Research Article

Effect of Quercetin Against Roundup® and/or Fluoride Induced Biochemical Alterations and Lipid Peroxidation in Rats

M. A. Bader [a*], M. Sultana [a], R. Raina [a], S. Prawez [a], N. K. Pankaj [a], R. N. Reshi [b], R. R. Ahanger [c], M. Y. Dar [d].

[a]Division of veterinary pharmacology and toxicology, Faculty of veterinary sciences and animal husbandry R S Pura, SKUAST, Jammu, India.
[b]Division of Veterinary Gynaecology & Obstetrics, Faculty of veterinary sciences and animal husbandry R S Pura, SKUAST, Jammu, India.
[c]Division of Veterinary Parasitology, Faculty of veterinary sciences and animal husbandry R S Pura, SKUAST, Jammu, India.
[d]Division of Veterinary Anatomy, Faculty of veterinary sciences and animal husbandry R S Pura, SKUAST, Jammu, India.

***Corresponding author's E-mail:** mudasir1135@gmail.com

Accepted on: 28-08-2015; **Finalized on:** 30-09-2015.

ABSTRACT

The study investigating the toxic effects of Roundup® (glyphosate) and/or fluoride following repeated oral administration in rats for 28 days (sub acute toxicity) and the modulatory effect of quercetin on such toxicity was taken. Roundup @ 500mg/Kg body weight and fluoride @ 10 ppm on repeated oral administration either alone or in combination caused significant increase in levels of AST, ALT and ALP as compared to control group. In fluoride treated group significant increase in the levels of ACP, ALP and AST values was observed. Alterations in biochemical parameters like AST, ALP and BUN were more pronounced with co-administration than individually treated fluoride or Roundup groups. Significant increase in MDA levels of Roundup and/or fluoride treated rats was observed in RBC's, liver and kidney. Cotreated rats have higher levels of MDA in erythrocytes and livers than Roundup or fluoride treated rats. Quercetin @ 10mg/kg body weight was successful in modulating the adverse effects due to repeated oral administration of Roundup and/or fluoride on most of the biochemical parameters. Pretreatment with quercetin reversed changes in lipid peroxidation (LPO) near to control values in erythrocytes but levels of cotreated toxic group were not completely reversed. In liver pretreatment with quercetin in Roundup treated group reversed altered changes near to control values while levels of fluoride and co-treated toxic groups were reversed non-significantly. In kidney pretreatment with quercetin in fluoride treated group reversed altered changes while LPO levels of Roundup and cotreated toxic groups were not normalized when compared to control group.

Keywords: Biochemical; Fluoride; Lipid peroxidation; Quercetin; Rats; Roundup.

INTRODUCTION

Pesticides are among the most widely used chemicals in agriculture. Their wide spread application in agricultural and veterinary field constitutes a major role in contamination of the environment. The use of pesticides to eliminate pests or to regulate crop growth has led to pesticide residues in soils, air, water, stored grains, crops and plants at concentration levels which exceed the legal limits[1,2]. Their residues in human food even in extremely small quantities are considered as potential risk to human health. Human beings and livestock interact with their environment and consequently are exposed to broad spectrum of synthesized chemicals present in the food they eat, water they drink and the air they breathe. Pesticides applied in agriculture never reach target organisms only but instead are also dispersed in air, water and soil. Pesticides permeate into the soil with rains and mix with ground waters contaminating them. Pesticides may drain into the streams, rivers, and lakes polluting the water sources and killing living organisms in water reservoirs[3]. Roundup, represents one of the most commonly applied herbicides in the world with applications in agriculture, forestry, industrial weed control, lawns, gardens and aquatic environments[4,5]. Roundup® is a glyphosate product which is formulated as a concentrate containing 41% glyphosate as isopropylamine and 15% as polyoxyethyleneamine. Studies indicate that the surfactant polyoxyethyleneamine or polyethoxylated tallow amine (both abbreviated POEA), used in some commercial glyphosate-based formulations may be more toxic by the oral route to animals than glyphosate itself[6-8].

Fluorosis is an endemic public health problem in 23 nations around the world including India, where it is endemic in 17 out of 32 States and Union territories[9]. A much elevated concentration of fluoride, ranging from more than 1.5 parts per million (ppm) to 20 ppm in surface, subsurface and thermal waters in nine States including J&K which is reported to have fluoride concentration ranging from < 0.2 to 18 ppm, is beyond the permissible limit fixed by the WHO for human beings[10]. In recent years, soft tissue involvement in fluorosis has attracted increasing attention with convincing evidence from fluorosis patients and animal studies demonstrating damage/involvement of soft/tissue/organ systems. Oxidative stress is being increasingly recognized as one of the possible mechanisms implicated in both fluoride and Roundup® induced toxicity. Quercetin, a flavonoid, has been reported to increase the genomic stability in rats, antioxidative defence system and decrease the incidence of cardiovascular and neoplastic diseases by up regulating antioxidant enzymes[11]. It may scavenge reactive oxygen species (ROS), chelate metal ions and acts as chain-breaking antioxidant by scavenging lipid peroxide radicals

or integrate into the lipid bilayer to prevent lipid damage[12]. With these reports in mind, we have investigated the toxicological biochemical alterations and lipid peroxidative damage in rats co-exposed to Roundup®, fluoride and the antioxidant role of quercetin against such induced changes.

