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Hyperlipidemic and Oxidative Stress Effects of Sodium Fluoride and The Ameliorative Role of Selenium and Curcumin in Male Mice

Biology

KEYWORDS : Sodium fluoride, Selenium, Curcumin extract, lipid profile, Oxidative stress

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ABSTRACT

Selenium and Curcumin longa L are full of natural antioxidants and have a lipid-lowering effect. The aim of this study was to investigate the effect of sodium fluoride on lipid profile and antioxidant enzymes and the possible protective effects of selenium and Curcumin extract against the toxicity and oxidative stress of sodium fluoride (NaF) that adversely affects antioxidant defense system and lipid profile. Mature male mice (weighing 35-45 g and each group of ten animals) were given sodium fluoride (10.3 mg/Kg bw) and/or Selenium (0.5 mg/Kg) + Curcumin extract (60 mg/Kg) daily intraperitoneally (I.P) for 4 weeks. In the present study, Sodium Fluoride exposure resulted in an increase in the Triglycerides, Cholesterol, LDL and v-LDL levels with respect to the control and significant decrease in HDL-c level. As a result, Sodium fluoride induced oxidative stress by decreasing levels of SOD, CAT, GPX, GST and increased MDA which are ameliorated by Curcumin extract and/or selenium to great extent.

1. Introduction:

Fluoride is abundant in the environment and exists only in combination with other elements as fluoride compounds, which are constituents of minerals in rocks and soil (Edmunds and Smedley., 1996). Sources of fluoride include natural fluoride in food stuffs and water (fluoridated water usually at 1.0 mg/L) (Beltran and Szpunar., 1988).

Sodium fluoride was the first fluoride compound used in the fluoridation of drinking water and it is still commonly used for that purpose to prevent dental caries.(National Toxicology Program (NTP) (1990) In addition to the well known effects of fluoride on the skeleton and on teeth, it exerts toxic effects on many other soft tissues and organs (Waldbott., 1978).

It is known that fluoride-induced increase in the generation of free oxygen radicals and decrease in antioxidant enzyme capacity play an important role in fluorosis (Bouaziz et al., 2006 and Bouaziz et al., 2007). Increase in tissue levels of lipid peroxidants, which are generated as a result of oxidative stress, is prevented by antioxidant defense systems, which are either endogenous and enzymatic or non-enzymatic (Halliwell.,2007). In circumstances where these systems are insufficient, antioxidants are used to prevent oxidative stress.

Fluoride in small doses has remarkable prophylactic influence by inhibiting dental caries while in higher doses it causes dental and skeletal fluorosis (Shanthakumari et al., 2004). However, detrimental effects of high-fluoride intake are also observed in soft tissues (Monsour and Kruger, 1985). Fluoride enter the body through drinking water, food, toothpaste, mouth rinses, and other dental products; drugs and fluoride dust and fumes from industries using fluoride containing salt and hydrofluoric acid (Shulman and Wells, 1997).

Selenium (Se), a human body essential trace element, displaying an antioxidant effective oxygen free radical scavenging, protects the organs and tissues from oxidative damage and improves the body's immune system (Liu et al., 2007; Zhou et al., 2009).

Among antioxidant micronutrients, Se plays an important role in a number of biological processes for humans and many other forms of life. Deficiency of this element induces cell death and has also been incriminated in the etiology of cardiovascular diseases (Saito et al., 2003; Wu and Huang, 2004).

Selenium is a structural component of several enzymes including glutathione peroxidase (GPx) and thioredoxine (Perottoni et al., 2004), which play a key role in the cellular oxidative defense and have been shown to be induced by oxidative stress (Lechner et al., 2002). In recent years, there has been a great deal of studies carried out on selenium metabolism (Shi et al., 2004).

Curcumin, the active compound in turmeric, because of its antioxidant and anti-inflammatory properties, has been demonstrated in the prevention and treatment of neurodegenerative disorders such as Alzheimer disease and multiple sclerosis (Cole et al., 2007).

Previous study showed that curcumin and turmeric treatment have countered the hyperglycemia-induced oxidative stress (Suryanarayana et al., 2005).

