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Effect Of Curcumin Oxidative Stress And Sperm Quality In Rats With Fluoride Toxicity.

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ABSTRACT

Fluoride is considered a highly stress causing element which is able to make oxidative stress which may cause damage to all body organs including the testis. Therefore, a natural antioxidant agent may be a candidate for prevention toxic effect of fluoride, hence in this study; the effect of Curcumin on oxidative stress produced by fluoride in the rat testis was evaluated. The study groups (10 rats/ group) included: normal control group, fluoride control group, Curcumin control group and fluoride + Curcumin group. Treatment continued for 60 days, after sacrifice, sperm parameters, plasma testosterone level and testicular MDA level were assessed. Testicular histopathological analysis was evaluated. The results of this study showed that fluoride caused a significant decrease in sperm count and increased abnormal forms, there was a significant decrease in testosterone level and increased level testicular MDA level which reflect oxidative stress and disturbed function of the testis. Testicular histopathology of fluoride group showed atrophy and disturbance of testicular architecture. All these finding were significantly improved with curcumin treatment, Curcumin treated group showed nonsignificant difference compared to normal in all parameters measured. We concluded that Curcumin prevented oxidative stress and testicular dysfunction in rats with fluoride toxicity.

Keywords: Curcumin, fluoride, MDA, testosterone, testis.

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INTRODUCTION

Fluorosis is considered a common disease of the neurodegenerative disease affecting people living in areas of high fluoride exposure[1],as Fluoride is considered a highly stress causing element which is able to make oxidative stress in reactive oxygen species (ROS). Sodium fluoride causes oxidative stress damage to all body organs and causes cognitive dysfunction in rats brain [2]. Varying concentrations of fluoridecauses free radical formation in individuals living in areas of high fluorosis [3-4].

Increasing fluoride intake with food inhibits several enzymes functions and leads to disturbance in metabolism and physiological functions of the body [5]. Accumulation of fluoride in body tissues leads to disturbance of activity and functions of antioxidant system in the body and increase lipid peroxidation, Antioxidants as glutathione, play an important role in regulating the redox state of cells [6].

With oxidative stress free radicals scavengers are decreased and therefore lipid peroxidation is increased. Recently, there are many studies evaluating effect of nutritional antioxidants as herbs in diseases due to oxidative stress. The mechanism of protective effects of these agents may be due to their ability to act as free radical scavengers and potentiating natural antioxidant defense mechanisms [7]. Curcumin is the active component of turmeric, and it may act as natural antioxidant and used in treatment of many conditions without notable adverse effects [8].

Although several studies studied the antioxidant effects of Curcumin [9-10], the Curcumin effects on oxidative stress and sperm quality in fluoride treated rats have not been evaluated.

Therefore, the present study evaluates the protective effects of Curcumin on fluoride induced dysfunction to the reproductive system of male rats.

MATERIALS AND METHODS

Study animals:

Male albino rats 180-200 g with 8 weeks old were included in the study. They were purchased from the Egyptian Organization for Biological Products and Vaccines .they were allowed free access to food and water ad libitum. They were kept under constant temperature with 12/12 h light/dark cycles and left to acclimatize for one week before starting the experiment. The rats care was according to the guide of Animal Care and Use Committee at the Suez Canal University and the National Institutes of Health guide for the care and use of laboratory animals (Maryland, USA).

Type of the study: this is an experimental study. It took place in pharmacology department of Faculty of Medicine, Suez Canal University.

Drugs and Chemicals: All drugs and chemicals were purchased from Sigma Aldrich, USA

Study groups:

- 1- The control group (n=10) treated with 0.9% NaCl for 4 weeks oral.
- 2- The fluoride control group =10) treated with (20mg/L) NaF for 60 days in drinking water [11]
- 3- The curcumin treated group (n=10) treated with (20mg/kg) Curcumin [11]
- 4- Fluoride + Curcumin group in the same doses of the drugs mentioned above ding. At the end of study, rats were anaesthetized by intraperitoneal injection Nembutol 30 mg per kg. The heart was exposed and blood samples were collected.

Rat testis were incised and fixed in 10% phosphate buffered formalin (pH 7.4) for histopathological studies.