MATERIALS AND METHODS

Experimental animals

Adult wistar rats (200-250 g body weight) of either sex procured from Indian Institute of Integrative Medicine (CSIR), Jammu were used in the study. The animals were maintained under standard managemental conditions. Prior to commencement of experiments, all the animals were quarantined and acclimatized in the laboratory conditions for a period of 2 weeks. The animals were provided with standard pelleted feed and water *ad libitium*. Daily cycle was rotated in twelve hours of light and darkness. All the experimental animals were kept under observation during entire period of study.

Plan of work

The study was conducted for a period of 28 days. The animals were divided into nine groups in a completely randomized design with six rats of either sex in each group. Group I served as control and no chemical was administered to them while Group II received Roundup® orally @ 1/10th LD_{50}[7,13,14]. Group III was administered fluoride in drinking water @ 10 ppm. J&K is reported to have fluoride concentration ranging from < 0.2 to 18 ppm [9], hence dose rate of 10 ppm fluoride was selected for this trial. Group IV was administered quercetin orally @ 10 mg/kg body weight[15], CMC (0.5%) was used as vehicle for quercetin administration. Group V was treated with Roundup® & quercetin, Group VI was administered Roundup® as oral gavage & fluoride in drinking water, Whereas Group VII was given water containing fluoride and quercetin as oral gavage. Group VIII was given fluoride in drinking water while Roundup® & quercetin as gavage. Group IX acted as Quercetin vehicle control and was administered 0.5% CMC orally. The protocol for conducting the experiment was duly approved by Institutional Animal Ethics Committee.

Xenobiotics used

Roundup® (41 % glyphosate EC) was commercially obtained from Monsanto India Ltd, Mumbai in 1 litre pack. Sodium fluoride (NaF) was obtained from SD Fine Chemicals Ltd. Mumbai while Quercetin was obtained from Sigma Aldrich Ltd., as Quercetin hydrate.

Sample collection and processing

At the end of experiment, the animals were sacrificed by cervical dislocation and blood was collected by cardiac puncture (4 ml) in aliquots containing heparin@10 I.U/ml as an anticoagulant.Prior to centrifugation 20 µl whole blood was used for hemoglobin estimation. Then blood was centrifuged for 10-15 minutes @ 3000 rpm and plasma was harvested for analysis of various biochemical parameters by the standard kit methods. The RBC's were washed with normal saline solution three times before preparing the RBC lysate. The erythrocyte sediment obtained after harvesting of plasma was utilised for obtaining lysate. Washing of RBC's was undertaken by diluting RBC sediment with normal saline solution (NSS) in the ratio of 1:1 on v/v basis and supernatant was discarded along with buffy coat and again NSS was added to it on v/v basis, mixed gently and centrifuged for 10 min at 3000rpm. This process was repeated thrice. After final washing 33 percent haemolysate (330µl washed RBC+ 670µl 0.1M PBS) was used for estimation of lipid peroxidation[16]. Organs like liver and kidneys were collected, cleaned from extraneous material. Tissues (one gram of tissue mixed with 9 ml chilled PBS) were homogenised, centrifuged and supernatant harvested for estimation of lipid peroxidation. Data collected during the experiment was subjected to analysis of variance (ANOVA) using statistical software SPSS and the significance was tested using Duncan Multiple Range Test at 5% ($P<0.05$) level.

RESULTS

Hemoglobin (Hb)

The results of repeated oral administration of Roundup® @ 500 mg/kg body weight, fluoride @10ppm and quercetin @ 10 mg/kg body weight alone and their combinations on Hb values for 28 days in wistar rats are shown in table **1.** The mean hemoglobin levels (g/dl) in group II (12.12±0.76), group III (12.55±0.62) and group VI (12.36±0.74) revealed a non-significant decrease in comparison to control group I (13.72±0.48) on 28th day of treatment. However there was no such alteration in quercetin pretreated groups V (13.08±0.61), VII (13.33±0.52) and VIII (12.78±0.43). In group IV and group IX, the mean Hb concentrations were 13.60±0.47 and 13.64±0.35 g/dl on 28th day of treatment.

