Journal of Applied Life Sciences International
1(1): 1-16, 2014; Article no. JALSI.2014.001

SCIENCEDOMAIN *international*
www.sciencedomain.org

Potential Protective Effects of Blackberry and Quercetin on Sodium Fluoride-inducedImpaired Hepato-renal Biomarkers, Sex Hormones and Hematotoxicity in Male Rats

Hayat A. A. Ismail[1], Reham Z. Hamza[2] and Nahla S. El-Shenawy[3*]

[1]Department of Biology, Faculty of Science, King Abdel Aziz University, Jeddah, Saudi Arabia.
[2]Department of Zoology, Faculty of Science, Zagazig University, Zagazig 44519, Egypt.
[3]Department of Zoology, Faculty of Science, Suez Canal University, Ismailia, 41522, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. All authors designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author NSES managed the analyses of the study. Author RZH managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

Received 16th June 2014
Accepted 14th July 2014
Published 30th July 2014

ABSTRACT

Aim: The present study was carried out to evaluate the potential protective role of blackberry juice (BBJ) and quercetin (Q) against sodium fluoride-induced liver damage and impairment of kidney function, as well as hematotoxicity in rats.
Study Design: After 2 weeks of acclimation, animals were divided into seven groups, ten rats each. All the groups were treated interaperitoneal (i.p.) for 30 successive days. Group 1 served as untreated control and received 1ml/Kg of distilled water i.p. daily**.** Group 2 was given i.p. BBJ at a dose of 1.6g/kgb.wt. containing 5mg anthocyanin. Group 3 was given quercetin at dose of 75mg/Kgb.wt. Group 4 was treated with 10.3mg/kg b.wt of NaF. Group 5 was given i.p. BBJ followed by NaF at the same doses. Group 6 was given quercetin followed by NaF and finally group 7 was treated with BBJ and Q then followed by NaF as mentioned previous doses.
Methodology: Some hematological parameters were determined. Serum aspartate transaminase, alanine transaminase and alkaline phosphatase activities were as well as

**Corresponding author: E-mail: elshenawy_nahla@hotmail.com;*

lipid profile and total protein were evaluated as biomarkers for liver functions. The levels of uric acid and creatinine in serum were estimated. Hormones of testosterone and inhibin B were assessed.
Results: Sodium fluoride (NaF) caused an elevation in serum transaminases and alkaline phosphatase and reduced serum total protein, testosterone, and inhibin-B levels as well as levels of uric acid and creatinine, and induced hematotoxicity. It increased all the parameters of lipid profile except the high density lipoprotein cholesterol level was decreased. The presence of Q or BBJ with NaF successfully mitigated liver and kidney functions, which was more pronounced with Q.
Conclusion: It can be concluded that concomitant administration of Q or BBJ with NaF may be useful in reversing the toxicity of NaF in male rats.

Keywords: Blackberry; quercetin; hematological parameters; liver and kidney functions; lipid profile; Inhibin-B; testosterone.

ABBREVIATIONS

Q, quercetin; NaF, sodium fluoride; BBJ, blackberry juice, b. wt., body weight; i.p., interaperitoneal.

1. INTRODUCTION

Fluoride is a naturally occurring contaminant in the water and it is essential for normal maintenance of teeth and bones. However, prolonged exposure to high concentration of fluoride is found to be deleterious to teeth, bones and other organs. Besides drinking water, Fluoride can enter the body through food, dental products, drugs and industrial emission [1]. The widespread occurrence of per fluorinated compounds received worldwide attention because their accumulation in animal and human body can cause potential impairment of their health [2]. It persists in potentially active concentration for hours [3]. Fluoride compounds are used in the preparation of insecticide formulations. Intoxications mostly arise from exposure to sodium fluoride (NaF), sodium fluorosilicate, fluosilic acid, or hydrogen fluoride [4].

Fluoride crosses the cell membrane very rapidly [5], and is distributed in the skeletal and cardiac muscle, liver, skin, and erythrocytes [1,6-7]. *In vivo* studies [8-10] have proven that fluoride to be a cell toxin. The high toxicity of NaF arises from its being a very reactive ion. A study indicated that endoplasmic reticulum stress and inhibition of protein synthesis produced by fluoride during enamel development were responsible for dental fluorosis in the incisors of mice given water containing 50–100ppm of fluoride daily for 30 days [11]. Administration of NaF (50mg/l) in drinking water for 6 months produced renal oxidative stress and apoptosis in rats [12] resulting in a decrease in urinary excretion of fluoride, as it was found in human beings [10]. This may contribute to an elevation of the concentration of fluoride in the serum of NaF-treated animals. The chronic toxic action of fluoride also has been investigated in the liver. Oxidative stress was found to be produced by fluoride (100ppm) in the liver of rabbits [11].

Thus, the chronic toxic effects produced by fluoride in the kidney [10] and liver [13] of human beings had been demonstrated in experimental animals [1]. Fluoride was determined to cause adverse effects in mice on erythrocyte and liver tissue

malondialdehyde (MDA) levels and superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities [14]. They also reported that serum cholesterol, triglyceride, glucose, total protein and albumin levels, and aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were affected. In NaF-treated male rats (10mg/kg/day, for 30–50 days), decreased sperm motility and a reduction in serum testosterone levels have been reported [15]. Ortiz-Perez et al. [16] noticed that fluoride may be a reproductive toxicant in both Sertoli cells and gonadotrophs of human and caused reduction of inhibin-B and testosterone levels.

The use of natural antioxidant phytochemicals has surfaced as an effective and safe dietary reference for liver disease [17]. A major class of phytochemicals found ubiquitously in fruits and vegetables are flavonoids, which are rarely found to be toxic, and are highly efficient against ROS-mediated injury [18]. Therefore, flavonoids are popularly considered for the optional prevention and treatment of liver diseases.

One of the most important natural diets with anti-oxidant properties is blackberries (*Morusnigra*). Berries are among one of the most widely consumed fruits in the human diet [19]. Berry fruits, wild or cultivated, are proved as a traditional and rich source of bioactive compounds, possessing important biological activities such as flavonoid pigments (anthocyanin), some minerals (Na, K, Ca, Se, Zn and P), vitamins (vitamin A, B complex, C and E) phenolic acids (garlic, p-coumaric, caffeic, ferulic) and phenolic polymers [20]. The anti-oxidant capacity of these berries was related to their constituents' particularly total phenolics and anthocyanins [21]. Furthermore, anthocyanin is one of the most important water-soluble flavonoid pigments that give blackberries their characteristic red to blue color [22]. Moreover, it was found that, the contents of vitamin C, vitamin E, selenium and zinc in fresh fruit of blackberry has a good effect on human body through protection the integrity of cells and internal structure of cells, avoiding some enzymes and internal components of cells from being destructed. These contents have anti-oxidant and it can improve immunity, play an antagonistic role of protective agent from toxic substances [23].

Quercetin (Q), a flavonoid largely found in vegetable foods as broccoli, lettuce, apples, tomatoes, onions, tea and coffee with known anti-inflammatory and antioxidant properties [24,25]. Marcolin et al. [25] reported that quercetin decreases liver damage in mice with non-alcoholic steato-hepatitis which is a frequent condition in obese patients that may progress to end-stage liver disease. It has been reported that the biological effects of quercetin are as follow: Anti-cancer genic, antiviral, anti-ischemic, anti-inflammatory and antiallergenic, as well as preventive influence in atherosclerosis and coronary heart disease [26-28].

However there are little reports about the functional properties of the blackberry juice (BBJ) and its uses in alleviating the adverse effects of the chemical toxicity in co-administration with Q. Therefore, it seems of interest to evaluate the protective effect of BBJ against the toxicity of fluoride-induced free radicals in rats. For these reasons, the main objective of the present study was to evaluate the role of Q and BBJ on liver, kidney and hematology toxicity induced by NaF for 4 weeks. To achieve this aim, rats were given NaF and/or quercetin and blackberry for 4 weeks by interaperitoneal.

2. MATERIALS AND METHODS

2.1 Chemicals

Sodium fluoride (NaF) was purchased from Sigma Chemical Co., St. Louis, Mo., USA. The tested dose of NaF (10.3mg/kg b.wt) was chosen based on the previous studies of Zabulyte et al. [29]. Quercetin was purchased from Sigma Chemical Co., St. Louis, Mo., USA. It was given in a dose of 75mg/kg according to the previous study by Seiva et al. [30]. Fresh blackberry fruits were obtained from local market (Zagazig, Egypt), and then washed, homogenized and its juice was daily freshly prepared. The dose of blackberry (1.6g/kgb.w equal to 9ml/kgb.wt, which containing 5mg active constituent; anthocyanine) was used in this study according to the previous studies of Sauebin et al. [31] and Siriwoharn et al. [32]. All other chemicals used in the experiment were of analytical grade.

2.2 Experimental Animals

Seventy adult male Wistar albino rats (*Rattusrattus*) weighing 170-180g were purchased from King Fahed Medical Research Center in Jeddah (Kingdom of Saudi Arabia). The animals were housed in metal cages and bedded with wood shavings and kept under standard laboratory conditions of aeration and room temperature at about 25^{o}C with 12/12 light and dark cycle. The animals were allowed to free access of standard diet and water *ad libtum* throughout the experimental period. We have followed the European community Directive (86/609/EEC) and national rules on animal care that was carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals 8th edition. The animals were accommodated to the laboratory conditions for two weeks before being experimented.

2.3 Experimental Design

After 2 weeks of acclimation, animals were divided into seven groups, ten rats each. All the groups were treated interaperitoneal (i.p.) for 30 successive days. Group 1 served as untreated control and received 1ml/Kg of distilled water i.p. daily**.** Group 2 was given i.p. BBJ at a dose of 1.6g/kg b. wt. containing 5 mg anthocyanin. Group 3 was given quercetin at dose of 75mg/Kgb. wt. Group 4 was treated with 10.3mg/kgb. wt of NaF. Group 5 was given i.p. BBJ followed by NaF at the same doses. Group 6 was given quercetin followed by NaF and finally group 7 was treated with BBJ and Q then followed by NaF as mentioned previous doses.

2.4 Collection of Blood

Blood samples of the fasted rats were collected from the medial retro-orbital venous plexus immediately with capillary tubes (Micro Hematocrit Capillaries, Mucaps) under ether anesthesia [33]. Then, the blood was centrifuged at 3000rpm for 15 min and serum was collected for different biochemical analyses.

2.5 Hematological Parameters

Other heparinized tubes were used to collect the blood for determination some blood indices. A complete blood count including five major measurementswere determined using cell counter (Sysmex, model KX21N). White blood cells (WBC) were measured in thousands per cubic milliliter of blood. Red blood cells (RBC) were measured in millions per cubic mil-

limeter of blood. Hemoglobin (Hb) was measured in grams per deciliter (g/dL) of blood. The hematocrit value (PCV) is the percentage of red blood cells in relation to total blood volume was also determined. Mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were also calculated. Platelets were measured in thousands per cubic millimeter of blood.

2.6 Hepato-renal-biomarkers Determination

Serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) activities were determined with kits from Human diagnostic worldwide, Germany. The serum total cholesterol (TC) and triglycerides (TG) were determined by the method Carr et al. [34]. High density lipoprotein–cholesterol (HDL–c) was determined according to the methods of Warnick et al. [35]. Serum LDL–cholesterol (LDL–c) level was calculated according to Friedewald [36] formula:

$$\text{LDL-c} = \text{total cholesterol—(HDL-c + triglycerides)/5.}$$

Very low density lipoprotein cholesterol (VLDL-c) levels were calculated by using the following formula of Prakasam et al. [37]: VLDL-c = triglyceride/5.

The protein content was determined by the method described by the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules, CA. USA) using bovine serum albumin as the standard.

The levels of uric acid and creatinine in serum were estimated spectrophotometric using commercial diagnostic kits according to the manufacturer's instructions. The data were expressed as mg/dL.

2.7 Serum Testosterone and Inhibin-B Determination

Plasma testosterone levels were measured by electro chemi-luminescence by automat (Elecsys, Rochediagnostics) according to the method of Wheeler [38]. Briefly, the Elecsys testosterone assay is based on a competitive test principle using a monoclonal antibody specifically directed against testosterone. Endogenous testosterone released from the sample by ANS (8-anilino-1-naphthalene sulfonic acid) and norgestrel competes with the added testosterone derivative labeled with a ruthenium complex for the binding sites on the biotinylated antibody.

