Immunohistopathologic Study on the Ameliorative Effect of Propolis Against Fluoride Cytotoxicity on Rabbit Buck Fertility

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ABSTRACT
Fluoride is a highly electronegative anion with cumulative toxic effects causing reproductive dysfunction and increase in reactive oxygen species production in different organs including testis. Propolis is a honeybee product that has an antioxidant property. The aim of this study was to elucidate the protective effects of propolis against reproductive toxicity induced by sodium fluoride (NaF) in male rabbits (buck). Four experimental groups receiving a combination of sodium fluoride (10 mg/ kg body weight/day) and/or propolis (25 mg/ kg body weight/day) for 70-day was divided as follows: non treated (control), sodium fluoride alone, propolis alone and sodium fluoride + propolis. Sodium fluoride caused a decrease in testes and epididymis weights as well as serum testosterone and accompanied with disturbances in semen quality and quantity, compared to control group. Similarly, histopathological results revealed that NaF caused alterations in the testes, epididymis and prostate gland. There was intensive positive immunoreactivity for caspase-3 (dark brown granules) was observed in the cytoplasm of germinal cells, Sertoli cells and Leydig cells of NaF treated group compared with the control indicating the severity of its toxicity in testes. On the other hand, testicular cells of NaF in combination with propolis treated group showed apparent reduction of caspase-3 immunoreactivity to be more or less similar to the control group. Administration of propolis either alone or combined with NaF ameliorated these toxic effects. Testis of control group and propolis only revealed negative or low caspase-3 immunohistochemical reactivity. In conclusion, propolis reduced the oxidative stress toxicity induced by fluoride in the reproductive system of male albino rabbits.

Key words:
Sodium Fluoride, propolis; reproductive toxicity, immunohistopathology.

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1. INTRODUCTION
Fluoride (F) is a highly electronegative anion with cumulative toxic effects that causes a condition known as fluorosis (Fatma Khalil and Nora El-Sheikh, 2010). It is an endemic public health problem in nearly 22 nations around the world and its variability and presence depends upon the location (Bharti et al., 2017). Amal Ibrahim et al. (2013) stated that Egypt had problems with endemic fluorosis where the main pathway of fluoride exposure is the ingestion of tap water from contaminated ground water sources.

The main sources of F intake include drinking water, foodstuffs and smoke, pesticides, and F-containing dental products (Ahmad et al., 2012), as well as, it is a potent industrial toxicant (Jhala et al., 2008). According to World Health Organization (WHO), F exposure to animals above the 1.5 ppm, set as chronic fluoride toxicity (Susheela, 2006).

Fluoride consumption for a long period of time has many pathological effects as a result of increased oxidative stress on soft tissues like muscle, liver, gastrointestinal tract in addition to the reproductive and endocrine organs by the property of simple diffusion (Sahu et al., 2015).

Several clinical investigations and animal experiments suggested that F has adverse impacts on male reproductive function (Long et al., 2009;Kumar et al.,2010 and Hanaa El-Hallawany and Abeer El-Metwally,2011) that including structural and functional defects in spermatozoa (Chinoy et al., 1995) and decrease in
sperm count (Ghosh et al., 2002). Disturbances in reproductive hormone levels (Ortiz-Perez et al., 2003), alterations in the epididymis and accessory reproductive glands (Kumar and Susheela, 1995) and finally reduced fertility (Long et al., 2009) were also reported. In addition, spermatogonia undergo various processes to ultimately fertilize an oocyte, including spermatogenesis, capacitation, and the acrosome reaction. F has been shown to impair all three of these processes (Enaam Falih and Zainab Ibrahim, 2016).

Propolis, a resinous wax-like, beehive product is collected by honey bees from plant exudates and also known as bee glue (Majeda Alqayim, 2015). It is used by bees to repair and maintain their hives and had been used as a folk medicine from ancient times (Mahran et al., 2011 and Lamiaa Barakat et al., 2015). The propolis as found by Cardile et al. (2003) that contains a variety of chemical compounds as poly phenols (flavonoids, phenolic acids and alcohols), coumarins and steroids, which differ according to the geographical and botanical origins. For these constituents propolis has a wide range of biological activities, such as anti-inflammatory (Yoshizumi et al., 2005), antibacterial (Oris et al., 2005), anticarcinogenic (Aliyazicioglu et al., 2005) as well as protection against male infertility (Yousef et al., 2010).