Plasma aminotransferases

The results of repeated oral administration of Roundup® @ 500 mg/kg body weight, fluoride @10ppm and quercetin @ 10 mg/kg body weight alone and their combinations on plasma aminotransferases for 28 days in wistar rats are shown in table **1.** There was a significant ($P<0.05$) increase in the activity of plasma alanine aminotransferase (ALT) in groups II (66.81±2.84 IU/L) and VI (73.27±2.00 IU/L) as compared to control group I (52.08±3.68 IU/L), however levels of ALT were non significantly higher with co-administration of Roundup® and fluoride (Group VI) as compared to Roundup® (Group II) alone. Changes in ALT levels of group III (55.29±2.22 IU/L), group IV (53.74±3.30), group VII (55.41±1.48 IU/L) and group IX (53.70±2.27 IU/L) were non-significant in comparison to control group. Quercetin reversed altered changes in ALT as was evident from groups V (56.10±1.86 IU/L) and VIII (59.94±3.57 IU/L).

Table 1: Effect of repeated oral administration of Roundup, fluoride, quercitin alone and their combinations on different biochemical parameters in rats.

Parameter (Units)	Gr.I Control	Gr.II Roundup	Gr.III Fluoride	Gr.IV Quercitin	Gr.V Roundup +Quercitin	Gr.VI Roundup +Fluoride	Gr.VII Fluoride +Quercitin	Gr.VIII Roundup+ Fluoride+ Quercitin	Gr.IX CMC (0.5%) (Quercitin VehicleControl)
Hb g/dl	13.72±0.48^{a}	12.12±0.76^{a}	12.55±0.62^{a}	13.60±0.47^{a}	13.08±0.61^{a}	12.36±0.74^{a}	13.33±0.52^{a}	12.78±0.43^{a}	13.64±0.35^{a}
ALT IU/L	52.08±3.68^{a}	66.81±2.84^{bc}	55.29±2.22^{a}	53.74±3.30^{a}	56.10±1.86^{a}	73.27±2.00^{c}	55.41±1.48^{a}	59.94±3.57^{ab}	53.70±2.27^{a}
AST IU/L	95.59±4.38^{a}	111.08±5.9^{cd}	119.27±4.24^{de}	97.83±1.17^{ab}	102.38±2.14^{abc}	130.49±3.19^{f}	109.05±3.73^{bcd}	123.76±4.26^{ef}	99.23±2.06^{ab}
ALP IU/L	239.00±14.63^{a}	300.63±14.78^{c}	292.00±16.13^{bc}	236.79±14.67^{a}	242.93±12.55^{ab}	348.13±17.33^{d}	242.83±13.33^{ab}	264.05±23.65^{abc}	243.48±17.98^{ab}
ACP U/L	6.01±0.18a	6.10±0.25^{a}	6.88±0.14^{b}	6.07±0.18^{a}	6.06±0.28^{a}	7.25±0.14^{b}	6.04±0.26^{a}	6.63±0.25^{ab}	6.05±0.31^{a}
BUN mg/dl	42.90 ±0.37^{a}	44.15±0.53^{ab}	45.10±1.89^{ab}	43.93±1.03^{ab}	44.65±0.80^{ab}	46.44 ±0.92^{b}	44.03±0.64^{ab}	44.92±0.86^{ab}	43.68±0.58^{ab}
CR mg/dl	0.63±0.015^{a}	0.73±0.019^{a}	0.68±0.013^{a}	0.64±.029^{a}	0.67±0.077^{a}	0.74±0.014^{a}	0.67±0.038^{a}	0.69±0.029^{a}	0.63±0.016^{a}

Values given are mean ± SE of the results obtained from 6 animals unless otherwise stated.

Means with at least one common superscript do not differ significantly at 5% (P<0.05) level of significance

The values of plasma aspartate aminotransferase (AST) showed significant (P<0.05) increase in groups II (111.08±5.9 IU/L), III (119.27±4.24 IU/L) and VI (130.49±3.19 IU/L) as compared to control group I (95.59±4.38 IU/L). Fluoride treated rats (Group III) showed significantly higher levels of AST than Roundup® treated rats (Group II). Levels of AST were significantly higher with co-administration of Roundup® and fluoride (Group VI) as compared to Roundup® (Group II) and fluoride (Group III) alone. Pre-treatment with quercetin in group V (102.38±2.14 IU/L) decreased the values of AST near to control values. However quercetin treatment in group VII (109.05±3.73 IU/L) and VIII (123.76±4.26 IU/L) though decreased but failed to normalize the altered values. Alterations in AST levels in groups IV (97.83 ±1.17 IU/L) and IX (99.23±2.06 IU/L) were non-significant.