Inhibin B was assayed by using a commercial Enzyme-linked Immune Sorbent Assay (ELISA) kit (DSL-10-84100 Active, Beckman Coulter, Villepinte, France) according to the manufacturer's instructions. Each sample was diluted 2-fold in sample diluents solution prior to reactions.

2.8 Statistical Analysis

The SPSS 11.0 statistical software package programmer for Windows was used for statistical calculations. Data was given in the form of arithmetical mean values and ±standard error. Differences between groups were evaluated by Student t- tests and the level of statistical significance was set at $P \leq 0.05$.

3. RESULTS

In relation to the hematological parameters, male rats presented more pronounced changes when treated with NaF, in which a decrease in the Hb and HCT% of approximately 48.13 and 23.26%, respectively, was observed when compared to the control group (Table 1).

Results indicated that NaF caused a significant ($p<0.05$) increase in total leukocyte count (TLC), with simultaneous decrease in total erythrocyte count (TEC). However, there were insignificant alterations in platelet count (PLT) (Table 1). The present data did not show any significant changes in all tested parameters of RBCs or other hematological parameters of healthy rats that received BBJ.

Moreover, treatment the rats with Q alone did not affect tested hematological parameters except MCH% and MCV. However, the combinations of Q and BBJ with NaF alleviated the exerted hematotoxicity (Table 1).

The activities of AST, ALT and ALP were significantly increased in plasma of rats treated with NaF (Table 2). Treatment with BBJ alone did not cause any significant changes in the activity of these enzymes. While, the presence of BBJ with NaF minimized the observed alterations in examined enzymes activity induced by fluoride intoxication.

Treatment the rats with Q alone significant decreased the activities of ALS and ALP as compared to control by 12.06% and11.21%, respectively (Table 2). However, with the pretreatment of Q before NaF damage, the serum activities of ALT and AST were significantly decreased, compared to the NaF-intoxicated rats ($p<0.05$).

The NaF and Q caused greater decrease in ALT, AST and ALP activities than NaF and BBJ. The co-administration of rats with Q and BBJ before NaF decreased the activities of ALT and ALP more than NaF with BBJ (Table 2).

Sodium fluoride for30-days treatment every day caused significant abnormally in serum total protein. Quercetin alone or in combination with BBJ showed mitigating effects against NaF induced decrease in total protein content (Table 2). However, treatment the rats with BBJ alone or before NaF did not change in the level of protein as compared to control- and NaF-treated groups.

The treatment the rats with NaF caused significant increase in serum levels of total cholesterol, TG and LDL–c, and significant decrease in HDL–c (Table 3). Results revealed that there are significant changes in lipid profile of rats treated with BBJ alone as compared to control. However, the presence of BBJ with NaF can alleviate the adverse effects of NaF and the alterations of lipid profiles restored to the approximately the normal values.

Serum uric acid content reduced significantly in NaF treated rats by 54.95% as compared to control group (Fig. 1). However, i.p. administration of NaF along with Q or BBJ caused significant mitigation, as compared to NaF alone treated rats.

As compared to control, NaF treatment in rats for 30 days caused significant ($p<0.05$) rise in creatinine content by 2.55-fold (Fig.1). Co-treatment of Q and NaF or BBJ and NaF did not improve the creatinine levels, as compared to NaF treated mice. However the combination of Q and BBJ with NaF cause mitigation in creatinine level when compared to Q and NaF or BBJ and NaF.

Table 1. Changes in hematological parameters in male rats treated with blackberry, quercetin and sodium fluoride separately or in combination

Parameters	Groups						
	Control	Blackberry	Quercetin	Sodium fluoride (NaF)	NaF+blackberry	NaF+quercetin	NaF+blackberry+quercetin
RBCs(10^6/mm^3)	8.37±0.13	7.74±0.26	8.63±0.18	5.41±0.28[a]	6.43 ±0.20[b]	6.28±0.08[b]	7.24 ±0.10[c,d]
HB (g/dl)	12.84±0.29	13.10±0.17	14.09±0.13	6.66±0.41[a]	7.96±0.24[b]	9.15±0.24[b]	9.95 ±0.29[c]
HCT (%)	39.21±0.32	38.86±0.40	44.51±1.01[a]	30.09±0.47[a]	30.98±0.47	31.16±0.33	33.55 ±0.39[c,d]
MCV (fL)	46.83±1.09	50.44±1.63	51.53±1.25[a]	56.13±2.92[a]	48.27±1.20[b]	49.63±0.78	46.36±1.00[d]
MCH (Pg)	15.37±0.51	17.02±0.63	16.34±0.36	12.37±0.76[a]	12.39±0.40	14.56±0.37[b]	13.76±0.54
MCHC (g/dL)	32.71±0.56	33.71±0.18	31.69±0.45	22.05±1.13[a]	25.67±0.41[b]	29.33±0.48[b]	29.64±0.54[c]
WBCs (0^3/mm^3)	7.94±0.32	7.42±0.16	8.13±0.23	9.40±1.32	3.67±0.09[b]	4.78±0.17	4.90±0.28[c]
Platelets (0^3/mm^3)	549.43±3.40	569.16±5.42[a]	537.38±13.58	679.53±5.46[a]	614.48±7.37[b]	558.52±9.81[b]	588.33±4.03[c,d]

Values are expressed as means ± SE; n = 10 for each treatment group.; [a]significant difference as compared to control, [b]significant difference as compared to sodium fluoride group (NaF), [c]significant difference as compared to blackberry group and [d]significant difference as compared to quercetin group

Table 2. Changes in hepatic functions in male rats treated with blackberry, quercetin and sodium fluoride separately or in combination

Parameters	Groups						
	Control	Blackberry	Quercetin	Sodium fluoride (NaF)	NaF+blackberry	NaF+quercetin	NaF+blackberry+quercetin
ALT (U/L)	11.00±1.41	13.40±0.51	10.20±0.58	220.00±11.40[a]	99.40±6.66[b]	67.20±3.68[b]	68.60±4.47[c]
AST (U/L)	23.20±1.07	26.60±1.89	20.40±0.51[a]	118.80±19.77[a]	51.20±2.22[b]	38.00±1.14[b]	46.20±7.29
ALP (U/L)	23.20±0.97	21.80±0.86	20.60±1.08	94.00±7.62[a]	63.80±1.07[b]	60.40±3.39[b]	57.00± 2.63[c]
Total proteins (g/dL)	7.84±0.12	8.10±0.37	9.96±0.53[a]	4.03±0.17[a]	4.33±0.47	6.84±0.28[b]	7.02±0.22[c]