Curcumin, the main polyphenolic active compound found in Curcuma longa L. (Zengiberaceae family) rhizomes, is used in curries and mustards as a coloring and flavoring agent. Curcumin is generally recognized as safe by the food and drug administration (FDA). Studies on either animals (Qureshi et al., 1992) or humans (Lao et al., 2006) have found no toxicity associated with the consumption of curcumin even at very high doses. Curcumin is under preclinical trial for cancer prevention and anti-inflammation since it is suggested as a powerful anti-inflammatory, anti-cancer and antioxidant agent (Strimpakos and Sharma, 2008).

2. Material and Methods:

2.1. Animals

This study was performed on 70 young male mice, weighing about 35–45 g b.wt. Animals were obtained from the animal house of the King Fahd Center for Medical Research, King Abdul-Aziz University in Jeddah. They were breeding in a well ventilated room with the temperature ranging between 22 and 25 °C and maintained under standardized conditions away from any stressful conditions with 12/12 light and dark cycle with free access to humidity and were fed dry balanced meal for experimental animals provided by the General Organization for Grain Silos and Flour Mills in Jeddah, with a constant source of water. All experimental procedures and animal maintenance were conducted in accordance with the accepted standards of animal care per cage (Council of Europe, European convention for the protection of vertebrate animals 2006). We have followed the European community Directive (86/609/EEC) and national rules on animal care. One group served as control. Animals were weighed and randomly allocated into 6 groups (7 rats each) as following:

2.2. Chemicals

2.2.1 Sodium Fluoride

Sodium fluoride (NaF) was purchased from Sigma Chemical Co., St. Louis, Mo., USA. The tested dose of NaF (10.3 mg/kg b.wt) was chosen based on the previous studies of Zabulyte et al. (2007). A stock solution was prepared by dissolving of 100 g of NaF in 1000 ml of distilled water. The dose schedule was so adjusted that the amount of NaF administration per animal was as per their respective weight.

2.2.2 Selenium

Selenium was purchased from BDH Chemicals Ltd., England.

The tested dose of selenium (0.5 mg/kg) was chosen based on the previous studies of Ibtissem et al. (2011).

2.2.3. Curcumin extract

Fresh Curcumin was obtained from local market (Cairo, Egypt), then washed and was soaked in water for 24 hours and after that it was dried then homogenized by using electrical mixer and then the dose was prepared (60 mg/Kg) and this dose was chosen according to Abdul-Hamid and Moustafa . (2013).

2.3. Experimental protocols

The study was performed on 70 mature male mice, divided into 7 main groups; each group was consisted of 10 rats. The 1st Control group: Animal's received 1ml of distilled water orally daily for 30 successive days. The 2nd Sodium Fluoride (NaF) treated group: Animals were daily received NaF (10.3 mg/Kg) for 30 successive days intraperitoneally (I.P).The 3rd Selenium treated group: Animals were received selenium (0.5 mg/Kg) for 30 successive days intraperitoneally (I.P).The 4th Curcumin extract treated group: Animals were received Curcumin extract (60mg/kg) for 30 successive intraperitoneally (I.P).The 5th NaF + Selenium treated group: Animals were given sodium fluoride (NaF) (10.3 mg/Kg) for 30 successive days and then co-administered by selenium (0.5 mg/Kg) intraperitoneally (I.P). The 6th NaF + Curcumin treated group: Animals were given sodium fluoride (NaF) (10.3 mg/Kg) for 30 successive days and then co-administered Curcumin extract (60mg/Kg) for 30 successive days intraperitoneally (I.P). The 7th NaF+ Selenium+ Curcumin extract treated group: Animals were given NaF (10.3mg/Kg) and then co-administered with selenium (0.5mg/Kg) and then followed by Curcumin extract (60 mg/Kg) for 30 successive days (I.P).The substances were administered in the morning (between 09.30 and 10.30 h) to non fasted rats. The first day the animals were treated was considered experimental day 0.At the end of the 30 days of treatment, all animals were scarified and dissected.

2.3. Blood samples collection

Blood samples were collected after the end of the experiment from rats after fasting for 8 hours (as not to effect on lipid profile picture) from the retro-orbital vein, which is a simple, convenient and successful procedure that allows bleeding of the same animal more than one time with minimal stress (Scherners, 1967).After the end of 4th week, individual blood samples were drawn by orbital puncture (from eye plexus) using microhematocrit capillary tubes (Lancer, Athy, County-Kildare, Republic of Ireland), Serum was harvested from blood without EDTA and Plasma was harvested from blood with EDTA and subsequently used for the determination of reduced Glutathione, Glutathione peroxidase.