The investigations done included the following:

Sperm count and morphology:

The caudal epididymis was separated. The epididymis was cleaned of pad of fat, cell suspension was obtained from macerating the epididymis and washing it in pre-warmed (35°C) phosphate-buffered saline and the wash was filtered in 80µm pore-size mesh. The resultant filtrate was used in assessment of sperm parameters and characteristics.

A part of the sample was used for sperm count in a Neubauerhemocytometer. Another part of the suspension was used to detect head and tail abnormality. As it was smeared and stained with hematoxylin and eosin and examined under a light microscope (Olympus, Japan) at magnification of 40X [12].

Serum testosterone:

Blood samples were collected after sacrifice as mentioned before. Blood serum was separated. By using ELISA technique, testosterone level was detected and compared in study groups.

Lipid Peroxidation (LPO)

Malondialdehyde is considered a marker of lipid peroxidation. The amount of testicular MDA was measured using the Thiobarbituric acid (TBA) assay. The absorbance of the pink coloured trimethine condensation product was measured at 533 nm using a spectrophotometer. The results were expressed as nano mole MDA/gm weight of testis [13].

Testicular histopathology:

Tissue sections of 5 mm thickness were prepared and placed on slides. They were stained with hematoxylin and then examined under the light microscope. For each testis several sections containing 20-50 tubule sections were examined for manifestations of interstitial edema, tubule degeneration, and congestion [12].

Statistical analysis

Data were analyzed using SPSS (version 17.0). Results were expressed as means \pm SD on Microsoft Excel 8.0. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey's test post hoc analysis. Values were considered statistically significant at $P \leq 0.05$. There is no conflict of interest or funding agency for this work.

RESULTS

Administration of fluoride for 60 days caused a significantly decreased sperm count versus normal control group whereas Curcumin administration resulted in a significant increased number of sperms versus fluoride control group.

The treatment of the male rats with fluoride resulted in significantly increased number of abnormal sperms versus normal control group. Treatment with Curcumin caused significant decreased number of abnormal forms compared to fluoride group fig. 1.

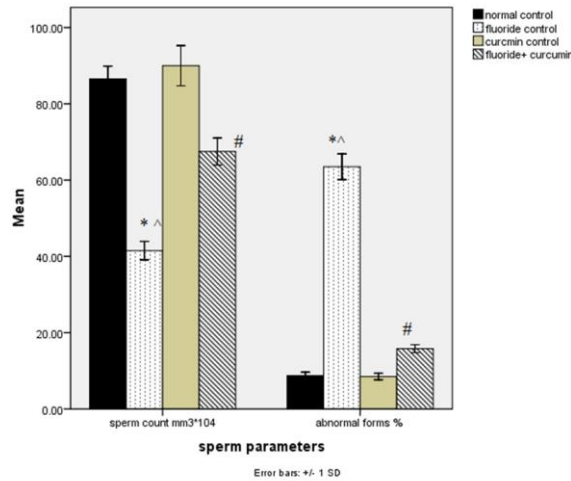


Fig 1: Mean±SD of sperm count and abnormal forms of sperms in study groups

P value is significant <0.05, * significant versus normal, ^ significant versus fluoride+curcumin, # significant versus fluoride

Assessment of level of plasma testosterone showed that there was significant decrease in testosterone level in fluoride treated group compared to normal and treatment with Curcumin resulted in significant increased level of testosterone. There was a significant increase of MDA level in testis of fluoride control group as compared to normal control group, and treatment with Curcumin caused a significant reduction of MDA versus fluoride treated group fig.2.

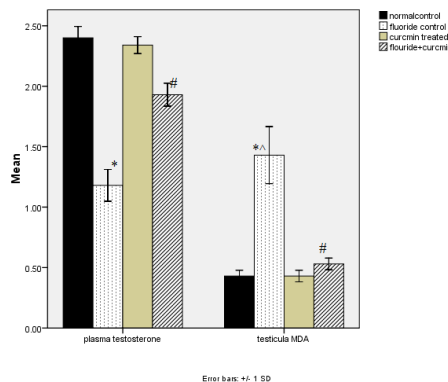


Fig 2: plasma level of testosterone and testicular MDA level in study groups.