Values are expressed as means±SE; n=10 for each treatment group.; ALT; alanine transaminase, AST; aspartate transaminase and ALP; alkaline phosphatase; [a]significant difference as compared to control, [b]significant difference as compared to sodium fluoride group (NaF) and [c]significant difference as compared to blackberry group

Table 3. Changes of serum lipid profile in male rats treated with blackberry, quercetin and sodium fluoride separately or in combination

Parameters (mg/dL)	Groups						
	Control	Blackberry	Quercetin	Sodium fluoride (NaF)	NaF+blackberry	NaF+quercetin	NaF+blackberry+quercetin
TC	70.20±1.77	87.20±4.26[a]	68.80±6.80	159.40±6.91[a]	129.80±5.80[b]	112.20±3.35[b]	93.00±6.53[c,d]
TG	62.40±1.03	71.20±2.89[a]	64.00±1.18	175.00±7.54[a]	97.20±4.85[b]	89.80±2.82[b]	80.80±2.82[c]
HDL-c	40.20±0.66	33.60±1.08[a]	35.60±0.81[a]	11.60±1.63[a]	21.60±0.51[b]	24.80±1.88[b]	31.00±1.00[c,d]
LDL-c	49.06±1.74	66.18±4.11[a]	48.88±6.89	122.08±7.14[a]	104.04±6.29	87.68±3.32[b]	70.12±6.39[c,d]
VLDL-c	12.48±0.21	14.24±0.58[a]	12.80±0.24	29.00±2.64[a]	19.44±0.97	49.28±31.18	16.16±0.56[c]
Risk Ratio	1.74±0.05	2.59±0.11[a]	1.94±0.21	15.07±2.53[a]	4.12±0.24[b]	3.45±0.21[b]	2.77±0.19[c,d]

Values are expressed as means ± SE; n = 10 for each treatment group. [a]significant difference as compared to control, [b]significant difference as compared to sodium fluoride group (NaF), [c]significant difference as compared to blackberry group and [d]significant difference as compared to quercetin group

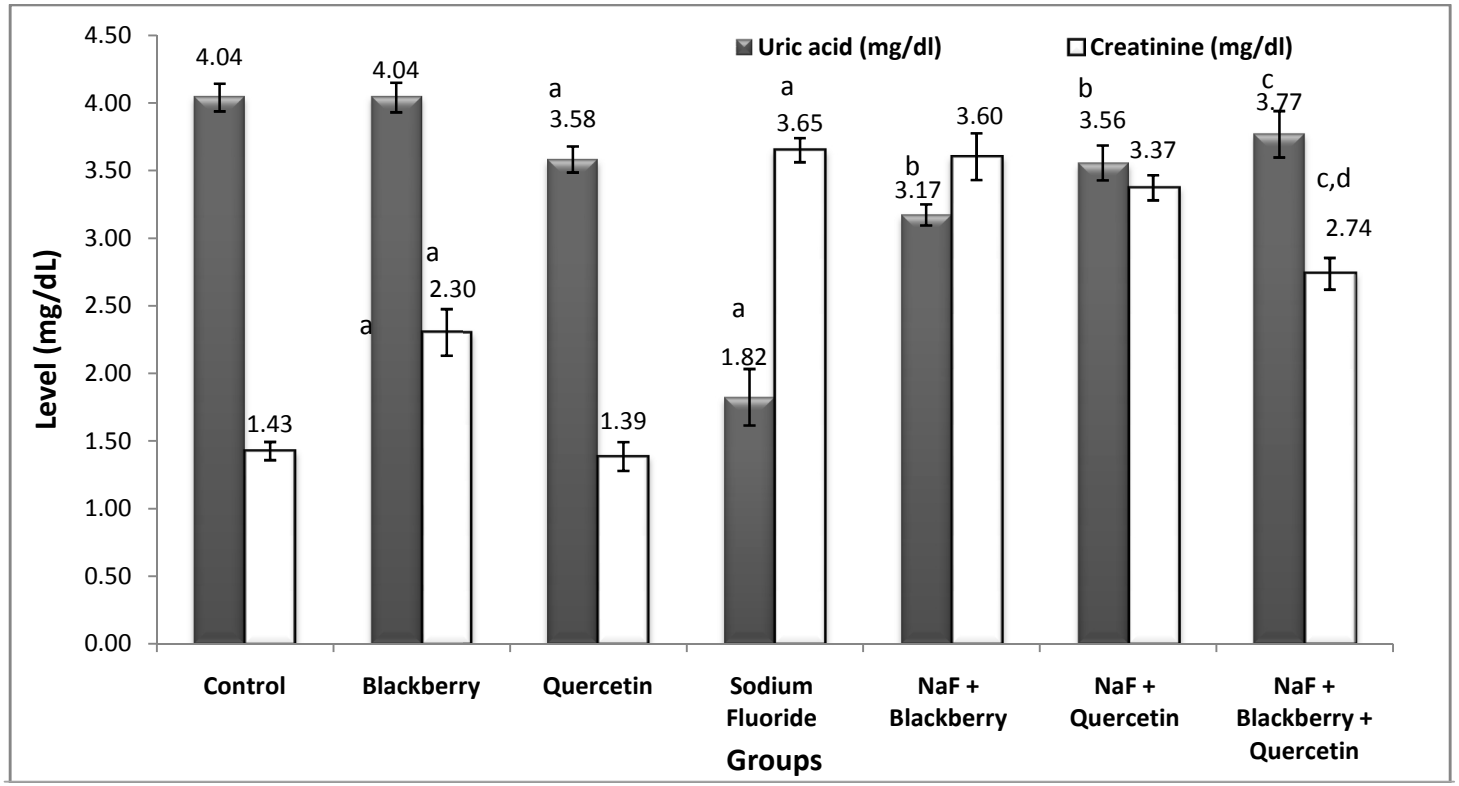

Fig. 1. Changes of serum creatinine and uric acid levels in male rats treated with blackberry, quercetin and sodium fluoride separately or in combination

Values are expressed as means±SE; n=10 for each treatment group.[a] significant difference as compared to control,[b] significant difference as compared to sodium fluoride group (NaF), [c] significant difference as compared to blackberry group and[d] significant difference as compared to quercetin group

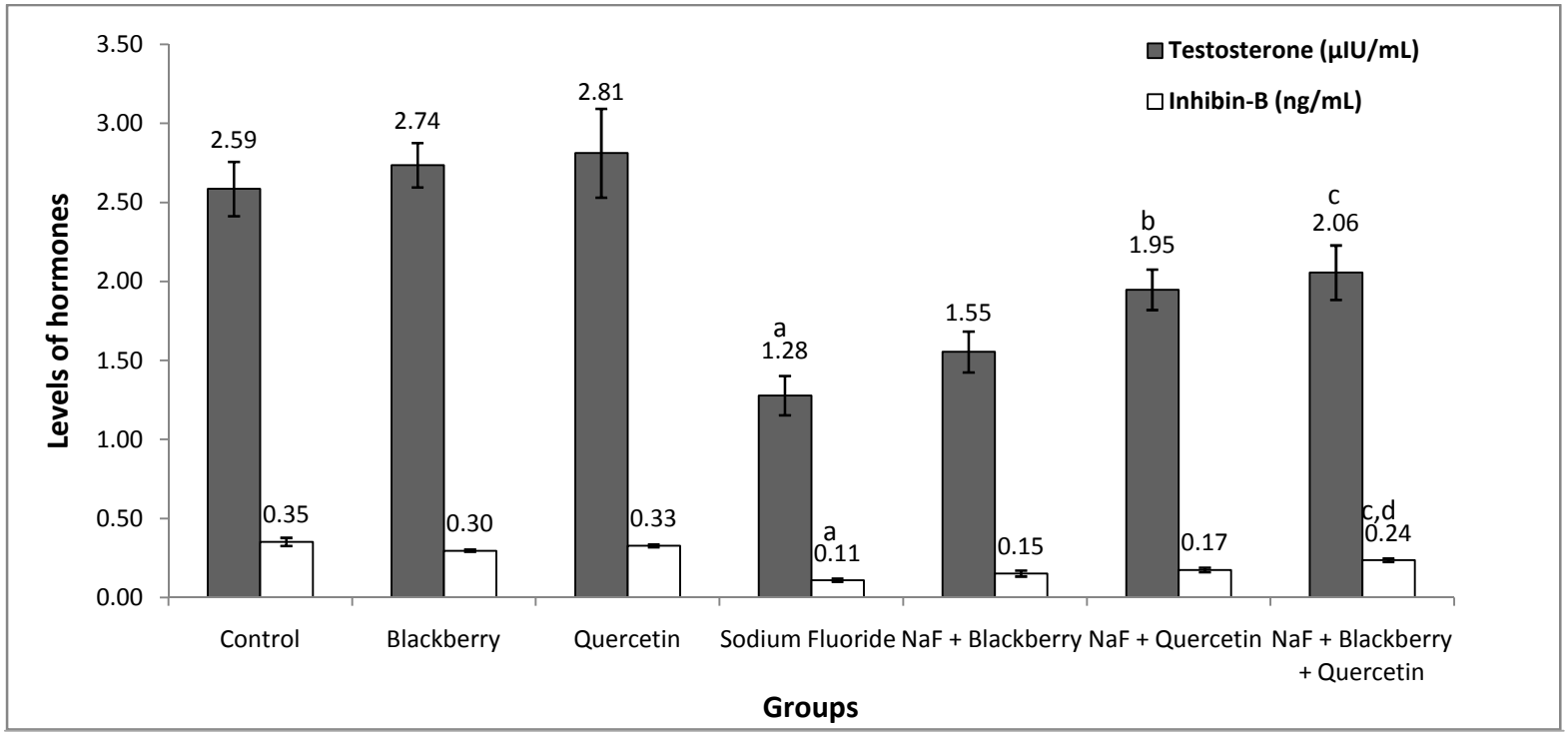

Fig. 2. Changes of serum testosterone and inhibin-B levels in male rats treated with blackberry, quercetin and sodium fluoride separately or in combination

Values are expressed as means±SE; n=10 for each treatment group.[a] significant difference as compared to control, [b] significant difference as compared to sodium fluoride group (NaF), [c] significant difference as compared to blackberry group and [d] significant difference as compared to quercetin group

As shown in (Fig. 2), NaF treatment decreased significantly ($p<0.01$) the plasma testosterone levels compared with control rats. The treatment of NaF-exposed rats with Q separately or in combination with BBJ entirely alleviated the NaF-induced decrease in plasma testosterone levels.

A significant reduction of inhibin-B was noticed in NaF-treated rats by 68.57% as compared to control group. There were not significant changes in the inhibin-B levels in healthy rats that had been treated with BBJ or Q. The Administration of Q in combination with NaF resulted in partial protection against NaF-induced inhibin-B changes. Meanwhile, treatment the rats with BBJ alone did not effect on the inhibin-B level. Concomitant administration of Q with BBJ against NaF resulted in satisfactory preservation of inhibin-B level.

4. DISCUSSION

Results obtained in the present study indicated that Q successfully alleviated hepatic and renal damage, maintained normal hematological parameters and mitigated the induction of sex hormones caused by NaF in male rats. In the present work, Q also protected from liver damages demonstrated by the decrease in plasma transaminases and ALP activities, with consequent restoration of plasma total protein. The observed hepatoprotective effect might be a consequence of the stabilization in the redox state and maintenance of the antioxidant capacity offered by Q [39]. It could be also attributed to calcium channel blocking activity exerted by Q. Calcium contents in liver cells are liable to be increased during the process of experimental liver damage, and calcium channel blocking drugs were found to inhibit the development of hepatic damage induced by different hepatotoxins [39,40].

We used the hematologic constituents as biomarkers for detection of hematotoxic effects of NaF. There is no data available about the effect of NaF alone or in combination with Q or/and BBJ on hematological parameters. Moreover, there is no data about the effect of BBJ in combination with Q on the hematological, hepato- and renal-biomarkers as well as the levels of sex hormones. Therefore it is difficult to compare between the present data and other previous study.

In the present study, NaF-treated rats exhibited significantly increases WBC and thrombocyte counts while RBCs and its related indices are decreased significantly as compared to control group. Quercetin with NaF had more potential effect on hematological parameters than BBJ. Co-administration of Q and BBJ against NaF hematotoxic more effective on RBCs count, HCT% and platelets number than each flavonoid separately with NaF. Ingestion of fluoride through drinking water (10, 50, 100ppm) for 12 weeks was found to affect hemoglobin synthesis in rats [41]. They found that fluoride was accumulated in the erythrocyte membrane and caused the formation of echinocytes. Since the life span of echinocytes is less than that of red blood cells, the early destruction of these red blood cells is likely to cause anemia.

The liver biomarkers were detected in the present study using some enzymes activity and ability to synthesis the protein.Among other biochemical parameters, the increase inAST, ALT and ALK activities were found to be relatedto damage in the liver and the change in hepatic functions. ALP activity increases in case of the damage of hepatic cellsand the obstruction of bile ducts arising from cellular reproduction [4]. Liver cells contain more AST than ALT, but ALT is confined to the cytoplasm in which its concentration is higher than that of AST [19]. Fluoride toxicities caused elevated in the activities of transaminases in rats and mice [42,43].

Abnormal protein metabolism is considered a sign of hepatotoxicity. In the present study, the administration of NaF resulted in a significant decrease in the concentration of total protein as compared to the control group. Previous study reported a similar reduction in protein content in NaF-treated animals and related it to inhibition of decarboxylation of branched chain amino acids and simultaneously promoting protein breakdown [44]. Sodium fluoride-generated free radicals down-regulate the activity of enzymes important in the polymerization of amino acids, thus inhibiting the process of elongation of peptides [45]. Free radicals are also a major source for DNA damage, which can cause strand breaks and base alteration in the DNA [46]. Therefore the reduction in protein content observed in the present study may be due to either direct effect of fluoride on protein synthesis or indirectly through DNA and RNA damage.

Treatment the animals with Q or BBJ improved the reduced levels of total proteins of NaF-intoxicated rats. This tendency to increase the level of protein contents could be ascribed to suppression of NaF-induced oxidative stress and liver damage with the subsequent improvement in liver synthetic function following Q or BBJ treatment. In the present study the efficacy of Q is more than BBJ in improving the total protein level. The co-administration of Q and BBJ with NaF is most effective than the effect of each flavonoid separately.

Sodium fluoride induced hepatotoxicity was reported earlier previous studies [1,4,14,19]. Abdel-Wahab [44] had reported elevated serum levels of TG, TC and total lipids in rats that were treated orally with NaF for 4 weeks. The results of the present investigation are in line with the above mentioned reports. They suggested that the abnormal activities of lipases enzymes seem to be one of the chief factors responsible for the rise in serum TG and TC. It appears that enzymes inhibited by fluoride, such as triglyceride lipase, unspecific esterase and pyrophosphates. Also, the obtained results of hyperlipidemia may be attributed to an increase in the synthesis of fatty acids in the liver or possibility due to incidence of liver cholestasis [47]. The observed abnormalities in lipoprotein profile may be due to over-production of very low density lipoprotein (VLDL-c) by the liver or to the decrease in removal of VLDL-c and LDL-c from the circulation.

Hyperlipidemia and hypercholesterolemia are reported as the major risk factors in life style related diseases such atherosclerosis and related cardiovascular complications [48]. It is well documented that a low level of HDL-c is indicative of high risk for cardiovascular disease; an increase in HDL-c level could potentially contribute to anti-atherogenicity [49]. Our study suggests that Q and BBJ have hypolipidemic effect and offer protection against NaF induced toxicity in liver by restoring the altered level of lipid. However, the Q was more potent effect on lipid profile parameters than BBJ. Quercetin significantly reduced the activity and mRNA levels of various enzymes involved in hepatic fatty acid synthesis. It was suggested that a reduction in hepatic lipogenesis is the mechanism underlying the hypolipidemic effect of Q.

The presence of BBJ with NaF alleviated the harmful effects of NaF on most of the measured parameters. This effect may be related to the antioxidant properties of BBJ. Hassan and Abdel-Aziz [19] reported that the total pigment extract from BBJ exhibited strong antioxidant activity health benefits.

Renal injury due to fluoride intoxication could be assessed by measuring the serum uric acid and creatinine levels. The elevated levels of uric acid and creatinine and the diminished creatinine clearance in the fluoride-treated rats indicate the development of glomerular injury and renal dysfunction. Increase in the level of uric acid in mammals is related to increase in

protein catabolism, and the level of creatinine tends to display increase in renal disorders [4]. The serum concentration of creatinine is relatively constant under normal circumstances, unless glomerular filtration rate (GFR) changes, as a result of defective renal function [50]. The increased level of serum creatinine after NaF intoxication might be due to reduced ability of the kidney to eliminate the toxic metabolic substances. Fluoride is freely filtered through glomerular capillaries and then undergoes a variable degree of tubular reabsorption. Fluoride can impair the function of mitochondria, diminishing cellular respiration, enhance mitochondrial production of free radicals and consequently lead to cell death and renal dysfunction [50].

Pretreatment of fluoride-intoxicated rats with Q or BBJ with NaF did not bring back the normal level of serum creatinine concentration of control group. Co-administration of Q and BBJ with NaF enhanced renal filtration and increased the excretion of xenobiotic. This may be due to antioxidative action of Q [51]. Quercetin was able as well to attenuate renal impairment which is in accordance with Singh et al. [51], who demonstrated that Q produced a significant reduction in serum levels of creatinine and urea nitrogen in a model of ischemia/reperfusion induced renal injury in rats.

Fluoride-induced serum inhibin-B reduction would have been much greater in the present study. These effects of fluoride could be mediated by an increment in the hypothalamic–pituitary receptor affinity by inhibin-B and/or by a gonadotrophic post receptor attenuation of the response to reduced levels of inhibin-B. It has been demonstrated that the FSH inhibiting activity of inhibin-B resides within this region of the molecule [52]. Interestingly, fluoride has a great affinity for electropositive basic amino acids, and in fact, the binding of fluoride to this type of amino acids, has been shown in other proteins [53]. Thus, a direct interaction of fluoride with the inhibin-B molecule is possible and could explain the observed and discussed effects in both Sertoli cells and gonadotrophs. Ghosh et al. [54] found that there was significant diminution in the relative wet weight of the testis, prostate, and seminal vesicle in rats exposed to NaF 20mg/kg/day for 29 days by oral gavage. All the previous study confirmed that NaF caused reduction in the inhibin-B and testosterone.

Quercetin partially protected the inhibin–B and testosterone against the toxicity of NaF, while BBJ did not. There was potential effect between Q and BBJ to increase the levels of testosterone and inhibin-B by 1.61- and 2.18-fold as compared to rat that treated with NaF. Nabavi et al. [55] evaluated the protective potential of the polyphenols curcumin and quercetin on thyroid function in NaF intoxicated rats and they found the Q kept the thyroid hormones near to normal level.

5. CONCLUSION

Blackberry and quercetin successfully restored liver function and normalized kidney functions and improve the sex hormones reduction induced by NaF. The antioxidants also alleviated the hematotoxicity compared with NaF. However, quercetin exhibited a more pronounced protection towards liver function and hematotoxicity. Concurrent intake of BBJ or Q with NaF can mitigate its toxicity. Still, further investigations should be done to estimate the mechanism of phytochemicals to protect different tissues in rats.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Perumal E, Paul V, Govindarajan V, Panneerselvam L. Mini-review: A brief review on experimental fluorosis. Toxicol. Letters. 2013;223:236–251.
2. Domingo JL. Health risk of dietary exposure to perfluorinated compounds. Environ. Intern. 2012;40:187–195.
3. Duckworth RM. Pharmacokinetics in the oral cavity: Fluoride and other active ingredients. Monographs in Oral Sci. 2013;23:125–139.
4. Eraslan G, Kanbur M, Silici S. Evaluation of propolis effects on some biochemical parameters in rats treated with sodium fluoride. Pest. Biochem. Physiol. 2007;88:273–283.
5. Sireli M, Bülbül A. The effect of acute fluoride poisoning on nitric oxide and methemoglobin formation in the Guinea pig. Turk. J. Vet. Anim. Sci. 2004;28:591–595.
6. Akdogan M, Bilgili A, Karaoz E, Gokcimen A, Eraslan G, Ustuner E. The structural and biochemical changes of kidney tissue on fluorosis in rabbit, Turk. J. Vet. Anim. Sci. 2002;26:71–77.
7. Akdogan M, Bilgili A, Karaoz E, Gokcimen A, Yarsan E, Eraslan G. The structural and biochemical alternations in liver of rabbits, received flour with water for particular dose and period. FU. J. Health Sci. 2002;16:41–46.
8. Kaushik T, Shyam R, Vats P, Suri S, Kumria MML, Sharma PC, Singh SN. Glutathione metabolism in rats exposed to high-fluoride water and effect of spirulina treatment. Fluoride. 2001;34:132–138.
9. Reddy GB, Khandare AL, Reddy PY, Rao GS, Blakrisma N, Srivalli I. Antioxidant defence system and lipid peroxidation in patients with skeletal fluorosis and in fluoride-intoxicated rabbits. Toxicol. Sci. 2003;72:363–368.
10. Schiffl H. Fluoridation of drinking water and chronic kidney disease, absence of evidence is not evidence of absence. Nephrol. Daily. Transplan. 2008;23:411–415.
11. Denbesten P, Li W. Chronic fluoride toxicity, dental fluorosis. Monographs Oral Sci. 2011;22:81–96.
12. Yu RA, Xia T, Wang AG, Chen XM. Effects of selenium and zinc on renal oxidative stress and apoptosis induced by fluoride in rats. Biomed. Environ. Sci. 2006;19:439–444.
13. Chattopadhyay A, Agarwal S, Podder S, Bhattacharya S. Fluoride-induced histopathology and synthesis of stress protein in liver and kidney of mice. Arch. Toxicol. 2011;85:327–335.
14. Kanbur M, Eraslan G, Silici S, Karabacak M. Effects of sodium fluoride exposure on some biochemical parameters in mice: Evaluation of the ameliorative effect of royal jelly applications on these parameters. Food Chem. Toxicol. 2009;47:1184–1189.
15. Narayana MV, Chinoy NJ. Effect of fluoride on rat testicular steroidogenesis. Fluoride. 1994;27:7–12.

16. Ortiz-Perez D, Rodrıguez-Martı´nez M, Martınez F, Borja-Aburto VH, Castelo J, Grimaldo JI, de la Cruz E, Carrizales L, Dı´az-Barrigaa F. Fluoride-induced disruption of reproductive hormones in men. Environ. Res. 2003;93:20–30.
17. Hsiao G, Shen MY, Lin KH, Lan MH, Wu LY, Chou DS, Lin CH, Su CH, Shen JR. Antioxidative and hepatoprotective effects of *Antrodia camphorata* extract. J. Agric. Food Chem. 2003;51:3302–3308.
18. Garcia OB, Castillo J. Update on uses and properties of citrus flavonoids: New findings in anticancer, cardiovascular, and anti-inflammatory activity. J. Agric. Food Chem. 2008;56:6185–6205.
19. Hassan HA, Abdel Aziz AF. Evaluation of free radical scavenging and anti-oxidant properties of blackberry against fluoride toxicity in rats. Food Chem. Toxicol. 2010;48:1999–2004.
20. Facchini PJ, Bird DA, St-Pierre B. Can Arabidopsis make complex alkaloids? Trends Plant Sci. 2004;9:116–122.
21. Stoner GD. Food stuffs for preventing cancer: The preclinical and clinical development of berries. Cancer Prevent. Res. 2009;2(3):187–194.
22. Felgines C, Texier O, Besson C, Fraisse D, Lamaison JL, Rémésy C. Blackberry anthocyanins are slightly bioavailable in rats. J. Nutr. 2002;132:1249–1253.
23. Dunn WB, Ellis DI. Metabolomics: current analytical platforms and methodologies. Trends Anal. Chem. 2005,24(4):285–294.
24. Uzun FG, Demir F, Kalender S, Bas H, Kalender Y. Protective effect of catechin and quercetin on chlorpyrifos-induced lung toxicity in male rats. Food Chem. Toxicol. 2010;48:1714–1720.
25. Marcolin É, Forgiarini LF, Rodrigues G, Tieppo J, Borghetti GS, Bassani VL, Picada JN, Marroni NP. Quercetin Decreases Liver Damage in Mice with Non-Alcoholic Steatohepatitis. Basic Clin. Pharmacol. Toxicol. 2013;112(6):385–391.
26. Boots AW, Haenen GRMM, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. Eur. J. Pharmacol. 2008;585:325–337.
27. Hwang MK, Song NR, Kang NJ, Lee KW, Lee HJ. Activation of phosphatidylinositol 3-kinase is required for tumor necrosis factor-a-induced up regulation of matrix metalloproteinase-9: Its direct inhibition by quercetin. Int. J. Biochem. Cell B. 2009;41:1592–1600.
28. Zhang HS, Zhang M, Yu LH, Zhao Y, He NW, Yang XB. Antitumor activities of quercetin and quercetin-5',8-disulfonate in human colon and breast cancer cell lines. Food Chem. Toxicol. 2012;29:3451–3460.
29. Zabulyte D, Uleckiene S, Kalibatas J, Paltanaviciene A, Jascaniniene N, Stosik, M. Experimental studies on effect of sodium fluoride and nitrate on biochemical parameters in rats. Bull. Vet. Inst. Pulawy. 2007;51:79–82.
30. Seiva FRF, Chuffa LGA., Braga CP, Amorim JPA, Fernandes AAH. Quercetin ameliorates glucose and lipid metabolism and improves antioxidant status in postnatally monosodium glutamate-induced metabolic alterations. Food Chem. Toxicol. 2012;50:3556–3561.
31. Sauebin L, Rossi A, Serraino I. Effect of anthocyanin contained in a blackberry extract on the circulatory failure and multiple organ dysfunction caused by endotoxin in the rat. Planta Med. 2004;70:745–752.
32. Siriwoharn T, Wrolstad RE, Finn CE, Pereira CB. Influence of cultivar, maturity, and sampling on blackberry (*Rubus L*. Hybrids) anthocyanins, polyphenolics, and antioxidant properties. J. Agric. Food Chem. 2004;52:8021–8030.
33. Boussarie D. Hematology rongeurs et lagomorphes company. Bull. Acad. Vet. of France. 1999;72:209-216.