2.4. Lipid profile in the serum

Serum triglycerides were determined according to (Fossati and Prencipe, 1990), the quantitative determination of triglycerides level in serum was based on the fact that when samples of triglyceride incubated with lipoprotein in lipase (LPL), liberate glycerol and fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5- diphosphate (ADP) by glycerol kinase and ATP. Serum cholesterol was determined according to (Varley and Richmond, 1976). The method is based on the extraction and oxidation of cholesterol by acidic solution of ferric chloride, then subsequent addition of sulfuric acid to form a colored complex. Determination of HDL and LDL/VLDL: HDL: High density lipoprotein.LDL: Low density lipoprotein, VLDL: Very low density Lipoprotein, Serum HDL-cholesterol was determined according to Stein (1986) using Stanbio HDL cholesterol obtained from Stanbio Company.

2.5. Antioxidant enzyme determination

TBARS content was evaluated using the thiobarbituric acid (TBA) test as described by Ohkawa et al. (1979). After incubation of testis homogenate with TBA at 95 ˚C, TBARS reacts to form a colored complex. Absorbance was measured spectrophotometrically at 532 nm to determine the TBARS content. The specific activity is expressed as nmol/mg protein protein.

2.5.1. Measurement of superoxide dismutase (SOD)

SOD activity was measured according to the method described by Marklund and Marklund (1974) by assaying the auto oxidation of pyrogallol at 440 nm for 3 min. One unit of SOD activity was calculated as the amount of protein that caused 50% pyrogallol autooxidation inhibition. A blank without homogenate was used as a control for non-enzymatic oxidation of pyrogallol in Tris–EDTA buffer (50 Mm Tris, 10 mM EDTA, pH 8.2). The SOD activity is expressed as U/mg protein.

2.5.2. Measurement of catalase (CAT)

CAT activity was measured determined according to the method described by Aebi (1984) by assaying the hydrolysis of H_2O_2 and the resulting decrease in absorbance at 240 nm over a 3 min period at 25˚C. Before determination of the CAT activity, samples were diluted 1:9 with 1% (v/v) Triton X-100. CAT activity is expressed as mmol/mg protein.

2.5.3. Measurement of glutathione peroxidase (GPx)

GPx activity was measured using H2O2 as substrate according to the method described by Paglia and Valentine (1967). The reaction was monitored indirectly as the oxidation rate of NADPH at 240 nm for 3 min. A blank without homogenate was used as a control for non-enzymatic oxidation of NADPH upon addition of hydrogen peroxide in 0.1 M Tris buffer, pH 8.0. Enzyme activity was expressed as nmol/mg protein.

2.6. Statistical analysis:

Data were collected, arranged and reported as mean ± standard error of mean (S.E.M) of nine groups (Each group was considered as one experimental unit), summarized and then analyzed using the computer program SPSS/ version 15.0) The statistical method was one way analyzes of variance ANOVA test (F-test), and if significant differences between means were found, Duncan's multiple range test (Whose significant level was defined as ($P<0.05$) was used according to (Snedecor and Cochran.,1982) to estimate the effect of different treated groups.

3. Results

3.1.1. Effect of Sodium Fluoride, Selenium, Curcumin extract and their combinations on serum Triglycerides (TG): Table (1) and Fig. (8) demonstrates that the administration of Sodium Fluoride in its recommended dose for 30 successive days afforded significant increases ($P< 0.05$) in serum triglycerides when compared with control group. The combination of Selenium with the Sodium Fluoride decreased significantly the elevated triglycerides level. Whereas, the combination of curcumin extract with sodium fluoride elicited a significant elevation in serum triglycerides with respect to control group but showed a significant decrease as compared to sodium fluoride treated group, compared with control group. However, this effect was much lesser than that produced with sodium fluoride alone. Meanwhile, treatment of rats with Selenium alone afforded a significant decrease in serum triglycerides compared with control group.