Moreover, propolis is reported to inhibit the generation of superoxide anion. Furthermore, propolis has been determined to reverse the consumption of glutathione, which is synthesized in the liver and has radical scavenging activity (Castaldo et al., 2002).

Excessive exposure to fluorides can evoke several oxidative reactions that leading to induction of apoptosis, an active process of cell death in different systems (Fatma Agha et al., 2012). The characteristics of apoptosis include; a series of biochemical and morphological changes, such as caspase family activation, nucleosomal DNA fragmentation, cell volume loss, and chromatin condensation (Vaskivuo et al., 2000). Many of events that occur during apoptosis are mediated by a family of cysteine proteases called caspases (Kumar et al., 2004). Sequential activation of caspase 3 plays a central role in the execution-phase of apoptosis (Gu et al., 2011). Reactive oxygen species (ROS) have been implicated as potential modulators of apoptosis. The ability of oxidative stress to provoke apoptosis as a result of massive cellular damage has been associated with lipid peroxidation and alterations of protein and nuclei (Khandare et al., 2011).

The aim of the present study was to evaluate the possible ameliorative role of propolis against negative effects of chronic sodium fluoride (NaF) exposure on adult male rabbit fertility.

2. MATERIALS AND METHODS:

2.1. Animals:

Twenty healthy adult New Zealand white male rabbits were used for the study. All the rabbits were of the same age, with a weight range of 2–2.5 kg. They were housed in a well-ventilated animal house and each group caged separately, at a temperature of 29-32°C. The animals were exposed to 10–12 h of daylight under proper hygienic conditions and received food and water ad libitum. All animals were acclimatized for one week before being dosed. The present study was carried out in Animal Reproduction Research Institute (A.R.R.I.).

2.2. Chemicals:

2.2.1. Sodium Fluoride (NaF): Crystalline powder Natrium Fluoride (Sigma, Germany) was dissolved in tap water from El Gomhoria Company for Chemical and Medical Trading, Egypt.

2.2.2. Propolis: Egyptian propolis was commercial purchased Beeswax honeycomb processing. The propolis adjuvant was prepared as previously described (Shaapan et al., 2014). Briefly, the propolis was ground with absolute ethanol for 10 days. Then, the solvent was evaporated and the resulting dried matter was dissolved in phosphate buffer solution (PBS, pH 6.2), in a final concentration of 40 mg /ml. The crude propolis (25mg/kg body weight) was suspended in 5ml of distilled water and was orally given. The dose of propolis was determined by LD50 test according to Purohit et al. (2013).

2.3. Animal groups and dose administration:

It was applied according to Khandare et al. (2011). The rabbits were divided into four equal groups of five each.

1 - Control group: Animals were given water without any treatment.
2- NaF group: Animals were provided 10 mg NaF/kg b.w.
3 - Propolis group: Animals were given 25mg Propolis /kg b.w.
4 -NaF+ Propolis group: Animals were given 10 mg NaF/kg b.w. + 25mg Propolis /kg b.w.

All experimental animals were given the different treatment daily in drinking water for 70-days for completion of the spermatogenic cycle and maturation of sperms in epididymis. At the end of
the experiment, animals were humanly euthenized. Incision was given on the scrotum of the rabbit, the testes and epididymis were carefully exposed, removed and were subjected to the following semen analysis and histopathological studies.

2.4. Body and organ weights:
The weight of rabbit’s testes and epididymis were recorded.

2.5. Biochemical studies:
Blood samples: was collected directly from the retro-orbital venus plexus each two weeks and serum samples were prepared by centrifugation at 3000 rpm for 10 min. Serum was used for testosterone estimation according to the method described by Chen et al., (1991).

2.6. Semen analysis:
The sperm motility and sperm counts of cauda epididymis as well as sperm abnormalities were determined by routine procedure as described by Bataîneh and Nusier (2006).

2.7. Pathological studies:
2.7.1. Histopathological examination: Testes, epididymis and prostate glands were dissected and preserved in a 10% neutral formalin solution for fixation, then dehydrated through ascending grades of alcohol, cleared in xylene and embedded and blocked in paraffin. Sections of 3–5-µm thickness were taken and stained with hematoxylin and eosin as described by Suvarna et al. (2013) then examined by light microscope.