Plasma phosphatases

The results of repeated oral administration of Roundup® @ 500 mg/kg body weight, fluoride @10ppm and quercetin @ 10 mg/kg body weight alone and their combinations on the activity of plasma phosphatases for 28 days in wistar rats are shown in table **1**. A significant (P<0.05) increase in plasma alkaline phosphatase (ALP) level was observed in groups II (300.63±14.78 IU/L), III (292±16.13 IU/L) and VI (348.13±17.33 IU/L) as compared to control group I (239±14.63 IU/L). Levels of ALP were significantly higher with co-administration of Roundup® and fluoride (Group VI) as compared to Roundup® (Group II) and fluoride (Group III) treatment alone. Increase in the levels of ALP in Roundup® (Group II) and fluoride (Group III) treated groups differed non-significantly from each other.

Quercetin treated groups V (242.93 ±12.55 IU/L), VII (242.83±13.33 IU/L) and VIII (264.05±23.65 IU/L) showed the levels returning towards normal range. Changes in ALP levels of group IV (236.79.29±14.67 IU/L) and group IX (243.48±17.68) were non-significant in comparison to control group.

Observations in the activity of plasma acid phosphatase (ACP) showed significant increase in groups III (6.88±0.14 IU/L) and VI (7.25±0.14 IU/L) as compared to control group I (6.01±0.18 IU/L), but no significant difference was observed between III and VI group. Levels of ACP were reversed by quercetin pretreatment as evident from groups VII (6.04±0.26 IU/L) and VIII (6.63±0.25 IU/L). Non-significant alterations were observed in other groups in comparison to control group.

Plasma creatinine and Blood urea nitrogen (BUN)

The results of repeated oral administration of Roundup® @ 500 mg/kg body weight, fluoride @10ppm and quercetin @ 10 mg/kg body weight alone and their combinations on the activities of plasma creatinine and BUN for 28 days in wistar rats are shown in table **1**. Treated groups revealed non-significant (P<0.05) alterations in creatinine concentration as compared to control group. Creatinine levels of Roundup treated rats in group II (0.73 ± 0.019), fluoride treated rats in group III (0.68 ± 0.013) and co-treated rats of group VI (0.74 ± 0.014) showed elevated levels of creatinine compared to rats in control group I (0.63 ± 0.015) but these alterations were statistically non-significant.

BUN value showed significant increase in group VI (46.44 ± 0.92 mg/dl) following the co-administration of Roundup® and fluoride as compared to control value (42.90 ± 0.37 mg/dl), which was reversed to normal by quercetin pretreatment as evident from group VIII (44.92 ± 0.86 mg/dl) but this increase was non-significant in comparison to administration of Roundup® (44.15 ± 0.53 mg/dl) or fluoride treated (45.10 ± 0.1.89 mg/dl) groups (Group II & III). Other groups showed non-significant variations in BUN values as compared to control.

Lipid peroxidation

The results on lipid peroxidation following repeated oral administration of Roundup® @ 500 mg/kg body weight, fluoride @10ppm and quercetin @ 10 mg/kg body weight alone and their combinations for period of 28 days in wistar rats are shown in table **2**. Significant (P<0.05) increase in MDA levels in erythrocytes, liver and kidney was observed in groups II (4.31 ± 0.07, 46.79 ± 1.02 & 51.49 ± 0.98), III (3.83 ± 0.07, 52.90 ± 0.98 & 49.08 ± 0.77) and VI (4.54 ± 0.05, 61.28 ± 1.40 & 52.39 ± 1.19) as compared to control group I (3.41 ± 0.06, 36.51 ± 0.71 & 39.79 ± 1.13). Significant increase in LPO was observed in group VI (4.54 ± 0.05 & 61.28 ± 1.40) with the co-administration of Roundup® and fluoride as compared to Roundup® treated group II (4.31 ± 0.07 & 46.79 ± 1.02) and fluoride treated group III (3.83 ± 0.07 & 52.90 ± 0.98) in erythrocytes and liver while kidneys showed levels of 52.39 ± 1.19, 51.49 ± 0.98 & 49.08 ± 0.77 in groups VI, II and III respectively indicating a non-significant alteration. Roundup® treated rats (4.31 ± 0.07) have significantly higher values in erythrocytes than fluoride treated rats (3.83 ± 0.07) while it is vice versa in liver. The values for LPO levels in liver for group II and group III were 46.79 ± 1.02 & 52.90 ± 0.98 respectively. Pretreatment with quercetin in groups V (3.54 ± 0.07) and VII (3.46 ± 0.07) reversed altered changes in LPO near to control values (3.41 ± 0.06) in erythrocytes while levels of group VIII (3.91 ± 0.10) were reversed but non-significantly. In liver pretreatment with quercetin in groups V (38.94 ± 1.34) reversed altered changes near to control values (36.51 ± 0.71) while LPO levels of groups VII (45.37 ± 1.55) and VIII (54.07 ± 0.55) were reversed but non-significantly in comparison to control values.