3.1.2. Effect of Sodium Fluoride, Selenium, Curcumin extract and their combinations on serum Total cholesterol: The administration of Sodium Fluoride, to rats for successive 30 days in their recommended doses elicited highly significant increase ($P<0.05$) in serum total cholesterol after the end of the experiment when compared with control group. Whereas the combinations of Sodium Fluoride and/or with either Selenium or Curcumin extract revealed a non significant change after four weeks post treatment except the combination of with curcumin extract which showed a significant elevation compared with control group yet this effect was much lesser than that produced with sodium fluoride alone when compared with control group Table (1) and Fig. (8).Meanwhile, Selenium induced non significant increase in serum triglycerides level compared with normal control group.

3.1.3. Effect of Sodium Fluoride, Selenium, Curcumin extract and their combinations on serum High Density lipoprotein (HDL-c): Table (1) and Fig. (9) illustrates the effect of different treatments on serum HDL-c of rats treated with either Sodium Fluoride or/ and with their combinations with both

Selenium and Curcumin extract. The results revealed that the administration of Sodium Fluoride elicited a significant decrease in serum HDL-c after 30 days post treatment when compared with normal control group, while administration of Selenium and Curcumin extract elicited a significant increase in serum HDL-c after 30 days post treatment when compared with normal control group The combinations of Selenium and/or curcumin extract with either Sodium Fluoride or elicited a significant decrease in serum HDL-C level when compared with control group except combination of sodium fluoride with selenium and curcumin which exhibited a slight decrease when compared with control group, yet this effect was much better than that produced with sodium fluoride alone.

3.1.4. Effect of Sodium Fluoride, Selenium, Curcumin extract and their combinations on serum low Density lipoprotein (LDL-c): Sodium Fluoride treated group showed a significant increase in serum LDL-c when compared with normal control group after four weeks post administration. Whereas, Selenium when given for 4 weeks each alone or in combination with sodium fluoride afforded a non significant decrease in serum LDL-C except group treated with the combination of curcumin extract with Sodium Fluoride which revealed a marked increase when compared with control group ,while showed significant decrease with respect to Sodium Fluoride treated group. The best combination was the combination between sodium Fluoride and selenium and curcumin extract as it showed a slight significant increase in LDL-C value as compared to normal control group but it was the better results which revealed the vital roles of both selenium and curcumin in reducing LDL-c value, Table (1) and Fig. (9).

3.1.5. Effect of Sodium Fluoride, Selenium, and Curcumin extract and their combinations on serum very low Density lipoprotein (vLDL-c): It was appearant from Table (1) and Fig. (9) that treatments of rats with Sodium Fluoride elicited a significant increase in serum vLDL-c after the end of the study when compared with normal control group. On the other hand, the results revealed that the administration of Selenium and/or curcumin extract induced a significant elevation in serum vLDL-c after the end of the experiment when compared with control group but the effect was much less intense. Meanwhile, the combination of either Selenium with Sodium Fluoride exhibited a non significant change in serum vLDL-C level compared with control group except group treated with combination of sodium fluoride and curcumin extract which showed a significant increase compared with control group.

3.1.6. Effect of Sodium Fluoride, selenium , Curcumin extract and their combinations on Catalase activity:Regarding the effect of Sodium Fluoride on catalase activity of normal rats, Sodium Fluoride afforded a marked decrease ($P<0.05$) in plasma catalase after the end of the study when compared with control group, whereas, non significant changes in the enzyme activity was recorded in selenium treated group. Treatment of normal rats with selenium alone exhibited non significant changes in plasma Catalase after the end of the experiment when compared with control group , Whereas , a significant decrease was reported in plamsa when administered Curcumin extract only compared with control group (Table 6 and Figs.17,18).While combinations of Sodium Fluoride with either Selenium or curcumin extract exhibited a significant decrease in plasma catalase activity after the end of the study as compared with normal control group.

3.1.7. Effect of Sodium Fluoride, selenium, Curcumin extract and their combinations on Superoxide dismutase (SOD) activity: The results of the study revealed that treatment of normal rats with Sodium Fluoride elicited a highly significant decrease ($P<0.05$) in plasma SOD level after the end of the study when compared with control group. Treatment of normal rats with either selenium or curcumin extract for 4 weeks elicited a non significant decrease in SOD activity of plasma after the end of the study except with curcumin extract which showed a slight significant decrease in SOD activity compared with control group. Whereas, the combinations of the curcumin extract and/or selenium with sodium fluoride afforded a slight decrease ($P<0.05$) in SOD activity of plasma compared with normal control group (Table 7 and Figs.19, 20). Meanwhile combination of sodium fluoride with Curcumin extract and selenium afforded slight decrease in SOD activity but the effect was much better than group treated with sodium fluoride only and other treatment combinations.