2.7.2. Histochemical examination: Periodic Acid Schiff (PAS) reaction was applied to demonstrate carbohydrates. In addition to, Masson’s Trichrome stain was used for connective tissue proliferation, Suvarna et al. (2013).

2.7.3. Immunohistochemical staining: Caspase-3 was used for detection of apoptosis in cells according to Fatma Agha et al. (2012). A standard Avidin-Biotin Complex method with alkaline phosphatase detection was carried out. Formalin-fixed paraffin-embedded sections were dewaxed in xylene and rehydrated through graded alcohol to distilled water. The sections were subjected to antigen retrieval by boiling in a microwave for 20 min in 0.01 M sodium citrate buffer (pH 6.0). The primary antibody to caspase-3 (Transduction Laboratories, Lexington, KY) was applied at a dilution of 1:1000 and incubated overnight at 4°C. After incubation, the slides were treated with biotinylated rabbit antimouse immunoglobulin (1:600 for 30 min; Dako Ltd., Ely, UK), washed as before, and then treated with streptavidin and biotinylated alkaline phosphatase according to the manufacturer’s instructions (Dako). The slides were then washed, and the signal was visualized using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. A negative control reaction with no primary antibody is always carried out alongside the reaction containing sample. The specificity of the caspase-3 antibody was confirmed by comparison with control antibodies.

2.8. Statistical analysis:
The mean ± S.D. Values were calculated for each group to determine the significance of intergroup difference. Each parameter was analyzed separately using two-way ANOVA analysis of variance. P values <0.05 were considered to be significant (Snedecor, and Cochran, 1982).

3. RESULTS AND DISCUSSION:
Chronic exposure to fluoride has bad impacts in livestock animals, experimental animals, as well as, humans (Bharti et al., 2017). Such deliterious effect inhibits the activities of antioxidant enzymes like superoxide dismutase, glutathione peroxidase and catalase that leading to overproduction of reactive oxygen species (ROS) at the mitochondrial level, resulting in damage of cellular components (Yılmaz and Erkan, 2015). Moreover, ROS results in oxidation of macromolecules, membrane phospholipid breakdown, lipid peroxidation, mitochondrial membrane depolarization and apoptosis (Bharti and Srivastava, 2009 and Nabavi et al., 2011). In addition, Chouhan et al. (2010) mentioned that ROS level occurring in a time- and dose-dependent manner with exposure to NaF.

In testis, a blood-testis barrier protects spermatogenic cells and subsequently, the process of spermatogenesis. But in prolonged F exposure, it may cross this permeable barrier and causing tubular necrosis (Long et al., 2009). Once it has crossed the barrier, F causes a lack of maturation and differentiation of spermatocytes, fragmentation of spermatozoa in the epididymis, leading to cessation of spermatogenesis (Sarkar et al., 2006).

The main cause of chronic fluorosis is a significant decline in DNA and RNA levels which leads to alterations in nucleic acid and protein metabolism in affected organ resulting in structural changes (Fatma Agha et al., 2012). This decreased protein synthesis has been attributed to a decrease in activity of antioxidant enzymes that catalyzing the key process of cellular metabolism (Chinoy et al., 1993).

3.1. Clinical signs:
Chronic exposure to F for a period of 70 days induced deleterious impacts in animals under experiment. First symptoms of chronic F toxicity were reduced feed intake as a result of loss of appetite that leads to emaciation and weakness. Also, transient diarrhea and/or constipation were seen in addition to, nervous manifestation included muscle tremor, pupilary dilatation and hyperesthesia as well as lethargy. Treated groups with propolis together with fluoride and propolis only didn’t show any of these symptoms.

These symptoms are in agreement with the finding of Bataineh and Nusier (2006); Lohakare et al. (2010) and Bharti et al. (2017) . Body weight reduction might be attributed to the decrease in feed consumption and transient diarrhea occurred which was considered as a first symptoms of chronic F toxicity. Moreover, Ulemale et al. (2010) claimed decrease in feed consumption to gastroenteritis as a formation of hydrofluoric acid.

3.2. Testis and epididymis weight:

In this study, there was a significant decrease the testes and epididymis weight in sodium fluoride group as compared with the control group and a significant increase in their weights in propolis only and sodium fluoride group treated with propolis groups as compared with control group as shown in table (1).