In kidney pretreatment with quercetin in groups VII (43.39 ± 1.72) reversed altered changes while LPO levels of groups V (45.95 ± 1.19) and VIII (46.33 ± 0.85) were reversed but not normalized when compared to control group (39.79 ± 1.13).

Table 2: Effect of repeated oral administration of Roundup, fluoride, quercetin alone and their combinations on Lipid peroxidation (n mole MDA formed/ml erythrocytes or g tissue) in wistar-rats.

Groups ↓	RBC	Liver	Kidney
Group-I (Control)	3.41 ± 0.06^{ae}	36.51 ± 0.71^{ab}	39.79 ± 1.13^{a}
Group-II (Roundup)	4.31 ± 0.07^{c}	46.79 ± 1.02^{c}	51.49 ± 0.98^{d}
Group-III (Fluoride)	3.83 ± 0.07^{b}	52.90 ± 0.98^{d}	49.08 ± 0.77^{cd}
Group-IV (Quercetin)	3.22 ± 0.07^{e}	35.18 ± 1.59^{a}	40.23 ± 1.36^{a}
Group-V (Roundup + Quercetin)	3.54 ± 0.07^{a}	38.94 ± 1.34^{b}	45.95 ± 1.19^{bc}
Group-VI (Roundup + Fluoride)	4.54 ± 0.05^{d}	61.28 ± 1.40^{e}	52.39 ± 1.19^{d}
Group-VII (Fluoride + Quercetin)	3.46 ± 0.07^{a}	45.37 ± 1.55^{c}	43.39 ± 1.72^{ab}
Group-VIII (Roundup + Fluoride + Quercetin)	3.91 ± 0.10^{b}	54.07 ± 0.55^{d}	46.33 ± 0.85^{bc}
Group-IX (CMC 0.5%) (Quercetin Vehicle Control)	3.35 ± 0.06^{ae}	38.77 ± 0.74^{b}	39.35 ± 2.68^{a}

Values given are mean ± SE of the results obtained from 6 animals unless otherwise stated.

Means with at least one common superscript do not differ significantly at 5% ($P<0.05$) level of significance.

DISCUSSION

The non-significant decrease in hemoglobin in our study corroborates with the other studies [13,14]. The production of reactive oxygen species may be the cause for change in hematological parameters. Exposure to toxicants glyphosate and fluoride might have led to the formation of methemoglobin by free radical generation[13,17]. In addition fluoride intoxication is also known to cause bone-marrow depression[18]. Glyphosate might have induced anemia by reducing the activity of Ferric reductase as it is required for iron absorption in the gut. In plants it is reduced by 50% within six hours of treatment, so it might have a similar effect in mammals, as evidence from plants support the idea that glyphosate may directly interfere with heme synthesis also[19]. Glyphosate inhibits 5Enolpyruvyl Shikimate 3Phosphate synthase (EPSP synthase) and it interferes with the reaction of the enzyme with pyridoxal 5'phosphate (catalyst), probably by binding at the same site, suggesting that glyphosate might have the same effect for δ aminolevulinic acid synthase (ALAS), the enzyme that catalyzes the first step of pyrrole synthesis.

Similar to the present study, increase in aminotransferases by single and co-supplementation of fluoride with pesticides was recorded by various other studies[5,13,20,21]. All the reported findings are consistent with the results obtained in our study. Similar to the results of our study, amelioration of induced biochemical alterations by quercetin pretreatment has been reported by various authors[22-24]. Increased levels of aminotransferases in blood have been used as an indicator of altered permeability of plasma membrane[25] and/or cellular damage[26]. Liver is predominant organ for metabolism of xenobiotics including pesticides and also the first organ exposed to ingested toxins due to portal blood supply[27]. Therefore, toxic responses occur more in liver as compared to other organs. Alterations of these enzymes are indicators of the liver health status.

Several studies reported similar increase in phosphatase activity in fluorotic cows[28,29] and rats[21]. Increased phosphatase activity following glyphosate treatment observed in the present study is also reported by others[13,30]. Phosphatases are important & critical enzymes in biological processes and are responsible for detoxification, metabolism and biosynthesis of macromolecules for different essential functions.

Any interference in these enzymes leads to biochemical and cellular impairment and lesions of the tissue[31]. The increase in alkaline and/or acid phosphatases following exposure to fluoride or Roundup alone and in combination as seen in present study indicate damage to liver and other organs at varying extents but exposure of rats to both Roundup & fluoride together severely damages the liver as indicated by significantly elevated ALP levels than the single administration.

Similar non-significant variations in creatinine and BUN following Roundup administration in mice and rats were reported[5,13].

Administration of 10 ppm NaF in mice for 21 days resulted in elevated levels of BUN and creatinine[32]. Increased levels of blood urea nitrogen as seen in the group VI is indicative of toxic potentiation, this may be correlated with increased level of protein catabolism in mammalian body and also with increased breakdown of tissue or dietary protein[33]. Increase in the BUN and creatinine levels suggest impairment in glomerular function and reduced ability of the kidney to eliminate the toxic metabolic substances[32,34].

Lipids are one of the most susceptible targets of free radicals[35]. Such increase in lipid peroxidation has also been reported in children during fluoride toxicity and also in fluoride treated rats[36]. Increased lipid peroxidation has also been reported[37] in rats exposed to fluoride and ethanol. A similar increase in lipid peroxidation in rats treated with sodium fluoride and katron was also observed[38]. Increase in lipid peroxidation of the

erythrocytes in rats by exposure to fluoride and/or chlorpyrifos was also reported[39]. Similar increased value of LPO and decreased antioxidant enzyme activities have been reported in glyphosate treated rats[40,41]. Pretreatment of quercetin prevented lipid peroxidation in lindane treated rats, there was normalization of increased triglyceride and cholesterol levels[42]. Reports of protection of lipid peroxidation due to glyphosate by vitamin C and E are also available[43].

Most of these studies are in conformity with the results obtained in the present study. Reports indicate potential of pesticides to induce lipid peroxidation[44]. LPO, which is initiated by ROS, is the process of auto-oxidation of polyunsaturated fatty acid in response to toxic substances[45]. Lipid peroxidation has been extensively used as a marker of oxidative stress[46]. It is well known that MDA is a terminal product of lipid peroxidation, so the content of MDA can be used to estimate extent of lipid peroxidation. Increased lipid peroxidation as seen in the present study suggests that fluoride and Roundup alone and in combination induce oxidative stress and there is potentation of this effect in erythrocytes and liver by co-administration while quercetin pretreatment has a protective role.

Pre treatment with quercetin reversed the changes comparable to control value which depict its modulating effect on altered biochemical levels. Antioxidants have the capacity to scavenge free radical directly or interfere with the generation of free radical events. Antioxidant enzymes that scavenge intermediates of oxygen reduction provide the primary defence against cytotoxic oxygen radical. Quercetin inhibits resorption of xenobiotics in renal tubules thereby accelerates the elimination of toxicants[47]. Furthermore, quercetin has been reported to mediate cytoprotection through induction of heme oxygenase (HO)-1, which has a potent antioxidant property[48]. It acts as hepato-protective by scavenging ROS and minimizing the oxidative stress thus normalized the altered enzyme levels. This explains the modulating effect of quercetin on changed hematological parameters. The structure of quercetin plays an important role in its antioxidant effect. The o-dihydroxy-structure in the B-ring has been observed to confer higher stability to the radical form and to participate in electron delocalization. Flavonoids are known to anchor on the polar head of the main phospholipids. Hence, quercetin distributed on the surface of the lipid bilayers as well as in the aqueous phase could scavenge free radicals and act as antioxidants.

The kidney has a remarkable ability to compensate for a loss in functional renal mass, consequently, chemically induced changes in renal function may not be detected until these compensatory mechanisms are overwhelmed by significant nephron loss and/or damage[49]. As the duration of treatment was less (28 days), no significant elevation of creatinine and BUN was observed in treated groups except marked elevated BUN levels in co-treated group VI probably due to more severe damage.

CONCLUSION

Significant alterations in MDA levels and biochemical parameters were observed with exposure of toxicants alone or in combination but alterations were more pronounced in co-administered toxic groups.

Quercetin in the present study appears to be a non-toxic substance that leads to the normalization of changes caused due to Roundup and/or fluorine compounds and can be exploited for protection of workers exposed to excessive emissions of these chemicals, however amelioration by quercetin alone is not completely protective.

Acknowledgement: Authors are highly thankful to the Vice Chancellor, SKUAST-Jammu, Dean, FVSc & AH and Head, Division of pharmacology and Toxicology FVSc & AH, R. S. Pura, Jammu for providing necessary facilities for conducting the experiment.