3.1.8. Effect of Sodium Fluoride, selenium, Curcumin extract and their combinations on Malondialdhyde (MDA) activity: The MDA content of the serum was elevated ($P<0.05$) in response to treatment of normal male rats with Sodium Fluoride for 4 weeks compared with normal control group. The same previous response was reported with selenium, curcumin extract combinations with Sodium Fluoride compared with control group (Table 8 and Figs.21,22) but the effect was much less intense. Meanwhile, groups treated with either selenium or curcumin extract induced non significant changes in serum MDA level as compared to normal control group.

3.1.9. Effect of Sodium Fluoride, selenium, Curcumin extract and their combinations on Glutathione reductase (GR) activity: It was apparent from Table (9) and (Figs.32,24) that treatment of rats with Sodium Fluoride, alone afforded a significant decrease ($P<0.05$) in serum reduced glutathione after the end of the study when compared with normal control group. On the other hand, the results revealed that Selenium induced a non significant change in reduced Glutathione content of serum as compared to normal control group with slight significant increase in the reduced glutathione content of serum in Curcumin extract treated group compared with control group.

3.1.10. Effect Sodium Fluoride, selenium, Curcumin extract and their combinations on Glutathione Peroxidase activity: The plasma Glutathione peroxidase level was significantly reduced ($P<0.05$) in all groups treated with Sodium Fluoride alone and in combination with selenium, curcumin extract for successive 30 days when compared with normal control group. Whereas, non significant increase ($P<0.05$) was recorded in the enzyme activity of Plasma of group treated with selenium when compared with control group. Together with a significant decrease in the enzyme activity in Plasma in response to treatment with Curcumin extract as compared with normal control group. The enzyme activity in the plasma was markedly decreased in response to treatment with either Sodium Fluoride or it's combinations with selenium and/or curcumin extract compared with control group. Beside a non significant increase in response to treatments with selenium. Whereas, a significant decrease was reported in response to treatments with all combinations used except combination of Sodium Fluoride with selenium and curcumin which showed a slight decrease compared with normal control group (Table 10 and Figs.25, 26).

4. Discussion

The present study was an attempt to evaluate the toxic effect of sodium fluoride and possible ameliorative role of selenium or Curcumin extract as it is well known that selenium and Curcumin extract have been reported to be effective antioxidant, therefore, the present study aimed to elucidate the possible ameliorative role of Selenium and/or Curcumin extract in alleviating the toxicity of sodium fluoride when given to normal rats. The following parameters: lipogram picture (Triglycerides, total cholesterol, HDL–C, LDL-C, VLDL-C) as well as antioxidant parameters (SOD, CAT, GPX, MDA and GR) parameters in serum and plasma.

Furthermore, the treatment with NaF caused changes in lipid profile and this is in accordance with the obtained results by Czerny et al. (2000). They suggested that the abnormal activities of lipases enzymes seem to be one of the chief factors responsible for the rise in serum triglycerides and cholesterol. 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 inci-

dence of liver cholestosis (Owings and Georgeson, 2000).

On serum triglycerides, the administration of Sodium fluoride in its recommended doses for 30 successive days afforded significant increases (P< 0.05) in serum triglycerides when compared with control group. The combination of Selenium with the Sodium Fluoride decreased significantly the elevated triglycerides level. Whereas, the combination of curcumin extract with sodium fluoride elicited a significant elevation in serum triglycerides with respect to control group.

On serum total cholesterol, The administration of Sodium Fluoride, to rats for successive 30 days in their recommended doses elicited highly significant increase (P<0.05) in serum total cholesterol after the end of the experiment when compared with control group. On serum HDl , LDl and vLDl-c The results revealed that the administration of Sodium Fluoride elicited a significant decrease in serum HDL-c after 30 days post treatment when compared with normal control group, while administration of Selenium and Curcumin extract elicited a significant increase in serum HDL-c after 30 days post treatment when compared with normal control group.

The observed abnormalities in lipoprotein profile may be due to over-production of very low density lipoprotein (VLDL) by the liver or to the decrease in removal of VLDL and LDL from the circulation (Tsutsumi et al., 1995). The liver is the site of cholesterol and triglycerides synthesis.

Fluorosis is a well-defined clinical entity characterized by toxic effects of high-fluoride intake on teeth, bones and soft tissues (Krishnamachari, 1996). Increased oxygen radical generation and lipid peroxidation have been implicated in the pathogenesis of many diseases and toxic action of a wide range of compounds (Bouaziz et al., 2007).

Increased generation of reactive oxygen species (ROS) is implicated in the pathogenesis of many diseases and in the toxicity of a wide range of compounds (Halliwell and Gutteridge, 1985). Lipid peroxidation represents one of the most frequent reactions resulting from free radical's attack on biological structures (Stohs, 1995).

The decreased GSH along with an increased activity of GSH-Px in the fluoride-treated group suggests increased conversion of GSH to GSSG to combat lipid hydroperoxides or H2O2. Previous studies in this field reported decreased GSH and GSH-Px in various tissues of experimental animals subjected to chronic fluoride toxicity (Sharma and Chinoy, 1998).

In harmony with the present results, A decrease in the activity of free radical scavenging enzymes, SOD and GSH-Px, was found in people living in areas of endemic fluorosis (Li and Ca, 1994). A similar inhibitory effect of fluoride on SOD in germinating mung-bean seedlings support the above findings and indicate the possibility of greater toxicity if fluoride can impair the free radical scavengers (Rzeuski et al., 1998).

In another study and in accordance with the present findings, A decreased GST, SOD and catalase activities in rat brain upon ingestion of sodium fluoride (20 mg/kg body weight/day, ip) for 14 days were observed (Vani and Reddy, 2000). Liu et al. (2003) suggested the mechanism of fluoride injuring soft tissues that it causes excessive production of lipid peroxidation (LPO) and oxygen free radicals, leads to the ability of scavenging free radicals and antioxidation being reduced. These radicals can seriously damage biological membrane structure, functions of cells, and biomacromolecules, such as proteins and nucleic acids, furthermore, damage the entire soft tissues.

Selenium treated group has greatly ameliorated the antioxidant enzymes capacities and our findings are greatly in accordance with (Kipp et al.,2009) who reported that feeding a moderate selenium-deficient diet to mice resulted in a consistent down-regulation of the plasma selenium level and GPx activity.

As previously reported, GPx activity was also strongly reduced in the liver and colon of these animals. In addition, GPx activity was lower in splenic leukocytes (Hoffmann et al., 2010). Accordingly, our results revealed that sodium fluoride has reduced the GPX activities.

The present results come in harmony with (Lechner et al., 2002) who reported that selenium is a structural component of several enzymes including glutathione peroxidase (GPx) and thioredoxine, which play a key role in the cellular oxidative defense and have been shown to be induced by oxidative stress. In recent years, there has been a great deal of studies carried out on selenium metabolism (Shi et al., 2004).

Also, curcumin can effectively block thiol depletion. Results of Tirkey et al. (2005) indicated that curcumin improved renal GSH levels in treated rats. Moreover, the presence of curcumin with sodium arsenite alleviated its toxicity and ameliorated SOD and CAT levels, and this is in agreement with the results of Tirkey et al. (2005) who showed that treatment with curcumin improved the levels of renal.

SOD and CAT to reach the control level. Furthermore, curcumin increase endogenous antioxidant defense enzymes (Thiyagarajan and Sharma, 2004). Dinkova-Kostova and Talalay (1999) reported that the protective effects of curcumin as an antioxidant are attributed to the presence of the hydroxyl groups at ortho-positions on the aromatic rings and the b-diketone functionality.

Table (1): Effect of Sodium fluoride (10.3 mg/kg), Selenium (0.5 mg/ Kg), Curcumin extract (60 mg/Kg) and their combinations on Lipid profile in male mice (mean ± SE). (N = 7).

Groups	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	HDL (g/dl)	LDL(g/dl)	VLDL(g/dl)
Control group	63.56± 1.22^{g}	60.80± 2.17^{g}	45.00± 3.00^{ab}	10.28± 1.28^{fg}	12.71± 1.59^{g}
Sodium fluoride	195.20± 2.54^{a}	136.40± 1.9^{a}	26.80± 1.20^{g}	60.56± 3.84^{a}	39.04± 1.24^{a}
Selenium	65.80± 4.43^{fg}	64.00± 1.87^{fg}	42.60± 2.50^{b}	10.16± 3.07^{g}	13.16± 1.34^{f}
Curcumin extract	83.00± 4.51^{d}	70.00± 3.74^{de}	36.40± 2.24^{f}	14.60± 2.84^{c}	16.60± 1.25^{d}
Sodium fluoride + Selenium	92.64± 2.97^{c}	75.40± 4.41^{c}	39.70± 1.70^{d}	12.98± 3.77^{de}	18.52± 0.34^{cb}
Sodium fluoride +Curcumin extract	101.40± 2.73^{bc}	82.60± 1.12^{b}	38.00± 8.37^{ef}	15.80± 2.30^{b}	20.28± 1.67^{bc}
Sodium fluoride + Selenium+ Curcumin extract	69.00± 2.21^{e}	67.80± 4.60^{e}	40.40± 1.70^{cd}	12.10± 3.62^{e}	13.82± 0.50^{efd}

Means within the same column in each category carrying different litters are significant at (P ≤ 0.05) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

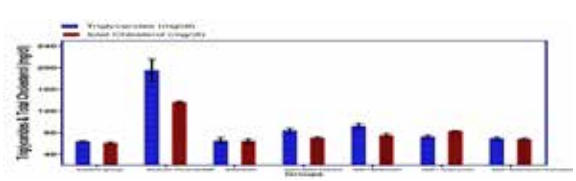

Fig. (1): Effect of Sodium fluoride (10.3 mg/kg), Selenium (0.5 mg/ Kg), Curcumin extract (60 mg/Kg) and their Combinations on Triglycerides & total cholesterol (mg/dl) in male mice.

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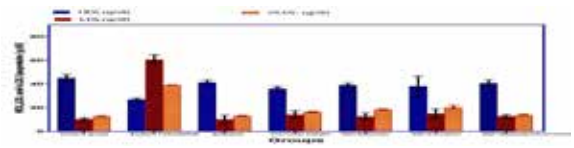

Fig. (2): Effect of Sodium fluoride (10.3 mg/kg), Selenium (0.5 mg/ Kg), Curcumin extract (60 mg/Kg) and their Combinations on High density lipoprotein , Low density lipoproteins and very low density lipoproteins (g/dl) in male mice.

Table (2): Effect of Sodium fluoride (10.3 mg/kg), Selenium (0.5 mg/ Kg), Curcumin extract (60 mg/Kg) and their combinations on Catalase in male mice (mean ± SE). (N = 7).

Groups	Plasma Catalase (U/g)	Plasma SOD (U/g)	Plasma MDA (nmol/g)	Serum Glutathione reductase (U/g)	Plasma Glutathione Peroxidase (U /g)
Control group	159.92 ±2.21^{ab}	108.06 ±3.70^{ab}	16.50 ±0.63^{f}	15.60 ±0.66^{a}	34.53 ±0.67^{b}
Sodium fluoride	67.60 ±7.16^{g}	22.91 ±3.77^{g}	105.47 ±1.60^{a}	7.62 ±0.47^{f}	5.61 ±0.47^{f}
Selenium	158.07 ±0.99^{b}	100.07 ±2.26^{b}	15.30 ±1.64^{f}	15.91 ±1.34^{a}	34.90 ±1.19^{ab}
Curcumin extract	150.53 ±1.08^{c}	95.80 ±3.92^{c}	18.04 ±0.45^{ef}	13.67 ±0.73^{b}	32.14 ±0.45^{c}
Sodium fluoride +Selenium	107.93 ±1.45^{ef}	81.27 ±1.90^{e}	75.39 ±0.16^{c}	10.37 ±0.38^{e}	15.33 ±0.39^{d}
Sodium fluoride +Curcumin extract	103.37 ±2.47^{f}	72.90 ±2.20^{f}	76.52 ±0.67^{bc}	11.20 ±0.68^{d}	16.46 ±0.84^{d}
Sodium fluoride + Selenium+ Curcumin extract	115.75 ±2.25^{d}	92.77 ±10.79^{d}	52.18 ±0.56^{d}	12.20 ±0.37^{c}	28.37 ±0.45^{e}