Similarly, in another studies, rabbits fed on fluoride were having a significant decrease in testes and epididymal weight (Kumar and Susheela ,1995 and Kumar et al., 2010) as well as in mice (Chinoy and Sharma ,1998). While, our findings differed from those of Ghosh et al. (2002) who reported an increase in the relative testis weight of (20 mg/kg F for 29 days), which might be due to a compensatory change or due to fluid accumulation in the testes . The reported decrease in the reproductive organ weights could be due to Leydig cell atrophy that led to decrease in testosterone level which was noticed in the present study that may be resulted from the oxidative damage induced in rabbit testes.

However, we found that, propolis was capable of restoring the testes and epididymis weights in treated group administered sodium fluoride and propolis. These results were in line with the reported results of Yousef et al. (2010) who noticed that, the propolis had an effective role in the protection against the reproductive toxicity of fluoride in rabbits.

3.3. Serum testosterone estimation:

Testosterone is an important hormone that regulates spermatogenesis. Our results indicated that F induced significant decrease in testosterone level as compared to control group, while orally treatment with sodium fluoride and propolis caused a significant elevation in testosterone concentration as compared with control group as shown in table (1). These results were in agreement with that reported by Elbetieha et al. (2000). Our opinion suggested that the adverse effect of NaF on male rabbit fertility might be as a result of F accumulation in the testes that lowered testosterone concentrations and this was supported by the study of Bataineh and Nusier (2006). This decline in hormone levels influenced the weight of reproductive organs and affected the process of spermatogenesis which mimic the results of Koyama et al. (2000) and Archunan et al. (2004). Moreover, F interferes with spermatogenesis by G-protein that used by luteinizing hormone (LH) which is an important regulator of testosterone production in Leydig cells, thereby impairing spermatogenesis (Long et al., 2009). Moreover, Sertoli cell is dependent on G-protein signaling as Sertoli cells play an important role in spermatogenesis (Tsai et al., 2006) and other reproductive functions (Long et al., 2009).

On the other side, our results showed that treated rabbits with fluoride with propolis alleviated the testosterone levels nearly to the control level. This means that propolis increased the process of steroidogenesis and hence testosterone production.

3.4. Semen analysis:

The sperm quality is one of the important indexes of male reproductive function (Wan et al., 2006). Changes in sperm quality induced by fluoride have been demonstrated in vivo and in vitro in many species, including the rat, mouse, rabbit and even human as well, interfere with the anatomical structure and physiological activity of testes, epididymis, and associated duct system (Susheela and Kumar, 1991).

As showed in Table (2), the sperm count, sperm motility and sperm abnormalities in the cauda epididymis of NaF-treated rabbit group was significantly reduced. While in group of NaF with propolis significant improvement was noticed. In group given propolis only the recovery was most significant that was nearly normal and showed no significant difference with control group.

The present study indicated that the higher the fluoride exposure, the higher the effect on sperm counts and abnormalities as reported results in rats, mice and rabbits in studies performed earlier (Chinoy and Sharma 1998; Ahmad et al., 2012 and Mathur, 2013). Moreover, Dvoráková-Hortová et al. (2008) showed NaF exposure-related degenerative
changes mainly affecting shape, motility, viability, and capacitation of spermatozoa while, Collins et al. (2001) indicated that fluoride did not affect sperm quality in rats.

In addition, there was a significant decrease in sperm motility as compared with the control which came in accordance with results of Bataineh et al. (2006) and Kumar et al. (2010) in rat and mice, respectively. An earlier study demonstrated that human spermatozoa lost their motility in vitro in the presence of 250 mM fluoride /20 mins and, similarly, 30 mM fluoride made the bull sperms immotile / 2 min in vitro (Chinoy et al., 1995).

However, Zakrzewska et al. (2002) attributed the fluoride toxicity to its direct effect on the motile apparatus. Another cause could be decline in the fructose level, which provides energy for motility, in the seminal vesicle and vas deferens due to alteration in carbohydrate as reported by Kumar et al. (2010). Fluoride may act by inhibiting many enzymes: the first mode of action is that fluoride binds with co-factors like Mg, Ca, Zn and Se and thus inhibits glycolysis, respiration and motility of sperms (Zakrzewska et al., 2002). Another reason for decreased sperm motility was related to decreased level of androgen carrier proteins involved in sperm motility (Chinoy et al., 1997). Furthermore, structural defects were observed in the flagellum, acrosome and nucleus of spermatids and epididymal spermatozoa of fluoride-treated rabbits (10 mg/kg/day for 18 months) leading to abnormal motility (Kumar and Susheela 1995).