34. Carr T, Andressen CJ, Rudel LL. Enzymatic determination of triglyceride, free cholesterol and total cholesterol in tissue lipid extracts. Clin. Chem. 1993;26:39–42.
35. Warnick GR, Benderson J, Albers JJ. Selected methods of clinical chemistry. Amer. Assoc. Clin. Chem. 1983;10:91–99.
36. Friedewald WT, Levy RI, Fredrickson DS. Estimation of concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem. 1972;18:499–502.
37. Prakasam A, Sethupathy S, Pugalendi KV. Hypolipidaemic effect of *Casearia esculenta* root extracts in streptozotocin-induced diabetic rats. Pharmazie. 2003;58(11):828–32.
38. Wheeler MJ. The determination of bio-available testosterone. Ann. Clin. Biochem. 1995;32:345–357.
39. Yousef MI, Omar SAM, El-Guendi MI, Abdelmegid LA. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. Food Chem. Toxicol. 2010;48:3246–3261.
40. Thialbut N, Peytavin G, Glaude JR. Calcium channel blocking agents protect against acetaminophen-induced cytotoxicity in rat hepatocytes. J. Biol. Toxicol. 1991;6:237–238.
41. Chouhan S, Lomash V, Flora SJ. Fluoride-induced changes in haem biosynthesis pathway, neurological variables and tissue histopathology of rats. J. Appl. Toxicol. 2010;30:63–73.
42. Guo-Ving X, Sun G, Shun Y. Oxidative stress from fluoride induced hepatotoxicity in rats. Fluoride. 2003;36:25–29.
43. Qujeq D, Laghaie B, Gholipour A, Solimani N, Hassenzadeh S. Effects of sodium fluoride on total serum protein levels and transaminase activity in rats. Biomed. Pharmacol. 2002;56:169–172.
44. Abdel-Wahab WM. Protective effect of thymoquinone on sodium fluoride-induced hepatotoxicity and oxidative stress in rats. J. Basic Appl. Zool. 2013;66:263–270.
45. Hordyjewska A, Pasternak K. Influence of fluoride on organism of human. J. Elementol. 2004;9(4):883–987.
46. Trivedi MH, Verma RJ, Chinoy NJ. Ameliroation by black tea of sodium fluoride-induced effects on DNA, RNA and protein content of liver and kidney on serum transaminase activities in Swiss albino mice. Fluoride. 2008;41(1):61–66.
47. Owings E, Georgeson K. Management of cholestasis in infants with very low birth weight. Sem. Pediatr. Surg. 2000;9(2):96–102.
48. Chovančíková M, Šimek V. Effects of high-fat and *Chlorella vulgaris* feeding on changes in lipid metabolism in mice. Biologia, Bratislava. 2001;56(6):661-666.
49. Assmann G, Nofer J-R. Atheroprotective effects of high-density lipoproteins. Ann. Rev. Med. 2003,54:321-341.
50. Thangapandiyan S, Miltonprabu S. *Epigallocatechin gallate* supplementation protects against renal injury induced by fluoride intoxication in rats: Role of Nrf2/HO-1 signaling. Toxicol. Reports. 2014;1:12–30.
51. Singh D, Chander V, Chopra K. The effect of quercetin, a bioflavonoid on ischemia/ reperfusion induced renal injury in rats. Arch. Med. Res. 2004:35:484–494.
52. Sewani CR, Bagdasarian MM, Ireland JJ, Bagdasarian M. Display of an inhibin epitope in a surface-exposed loop of the *E. coli* heat-labile enterotoxin B subunit. Vaccine. 1998;16:1611–1619.
53. Edwards SL, Poulos TL, Kraut J. The crystal structure of fluoride-inhibited cytochrome-c peroxidase. J. Biol. Chem. 1984;259:12984–12988.

54. Ghosh D, Das Sarkar S, Maiti R, Jana D, Testicular toxicity in sodium fluoride treated rats: Association with oxidative stress. Reprod. Toxicol. 2002;16(4):385.
55. Nabavi SF, Moghaddam AH, Nabavi SM. Protective effect of curcumin and quercetin on thyroid function in sodium fluoride intoxicated rats. Res. Rep. Flu. 2011;44(3):147–152.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=619&id=40&aid=5590