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

Fig.(3): Effect of Sodium fluoride (10.3 mg/kg), Selenium (0.5 mg/ Kg), Curcumin extract (60 mg/Kg) and their combinations on Catalase activity (in plasma) in male mice

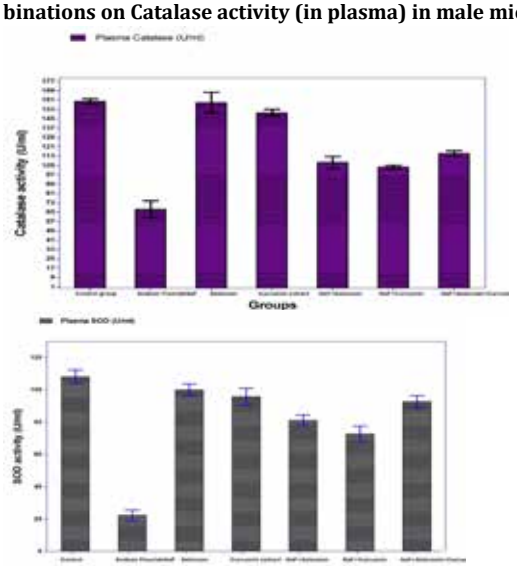

Fig.(4): Effect of Sodium fluoride (10.3 mg/kg), Selenium (0.5 mg/ Kg), Curcumin extract (60 mg/Kg) and their combinations on SOD (in plasma) activity in male mice .

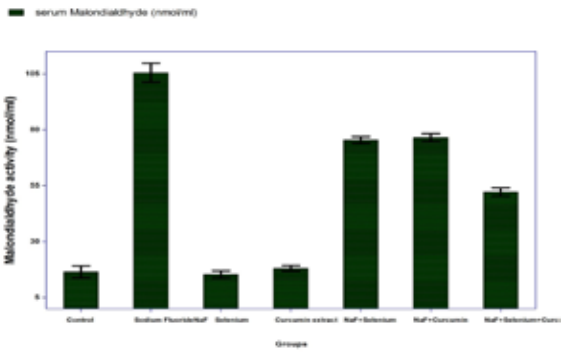

Fig. (5): Effect of Sodium fluoride (10.3 mg/kg), Selenium (0.5 mg/ Kg), Curcumin extract (60 mg/Kg) and their combinations on Malondialdhyde (MDA) (in plasma) in male mice.

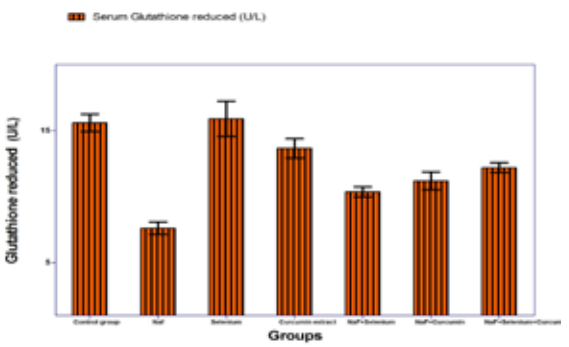

Fig. (6): Effect of Sodium fluoride (10.3 mg/kg), Selenium (0.5 mg/ Kg), Curcumin extract (60 mg/Kg) and their combinations on Glutathione reductase (in serum) in male mice.

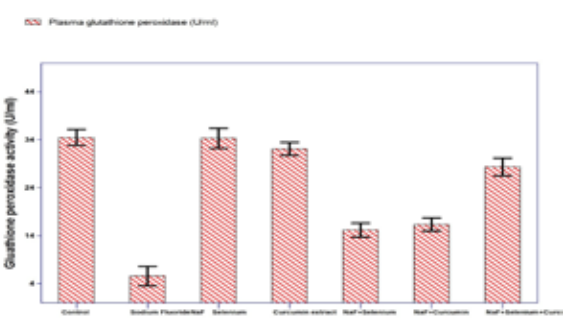

Fig.(7): Effect of Sodium fluoride (10.3 mg/kg), Selenium (0.5 mg/ Kg), Curcumin extract (60 mg/Kg) and their combinations on Glutathione peroxidase (in plasma) in male mice.

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