3.5. Pathological results:
3.5.1. Histopathological and histochemical results:

In the present study, the microscopic examination of the testes ,epididymis and prostate gland of the control rabbits showed normal structure and normal spermatogenesis with different stages of differentiation and maturation (Fig.1). Moreover, the testicular tissues revealed high positive reaction for PAS.

By contrast, the treated NaF group animals showed many pathological alterations including necrosis and a prominent decline in the number of spermatogonial cells resulting in epithelial disorganization and absence of all internal layers except scattered cells of spermatogonia layer along the basement membrane, leading to complete cessation of spermatogenesis and the tubular lumen showed a few scattered clusters of spermatozoa with luminal cellular debris. Vascular degeneration of the spermatogenic and Sertoli cells as well as leydig cells necrosis that appeared with deeply eosinophilic cytoplasm and rounded nuclei. Moreover, there was thick and irregular basement membrane (Fig.2&3). In addition to, marked inflammatory infiltration in the interstitial tissue of the seminiferous tubules was observed. Congestion of interstitial blood vessel and thickening of tunica albuginia (Fig.4) were seen that gave positive reaction with Masson’s Trichrome stain and resulting in testicular atrophy was observed. These testicular tissues showed a weak reaction with PAS (Fig.5).

While the epididymal epithelium was desquamated and severe hyalinization of epididymal tissue with focal inflammatory cell infiltrations were observed. The tubules were deshaped with loss of cilia of the epithelial lining and possessed little or no spermatozoa (Fig.6). In cauda epididymis, the epithelial cell height was reduced with nuclear pyknosis, denudation of cells.

The prostate gland showed evidence of variable degree of acinus epithelial hyperplasia accompanied with severe dilation resulted in giant acini and papillary projection formation. In addition to, there were focal and diffuse inflammatory infiltrations (Fig.7). Some acini were filled with eosinophilic pretentious material.

The histological picture of treated group with propolis only was similar to control group in which the denser luminal space was filled with spermatozoa as well as enhancement of zone of mitosis (Fig.8).

On the other side, the microscopic examination of the treated rabbits with propolis revealed an improvement in histological picture of fluoride toxicity of testis sections. The later showed normal seminiferous tubules epithelium with distinct nuclei and mature sperm bundles in their lumen, with normal basement membrane as well as normal interstitial cells of Leydig cells and lack of congestion .All along the basement membranes, in each seminiferous tubule, spermatogonia were arranged in concentric layers (typically representing the zone of mitosis), followed by similar concentric layers of spermatocytes immediately inward to the spermatogonial layers (representing the zone of meiosis), while the core area contained differentiating spermatozoa (identified as the zone of spermiogenesis) (Fig.9). A marked improvement in the positive PAS reaction in the testicular was noticed. In addition to fluoride improved the epididymal (Fig.10) and prostate picture that resemble to normal structure.
Thus, our histopathological results confirmed the observations of other authors (Ghosh et al., 2002; Hanaa El-Hallawany and Abeer El-Metwally, 2011; Tiwari and Pande, 2011 and Ahmad et al., 2012) in mice and Susheela et al. (2006) in rabbits. Moreover, the NaF exposure showed severe loss of Leydig cells as a result of the oxidative stress that affected on steroidogenesis and testicular apoptosis (Sarkar et al., 2006 and Enaam Falih and Zainab Ibrahim, 2015). On the other hand, Sprando et al. (1998) showed that rats fed on a 250 ppm fluoride /10 weeks showed no distinguishable change in testicular histology and this might be due to a lower level of fluoride.

The necrosis of seminiferous tubules was in acceptance with (Yang et al., 2002) who explained that fluoride stimulated free radicals which increased lipid peroxide (LPO) levels, and decreased the activities of glutathione peroxidase (GSH-Px) and ATPase in testis and epididymis that picked up by mitochondria producing swelling and distortion of mitochondrial cristae, uncoupled energy metabolism, inhibited cellular respiration, and altered calcium kinetics. Moreover, Fatma Agha et al., (2012) suggested that the oxidative stress results from the loss of equilibrium between oxidative and antioxidative mechanisms that can produce DNA fragmentation, resulting in apoptosis. The same finding was attributed by Shashi et al., (2010) who explained that fluoride toxicity leads to loss of selective permeability of the cell membrane, resulting in dilatation of cytoplasmic component secondary to intracellular fluid and electrolyte redistribution.