P value is significant <0.05, * significant versus normal, ^ significant versus fluoride+curcumin, # significant versus fluoride

Normal group testis showed normal somniferous tubules with normal arrangement pattern of spermatogenic cells and interstitial cells, fluoride control group showed decreased number of spermatogenic cells and height and increase interstitial tissue between tubules, there was loss of most spermatogenic cells, degenerative changes, and disturbed architecture of somniferous tubules and edema of interstitial tissue. All these changes were improved with Curcumin treatment, and showed normal histopathology of testis fig.3.

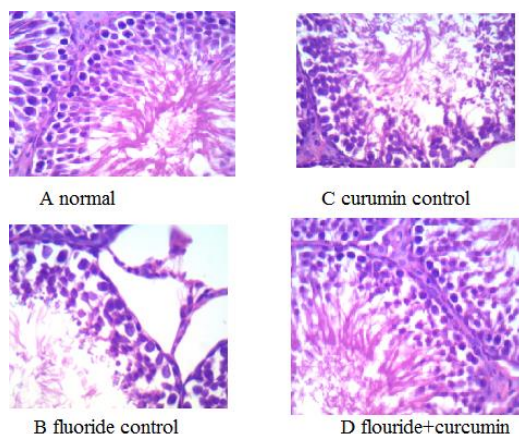


Fig 3: histopathology of testis of study groups.

DISCUSSION

There are very limited data available on the effects of Curcumin on seminal parameters in fluorosis, hence in this study, these favorable effects were studied to help us prevent complications of this condition. In the present study, we evaluated the effects of fluoride administration on testis of male rats and possible protective effect of curcumin administration. We studied the effect of these agents on sperm count, abnormal forms of sperms and histopathology of testis. We also studied the effect on serum testosterone and testicular MDA level.

The results of this study showed that fluoride caused a significant decrease number of sperms and increased abnormal forms compared with normal and this was supported by the histopathological changes in testis of fluoride control group which showed hypertrophied cells, necrosis of seminiferous tubules, destruction of testicular architecture and presence of inflammatory cells, all these changes were significantly decreased by Curcumin changes.

Oxidative stress is considered a chemical reaction inside the body tissues which causes increase production of reactive oxygen species [14]. The process of oxidative stress causes disturbance and alternation of the vascular epithelium cells and disruption of vascular integrity leading to the deprivation of oxygen and accumulation of toxic metabolites [15].

The fluorosis is a one of the degenerative disease caused by increased amount of fluoride in drinking water or food. Chronic exposure to fluoride disturbs the functions of the membrane lipids in animal studies [16].

Sodium fluoride causes oxidative stress and damages membrane lipid, proteins and antioxidant enzyme defence system [17].

This study found that the administration of sodium fluoride lead to a significant increase of MDA level in the testis in fluoride control group indicating increased oxidative stress.

Lipid peroxidation LPO is considered a frequent reaction caused by attacks of free radicals to biological structures. Earlier studies have recorded increased LPO levels in the liver and kidney of fluorotic rats. NaF decreases antioxidant defense mechanism and causes lipid peroxidation and disrupts cell membrane fatty acid [18].

Natural antioxidants, such as quercetin, resveratrol, catechin, β -carotene, Curcumin, vitamins C and E have gained great attention in recent years because they are able to support body system against several diseases [19]

Curcumin has multiple favorable prosperities therefore may have therapeutic potential for the prevention of several pathological conditions. Previous studies stated that Curcumin decreased lipid peroxidation and increased cellular redox control [20]

The ability of Curcumin to prevent many pathological condition may be attributed mainly to their antioxidant prosperities of Curcumin as it is a direct free radical scavenger and have metal chelating activity [21].

Curcumin is a perfect antioxidant agent and it stops lipid peroxidation in liver and kidney [22]. Curcumin supports and increases the activities of antioxidant enzymes like superoxide dismutase, Catalase and glutathione peroxidase and maintain their level at higher level. It also has the ability to scavenging oxygen free radicals as superoxide anions, singlet oxygen, NO and hydroxyl radicals which initiate LPO and cause the process of oxidative damage [23]. Curcumin also has indirect antioxidant effects by modulating antioxidant enzyme activities.

We concluded that NaF causes dysfunction of the test is demonstrated by decreased sperm count and increased abnormal forms. It causes abnormal histopathology of the testis and decreased testosterone levee; and increased MDA level reflecting increased oxidative stress. The findings of the study showed the possible protection role of Curcumin, as an easily available and cheap natural compound, which can prevent testis damages after exposure NAF.

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