REFERENCES

1. Susse S, Muller H, Pesticide analysis by micellar electrokinetic capillary chromatography, Journal of Chromatography, 730, 1996, 337-343.
2. Hala H, Elbardy BE, Effect of chlorfenapyr on some biochemical and haematological parameters and its residues in tissue of male albino rats, Egypt Journal of Applied Sciences, 21(11B), 2006, 674-689.
3. Pandey G, The Role of Biopesticides as a Challenge and Opportunities in the Field of Agriculture: A Review and Study of Green Chemistry, International Journal of Advance Research and Innovation, 3(1), 2015, 53-59.
4. Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P, Histopathological effects of Roundup, a glyphosate herbicide, on Nile tilapia (Oreochromis niloticus), Sci. Asia, 28, 2002, 121–127.
5. Tizhe EV, Ibrahim N, Fatihu M, Igbokwe I, George B, Ambali S, Shallangwaa J, Serum biochemical assessment of hepatic and renal functions of rats during oral exposure to glyphosate with Zinc, Comp Clinical Pathology, 6, 2013, 1740.
6. Williams GM, Kroes R, Munro IC, Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans, Regul. Toxicol. Pharmacol, 31(2), 2000, 117-165.
7. Bradberry SM, Proudfoot AT, Vale JA, Glyphosate poisoning, Toxicological Reviews, 23(3), 2004, 159-167.
8. Mesnage R, Berney B, Seralini GE, Ethoxylated adjuvants of glyphosate based herbicides are active principles of human cell toxicity, Toxicology, 10, 2013, 1016.
9. Susheela AK, Fluorosis management programme in India, Curr Sci, 77, 1999, 1250-6.
10. Sharma SK, High fluoride in groundwater cripples life in parts of India, Diffuse pollution conference Dublin, 2003.

11. Tieppo J, Vercelino R, Dias AS, Silva Vaz MF, Silveira TR, Marroni CA, Evaluation of the protective effects of quercetin in the hepatoppulmonary syndrome, Food Chem Toxicol, 45, 2007, 1140-1146.
12. Chander V, Singh D, Chopra K, Catechin, a natural antioxidant protects against rhabdomyolysis-induced myoglobinuric acute renal failure, Pharmacological Research, 48(5), 2003, 503–509.
13. Raquel j, Gabriel O, Locatelli CP, Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate Roundup, Interdisciplinary Toxicology, 5, 2012, 133–140.
14. Tizhe EV, Ibrahim N, Fatihu M, Igbokwe I, George B, Ambali S, Shallangwaa J, Haematogical changes induced by subchronic glyphosate exposure: ameliorative effect of zinc in Wistar rats, Sokoto Journal of Veterinary Sciences, 11(2), 2013, 28-35.
15. Duarte J, Perez PR, Vargas F, Ocete MA, Perez VF, Zaruelo A, Tamargo J, Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats, British Journal of Pharmacology, 133(1), 2001, 117-124.
16. Shafiq-ur-Rehman, Lead induced regional lipid peroxidation in brain, Toxicology letters, 21(3), 1984, 333-337.
17. Dudarev VP, Respiratory function of the blood. In: Seredenko, M. M. (ed.). Mechanisms of Development and Compensation of Haemic Hypoxia. 1987.pp. 200. Naukova Dumka, Kiev.
18. Blood DC, Radostits OM, Veterinary Medicine. 7th edn 1989. pp. 1258.ELSB/ Bailliere Tindall, London.
19. Ozturk L, Yazici A, Eker S, Gokmen O, Rmheld V, Cakmak I, Glyphosate Inhibition of Ferric Reductase Activity in Iron Deficient Sunflower Roots, New Phytologist, 177, 2008, 899-906.
20. Hanen B, Sabeur K, Kamel J, Tahia B, Fatma A, Feriel E, Najiba Z, Effects of sodium fluoride on the hepatic toxicity in adult mice and their suckling pups, Pesticide Biochemistry and Physiology, 86(3), 2006, 124-130.
21. Zabulyte D, Uleckiene S, Kalibatas J, Paltanaviciene A, Jascaniniene N, Stosik M, Bulletin of the Veterinary Institute in Pulawy, 51, 2007, 79-82.
22. Czerny B, Put A, Mysliwiec Z, Juzyszyn Z, The Influence of Quercetin on Some Biochemical Parameters in Rats Exposed to Environmental Contamination with Fluorine Compounds, Polish Journal of Environmental Studies, 9(3), 2000, 157-161.
23. David C, Rodrigues G, Bona S, Meurer L, Gonzalez J, Tunon M, Marrino N, Role of quercetin in thioacetamide-induced liver injury in rats, Toxicologic Pathology, 39, 2011, 949-957.
24. Waheed MPA, Mohammed HSM, Fenvalerate induced Hepatotoxicity and its amelioration by Quercetin, International Journal of Pharm Tech Research, 4, 2012, 1391-1400.
25. Ramazotto JL, Carlin R, Effect of DMSO on SGOT during hypothermia adrenalectomized rats, Life Sciences, 22, 1978, 329-336.
26. Drotman RB, Lawhorn GT, Serum enzymes as indicators of chemically induced liver damage, Drug & Chemical Toxicology, 1(2), 1978, 163-171.
27. Poppy JA, Cattley RC, Hepatobillary system In: Haschek, W. M. and Rousseaux, C. G. (eds.), Handbook of Toxicologic Pathology 1991; pp 279-315. Academic Press, Inc. San Diego.
28. Singh JL, Swarup D, Biochemical changes in serum and urine in bovine fluorosis, Indian Journal of Animal Sciences, 69(10), 1999, 776-778.
29. Muralidhara A, Sastry KNV, Plasma fluoride, Creatinine and alkaline phosphatase level in bovine dental fluorosis in Karnataka, National symposium on "Strategies for containment and control of economically important diseases of livestock". 2000. pp 8, Tirupati, India.
30. Benedetti AL, Vituri C de L, Trentin AG, Domingues MA, Alvarez-Silva M, The effects of subchronic exposure of wistar rats to the herbicide Glyphosate-Biocarb, Toxicology Letters, 153(2), 2004, 227-232.
31. Khan IA, Reddy BV, Mahboob M, Rahman MF, Jamil K, Effect of phosphorothionate on the reproductive system of male rats, Journal of Environmental Science and Health, 36(4), 2001, 445-456.
32. Khan I, Ranga A, Biochemical changes in Sodium Fluoride Induced kidney toxicity of Swiss Albino mice and it's Amelioration by Ascorbic Acid, International journal of advances in pharmacy, biology and chemistry, 3(4), 2014, 2277-4688.
33. Bush BM, Interpretation of laboratory results for small animal clinicians, 1991, Blackwell Scientific Publication, London.
34. George GS, Wakasi ME, Egoro E, Creatinine and urea levels as critical markers in end-stage renal failure, Research and Review, Journal of Medical and Health Sciences, 3(1), 2014, 41–44.
35. Rajani G, Purnima A, *In vitro* antioxidant and antihyperlipidemic activities of Bauhinia variegata Linn, Indian J Pharmacol, 41, 2009, 227-232.
36. Shivarajashankara YM, Shivashankara AR, Rao SH, Bhat PG, Oxidative stress in children with endemic skeletal fluorosis, Fluoride, 34, 2001, 103–107.
37. Inkielewicz I, Krechniak J, Gdansk, Lipid peroxidation and antioxidant enzyme activity in rats exposed to fluoride and ethanol, Fluoride, 39, 2006, 53-59.
38. Hussein MS, Abdou KA, Mahmoud AS, Abd-El-Rahaman, W, Toxic interaction of pyrethroid (Katron) and sodium fluoride on reproductive performance of male albino rats, Veterinary Medical Journal, 54, 2008, 219-241.
39. Baba NA, Raina R, Pawan KV, Sultana M, Prawez S, Nisar AN, Toxic effects of fluoride and chlorpyrifos on antioxidant parameters in rats: protective effects of vitamins C and E, Research report Fluoride, 46(2), 2013, 73–79.
40. Beuret CJ, Fanny Z, Maria SG, Effects of herbicide glyphosate on liver lipid peroxidation in pregnant rats and their foetuses, Reproductive Toxicology, 19(4), 2004, 501-504.

41. Vivian DCL, Claudia BRM, Toxicity and effects of glyphosate based herbicide on the Neotropical fish, Comp Biochem Physiol C, Toxicol Pharmacol, 2, 2007, 222-231.

42. Viswanadha VP, Gurusamy L, Nicholson PS, Rathinasamy B, Effect of quercetin against lindane induced alterations in the serum and hepatic tissue lipids in wistar rats, Asian Pac J Trop Biomed, 2, 2012, 910-915.

43. Gehin A, Guyon C, Nicod L, Glyphosate induced antioxidant imbalance in HaCaT: The protective role of vitamins C&E, Environ Toxic Pharmacol, 22, 2006, 27-34.

44. Raina R, Verma PK, Kusum, Kant V, Effect of repeated dermal application of α-cypermethrin on lipid peroxidation and antioxidant system in rats, Toxicology International, 16, 2009, 27-30.

45. Boots AW, Harnen GG, Bast A, Oxidant metabolism in chronic obstructive pulmonary disease, European Respiratory Journal, 22(46), 2003, 14–27.

46. Sayeed I, Parvez S, Pandey S, Bin-Hafeez B, Haque R, Raisuddin, Oxidative Stress biomarkers of exposure to deltamethrin in fresh water fish, Channa punctatus Bloch, Exotoxicology Environmental Safety, 56(2), 2003, 295-301.

47. Kuhnau J, The flavonoids, A class of semi-essential food components: their role in human nutrition, World Rev Nutr. Diet, 24, 1976, 117-191.

48. Chen TJ, Jeng JY, Lin CW, Quercetin Inhibition of ROS Dependent and -Independent Apoptosis in Rat Glioma C6 Cells, Toxicology, 223, 2006, 113–126.

49. Schnellmann RG. Toxic responses of the kidney. In: Klassen, C. D. (ed.), Casarett and Doull's Toxicology: The basic science of poisons, 2001, pp. 491-514, McGraw-Hill Companies Inc, NewYork, USA.

Source of Support: Nil, **Conflict of Interest:** None.