Sertoli cell degeneration related to decreased sperm quality and abnormal spermatozoa has been reported by Ozmen and Mor (2012) which supported our observations, in addition to the apoptotic appearance of Sertoli cells which are essential for spermatogenesis.

Yang et al. (2002) aimed the infiltration of inflammatory cells in testis and epididymis due to severe irritation of fluoride ions on the tissue parenchyma that causes apoptosis and necrosis of germinal cells

3.5.2. Immunohistochemical Results:

In this study, we investigate the roles of apoptosis and autophagy in testicular and epididymal toxicity of fluoride. Apoptosis is a physiological process of selected cell deletion. As an antagonist of cell proliferation, apoptosis contributes to keeping the cell number in testicular tissue and helps to remove superfluous and damaged cells, but excessive apoptosis could cause destruction of male reproductive function (Rania Elgawish and Heba Abdelrazek, 2014).

Caspases, or cysteine-aspartic proteases, are a family of cysteine proteases, which play an essential roles in apoptosis (programmed cell death), necrosis and inflammation as well as it also required in the immune system for cytokines production (Mathur, 2006). Failure of apoptosis is one of the main contributions to tumor development (Dias et al., 2000).

In this study, testis of control group (Fig. 11) and propolis only (Fig. 12) revealed negative or low caspase-3 immunohistochemical reactivity. However, few scattered cells exhibited faint light brown granules in stroma and less in the cytoplasm of germinal epithelium were detected. While there was intensive positive immunoreactivity for caspase-3 (dark brown) was observed in the cytoplasm of germinal cells of NaF treated group compared with the controls which reflecting the severity of NaF toxicity in testes. The apoptotic positive cells was seen to be prominent in germinal epithelium, Sertoli cells and Leydig cells stroma that was the possible cause of defective and insufficient sperm quality(Fig. 13&14). Slight caspase-positive reaction was also seen in spermatids.

On the other hand, testicular cells of NaF in combination with propolis treated group showed apparent reduction of caspase-3 immunoreactivity to be more or less similar to the control group (Fig. 15).

The obtained results came in accordance with Wang et al. (2009); Yilmaza and Erkan (2015) and Zhang et al. (2016). In this study, one of the other most severely affected types of cell was Leydig cells that are very important for testosterone and subsequently, spermatogenesis. The caspase positive reaction in Leydig cells correlated with decreased serum testosterone level which came in accordance with previous results of Ozmen and Mor (2012).

Mitochondria have a crucial function in initiating the cascade of caspase activation. Disruption of the outer mitochondrial membrane by apoptotic stimuli results in the release of cytochrome c into the cytoplasm where it initiates a cascade of caspase activation and results in apoptosis (Anuradha et al., 2001). Fluoride ion, although a nonoxidant ion, causes oxidative stress indirectly leading to an increase in lipid peroxide levels which may impair a variety of intra and extramitochondrial membrane transport systems that may contribute to apoptosis, such as mitochondrial ion-transport systems (Khandare et al., 2011).
Other factors responsible for the arrest of spermatogenesis might be the lack of available proteins necessary for cell division, growth and differentiation of germ cells as a result of 200–30 mg/L of fluoride, which may be attributed to the blockage of the G1 phase of the cell cycle. Another mechanism of apoptosis is increased levels of oxidants, which damages the DNA (Huang et al., 2007).

The protective effect of propolis against the reproductive toxicity of sodium fluoride was studied and this may be due to the activity of propolis as antioxidant in the present study. Chemically propolis may contain verities of compounds like resin, essential oils and waxes, and also contains amino acids, minerals, ethanol, vitamin A, B complex, E, and flavonoids. The primary mechanism of propolis may involve the scavenging of free radicals that cause lipid peroxidation as well as it may inhibit xanthenes oxidase, which is known to cause free radicals to be generated (Mahran et al., 2011).

