elevation in level of liver damage indices in our study agrees with the report of (55–57).

An investigation of renal function biomarkers revealed a markedly increased level of serum urea and creatinine, with concomitant decrease in serum Na^+ , K^+ , and HCO_3^- in group administered paraquat in comparison to the control. Paraquat has been reported in various studies to induce acute renal injury in both human and animal studies. In separate case reports involving human subject by Ito, Adejumo and colleagues, and Asl and coauthors, paraquat ingestion caused a remarkable elevation in creatinine, blood urea nitrogen, Na^+ , K^+ , and HCO_3^- and Cl^- level in a patient (58–60). Following paraquat ingestion, it is rapidly absorbed and distributed into such organs as liver, kidney and lungs where it elicits its toxicity. Kidney is the primary organ for its excretion, with the excretion occurring in a biphasic manner (58). This could explain the reason for making the kidney one of most vulnerable organ for paraquat toxicity. Following pumpkin fruit extract and resveratrol administration, the level of serum urea, creatinine and electrolytes were reversed, indicating the potential of these supplements in ameliorating paraquat-induced renal function impairment.

Conclusion

This investigation indicates that *C. pepo* fruit extract and resveratrol has the potential to alleviate oxidative damage by upregulating antioxidant status, acute inflammation, hyperlipidemia, hepatotoxicity and nephrotoxicity caused by paraquat ingestion.

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