Meanwhile, treatment of sodium fluoride group with propolis showed noticeable alleviation in histopathological changes and semen quality as well as testosterone level induced by NaF in the structures of testes and epididymis. Different studies confirmed that administration of antioxidants to animals intoxicated with NaF inhibits the alterations observed in exposure to fluorides, which clearly shows their preventive action (Chinoy and Patel, 2000; Krasowska et al., 2004 and Fatma Agha et al., 2012). Consequently, antioxidants could reduce cell death in different cellular structures caused by oxidants and free radicals, blocking apoptosis induced by changes in mitochondrial membrane permeability and subsequent release of cytochrome c and caspase activation (Fatma Agha et al., 2012).

Table (1): Effect of Sodium Fluoride on Testes, Epididymis Weight and Serum Testosterone in Male Rabbit Groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Testis weight (g)</th>
<th>Epididymal weight (g)</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.955±0.05</td>
<td>0.60 ± 0.20</td>
<td>0.840±0.021</td>
</tr>
<tr>
<td>NaF group</td>
<td>0.702±0.02*</td>
<td>0.40 ± 0.01*</td>
<td>0.120±0.001 *</td>
</tr>
<tr>
<td>Propolis group</td>
<td>1.936±0.03*</td>
<td>0.62 ± 0.05*</td>
<td>0.846±0.015</td>
</tr>
<tr>
<td>NaF+ Propolis group</td>
<td>2.004±0.05*</td>
<td>0.58± 0.02*</td>
<td>0.827±0.030</td>
</tr>
</tbody>
</table>

- Means with stars are significantly different (P <0.05). - All data are expressed as means ± SEM.

Table (2): Effect of sodium Fluoride on Semen Quality in Male Rabbit Groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Sperm Count x10⁷/ epididymis</th>
<th>Sperm Motility (%)</th>
<th>Sperm abnormalities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>260.0±6.52</td>
<td>55.25±2.06</td>
<td>36.25±2.06</td>
</tr>
<tr>
<td>NaF group</td>
<td>170.4±6.30*</td>
<td>27.50±3.23*</td>
<td>53.00±3.14*</td>
</tr>
<tr>
<td>Propolis group</td>
<td>262.0±6.55*</td>
<td>58.50±3.33*</td>
<td>37.75±2.17*</td>
</tr>
<tr>
<td>NaF+ Propolis group</td>
<td>256.3±12.5</td>
<td>53.25±2.17</td>
<td>34.50±3.53*</td>
</tr>
</tbody>
</table>

Means with stars are significantly different (P <0.05). - All data are expressed as means ± SEM.
Fig. 4: Testis of rabbit treated with NaF only showing marked proliferation of fibrous connective tissue (Masson's Trichrome stain, X100). (H&E, X100).

Fig. 5: Testis of rabbit treated with NaF only showing weak PAS reaction and marked thickening of basement membranes of seminiferous tubules (PAS stain 100X).

Fig. 6: Caput epididymis of rabbit treated with NaF only showing epithelial and vacuolization and infiltration of mononuclear cells (H&E, X100).

Fig. 7: Prostate of rabbit treated with NaF only showing dilation of acini with papillary projection and focal inflammatory cell aggregations (H&E stain, 40X).

Fig. 8: Testis of rabbit treated with propolis only showing normal histological structure of seminiferous tubules (H&E, X100).

Fig. 9: Testis of rabbit treated with NaF + propolis showing normal histological structure of most seminiferous tubules (H&E, X100).

Fig. 10: Epididymis of rabbit treated with NaF + propolis showing normal histological structure of its tubules (H&E, X100).

Fig. 11: Immunohistochemical staining of activated caspase-3 in testis of control rabbits showing weak immunostaining for caspase-3 (caspase-3 immunostain, X 40).

Fig. 12: Immunohistochemical staining of activated caspase-3 in testis of rabbits treated with propolis only showing weak immunostaining for caspase-3 in the cytoplasm of few spermatogenic cells (caspase-3 immunostain, X 40).
4. CONCLUSION:

The results of the present study showed that Sodium fluoride induced histopathological testicular damages, deterioration in testosterone levels and quantity and quality of semen changes in rabbit bucks. The administration of propolis with Sodium fluoride showed marked improvement effects against Sodium fluoride reproductive toxicity. Such improvement could be attributed to the activity of propolis as an antioxidant capacity against male infertility. Therefore, using diet rich in propolis could be a beneficial way to overcome the reproductive toxicity of Sodium fluoride.

5. REFERENCES:


