

# The Effects of Dietary Egyptian Propolis and Bee Pollen Supplementation against Toxicity of Sodium Fluoride in Rats

Fatma A. Khalil and Nora M. El-Sheikh

Biochemistry and Nutrition Department, Women's College, Ain Shams University, Cairo, Egypt.

**Abstract:** Propolis and bee pollen are substances produced by honey bees its components are strong antioxidant and free radical scavengers. The present study aimed to study the protective effects of propolis and bee pollen supplementation against toxicity of sodium fluoride in rats. After the end of experimental period, the rats sacrificed and biochemical analysis were carried out. The results showed that the administration of fluoride (F) alone causes significant increase of malondialdehyde (MDA) level and significant decrease of antioxidant system as erythrocyte superoxide dismutase (SOD) activity and reduced glutathione (GSH) levels in blood and brain. Also F causes significant increase alkaline phosphatase (ALP) activity, urea, creatinine, sodium and potassium levels. And significant decrease total protein, calcium, magnesium and phosphorus levels as compared to control group ( $P < 0.05$ ). Whereas administration of propolis or bee pollen with F led to significant decrease in MDA level and significant increase in SOD activity, GSH levels in blood and brain. And significant decrease ALP activity, urea, creatinine, sodium and potassium levels in serum. The propolis or bee pollen enhanced total protein, calcium, magnesium and phosphorus levels in serum as compared to F group alone. In conclusion; supplementation of natural antioxidant (propolis or bee pollen) during Fluoride administration, facilitate reduction of the toxic effects and enhanced the antioxidant system, the levels of minerals is serum. [Journal of American Science. 2010;6(11):310-316]. (ISSN: 1545-1003).

**Keywords:** Propolis, bee pollen, sodium fluoride, rats, antioxidant system minerals.

## 1. Introduction

Fluoride (F) is highly electronegative anion with cumulative toxic effects, from prolonged ingestion that can lead to the pathogenesis known as fluorosis a condition especially persistent in third world countries, where populations have little choice as to the main source of their often times-F-contaminated drinking, other sources include private water supplies, dietary ingredients, dental products, industrial emissions, and/or occupational exposure, which can cause an individual's total F intake to exceed safe dose (Ozsvath, 2009).

Fluoride crosses the cell membrane very rapidly and distributed from the plasma to all tissue and organs (Bouaziz et al., 2006).

Propolis and bee pollen are natural substances collected by honey bees from buds and trees. Propolis a sticky substance that have bees manufacture by mixing their own waxes with resinous sap (Yoshimi et al., 2009). The main chemical classes found in propolis are flavanoids, phenolic and various aromatic compound. However, propolis contains many of B-complex vitamins, important mineral and trace elements, caffeic acid phenethyl ester (CAPE), an active component of propolis, exhibits antioxidant properties.

Nowadays propolis is used in many medical formulas and food supplements for improving health, preventing and treating infections, inflammatory

diseases and effects of toxic substances (Attalla and Ayman, 2008).

Bee pollen is rich in carotenoids, flavonoid and phytosterols. The exact profile varies depending on the plant sources and growing conditions, however, beta-carotene, beta sitosterol, isohammetin, kaempferol, lycopene, quercetin and rutin are consistently (Campos et al., 2003). The antioxidant activity of flavanoides present in propolis and bee pollen has been shown to be capable of scavenging free radical (Survswaran et al., 2007).

The aim of this study was to evaluate the antioxidant effects of dietary Egyptian propolis and bee pollen supplementation against toxicity of sodium fluoride in rats.

## 2. Materials and Methods

### Materials

Sodium fluoride (AR, BDH) was used as the source of fluoride. The sodium fluoride was added to the standard diet at 1 g/kg diet (Bellack and Schoube, 1968).

The propolis and bee pollen used in the present study originated from the hive in Cairo, Egypt. These samples were harvested in September 2009. Bee pollen was obtained as yellow granules, while propolis was derived in the form of yellow-brown powder.

**Experimental animals:**

Adult male albino rats weighing ( $130 \pm 13.9$  g) were kept in [12:12 h (light:dark) photo period] and temperature ( $22 \pm 0.5^\circ\text{C}$ ) controlled room maintained at constant relative humidity of 65-70% and fed standard diet and water *ad libitum*.

**Diet:**

The standard diet was prepared according to (Revees et al., 1993).

**Experimental design:**

All animals fed on the standard diet and the animals were divided into 6 groups (10 animals in each group):

Group (1): Control rats (without any treatment).

Group (2): Rats administrated with sodium fluoride alone 1 g/kg diet (F groups).

Group (3): Rats administrated F and treated with propolis powder in diet 0.1%.

Group (4): Rats administrated F and treated with propolis powder in diet 0.2%.

Group (5): Rats administrated F and treated with bee pollen in diet 1%.

Group (6): Rats administrated F and treated with bee pollen in diet 2%.

After the end of the experimental period (42 days), all animals were fasted overnight and sacrificed. The two blood samples were collected from each animal, with and without anticoagulant for the following biochemical analysis. Reduced glutathione (GSH) was measured in blood and brain homogenate according to the method of Beutler et al. (1963). Malondialdehyde (MDA) level was measured in brain according to Satoh (1978).

Erythrocyte superoxide dismutase activity was determined in accordance with the method described by Sun et al. (1988). ALP activity in serum was determined by the method of Anon (1974), serum total protein level was determined by the method of Gornall et al. (1949). Serum creatinine and urea were determined by the methods of Bonsens and Taussky (1984) and Patton and Crouch (1977), respectively.

Serum sodium, potassium, calcium, magnesium and phosphorus were estimated by the colorimetric method of Berry et al. (1988), Sunderman and Sunderman (1958), Sarkar and

Chauvan (1967), Teitz (1983) and Drewes (1972), respectively.

**Statistical analysis:**

Results are expressed as mean  $\pm$  SD. The data were statistically analyzed following the one way analysis of variance [ANOVA, F test and least significant difference (L.S.D)] at ( $P < 0.05$ ) were carried out using SPSS version 11.5 (2002) SPSS Chicago / L, USA.

**3. Results**

From the results of Table (1) obtained it is evident that fluoride administration alone in group (2), caused significant increase of MDA level in brain and significant decrease in GSH levels in blood and brain, erythrocyte SOD activity as compared to control group ( $P < 0.05$ ). But the administration of propolis or bee pollen with fluoride significant decrease the MDA level in brain and significant increase GSH levels in blood and brain, erythrocyte SOD activity as compared to F group ( $P < 0.05$ ).

Table (2) shows that there was a significant increase in ALP activity, urea and creatinine. And significant decrease in total protein in F group as compared to control group ( $P < 0.05$ ). But the propolis or bee pollen enhanced the toxic effect of fluoride by a significant decrease in ALP activity, urea and creatinine. And significant increase in total protein in treated groups as compared to F group.

Table (3) and Figs. (1-5) shows the levels of serum cations in control group (G 1), fluoride group (G 2) and treated group (G 3 - G 6).

There was a significant increase in serum sodium and potassium levels and significant decrease in serum calcium, magnesium and phosphorus in F group as compared to control group ( $P < 0.05$ ). Whereas the administration of propolis and bee pollen improved the levels of cations in treated group as compared to F group ( $P < 0.05$ ).

**Table (1): Effects of propolis and bee pollen on lipid peroxide as (MDA) and antioxidant system in fluorotic rats.**

Parameters Groups	Brain MDA (nmol/ mg tissue)	Blood GSH (mg/dL)	Brain GSH (mg/g tissue)	Erythrocyte SOD activity (U/mL)
Group (1) (control)	0.70 ± 0.11 <sup>d</sup>	28.09 ± 1.91 <sup>a</sup>	14.99 ± 0.99 <sup>b</sup>	277.75 ± 5.70 <sup>a</sup>
Group (2) (F group)	3.08 ± 0.23 <sup>a</sup>	16.27 ± 0.94 <sup>e</sup>	8.04 ± 0.27 <sup>e</sup>	177.88 ± 10.13 <sup>d</sup>
Group (3) (F + propolis 0.1%)	0.51 ± 0.08 <sup>e</sup>	22.16 ± 1.20 <sup>c</sup>	13.25 ± 0.88 <sup>c</sup>	265.75 ± 6.78 <sup>b</sup>
Group (4) (F + propolis 0.2%)	1.10 ± 0.12 <sup>b</sup>	20.13 ± 1.18 <sup>d</sup>	9.75 ± 0.46 <sup>d</sup>	260.25 ± 4.68 <sup>bc</sup>
Group (5) (F + bee pollen 1%)	0.93 ± 0.10 <sup>c</sup>	27.70 ± 2.13 <sup>a</sup>	14.76 ± 0.91 <sup>b</sup>	279.38 ± 10.15 <sup>a</sup>
Group (6) (F + bee pollen 2%)	0.88 ± 0.08 <sup>c</sup>	25.38 ± 1.07 <sup>b</sup>	16.14 ± 1.43 <sup>a</sup>	270.75 ± 7.98 <sup>b</sup>
L.S.D.	0.135	1.49	0.91	7.93

Values are represented as mean ± SD. 10 rats each group.

Same letters (a, b, c, d) above each group indicate non significant difference between groups at (P < 0.05) in same column.

Different letters in the same column indicate significant difference between groups.

**Table (2): Effects of propolis and bee pollen on serum ALP activity, total protein, urea and creatinine in fluorotic rats.**

Parameters Groups	ALP activity (IU/L)	Total protein (g/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Group (1) (control)	167.56 ± 5.23 <sup>e</sup>	5.71 ± 0.52 <sup>a</sup>	33.93 ± 2.96 <sup>b</sup>	1.51 ± 0.08 <sup>b</sup>
Group (2) (F group)	323.13 ± 11.63 <sup>a</sup>	3.68 ± 0.11 <sup>d</sup>	63.57 ± 6.20 <sup>a</sup>	2.06 ± 0.14 <sup>a</sup>
Group (3) (F + propolis 0.1%)	258.00 ± 11.14 <sup>c</sup>	4.21 ± 0.18 <sup>c</sup>	36.68 ± 3.40 <sup>b</sup>	1.05 ± 0.04 <sup>d</sup>
Group (4) (F + propolis 0.2%)	269.75 ± 7.74 <sup>b</sup>	4.27 ± 0.11 <sup>c</sup>	34.68 ± 2.61 <sup>b</sup>	1.09 ± 0.08 <sup>d</sup>
Group (5) (F + bee pollen 1%)	266.63 ± 11.39 <sup>bc</sup>	4.36 ± 0.10 <sup>c</sup>	35.19 ± 2.01 <sup>b</sup>	1.22 ± 0.10 <sup>c</sup>
Group (6) (F + bee pollen 2%)	235.25 ± 9.21 <sup>d</sup>	4.83 ± 0.15 <sup>b</sup>	35.33 ± 2.32 <sup>b</sup>	1.08 ± 0.07 <sup>d</sup>
L.S.D.	9.76	0.25	3.64	0.09

Values are represented as mean ± SD. 10 rats each group.

Same letters (a, b, c, d) above each group indicate non significant difference between groups at (P < 0.05) in same column.

Different letters in the same column indicate significant difference between groups.

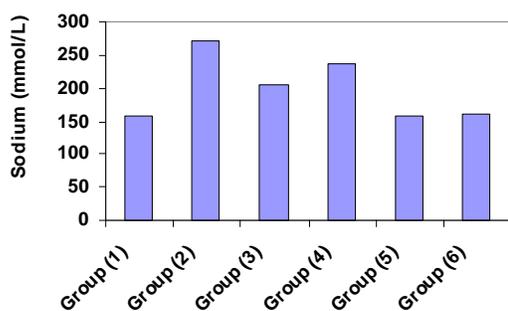
**Table (3): Effects of propolis and bee pollen on serum cations in fluorotic rats.**

Parameters Groups	Sodium (mmol/L)	Potassium (mmol/L)	Calcium (mmol/L)	Magnesium (mmol/L)	Phosphorus (mmol/L)
Group (1)	159.38±10.16 <sup>d</sup>	4.94 ± 1.12 <sup>d</sup>	3.48 ± 0.20 <sup>a</sup>	1.41 ± 0.20 <sup>bc</sup>	4.36 ± 0.11 <sup>a</sup>
Group (2)	272.75 ± 9.79 <sup>a</sup>	12.19 ± 1.40 <sup>a</sup>	2.34 ± 0.14 <sup>d</sup>	1.06 ± 0.08 <sup>d</sup>	3.01 ± 0.14 <sup>d</sup>
Group (3)	203.75±18.10 <sup>c</sup>	6.49 ± 0.30 <sup>c</sup>	3.09 ± 0.12 <sup>b</sup>	1.49 ± 0.08 <sup>bc</sup>	3.80 ± 0.09 <sup>b</sup>
Group (4)	237.38±11.65 <sup>b</sup>	8.05 ± 0.28 <sup>b</sup>	2.84 ± 0.14 <sup>bc</sup>	1.49 ± 0.08 <sup>bc</sup>	3.55 ± 0.16 <sup>c</sup>
Group (5)	158.13 ± 7.74 <sup>d</sup>	7.06 ± 0.61 <sup>c</sup>	2.98 ± 0.19 <sup>b</sup>	1.54 ± 0.05 <sup>b</sup>	4.28 ± 0.13 <sup>a</sup>
Group (6)	160.13 ± 9.82 <sup>d</sup>	8.33 ± 0.86 <sup>b</sup>	2.83 ± 0.09 <sup>c</sup>	1.66 ± 0.04 <sup>a</sup>	4.26 ± 0.14 <sup>a</sup>
L.S.D.	11.79	0.87	0.15	0.1	0.13

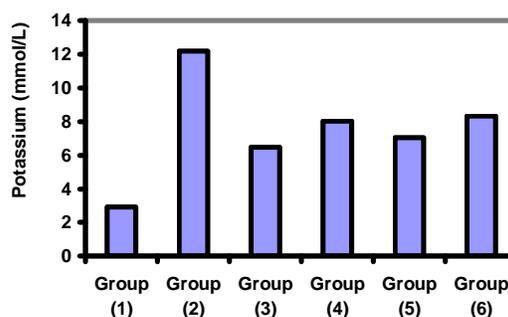
Values are represented as mean ± SD. 10 rats each group.

Same letters (a, b c, d) above each group indicate non significant difference between groups at (P < 0.05) in same column.

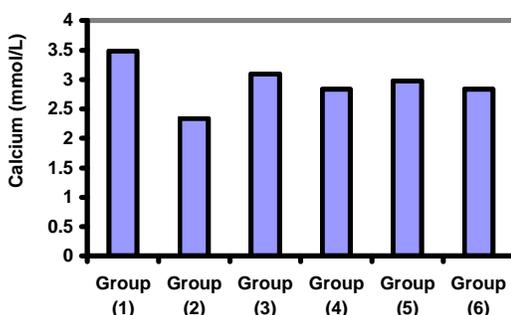
Different letters in the same Group column indicate significant difference between groups.



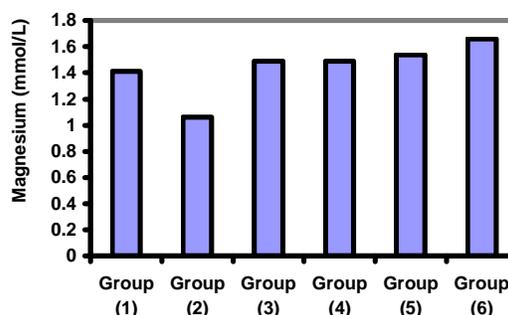
**Fig. (1): Effects of propolis and bee pollen on serum sodium in fluorotic rats.**



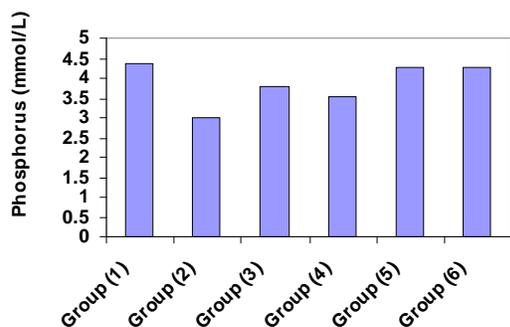
**Fig. (2): Effects of propolis and bee pollen on serum potassium in fluorotic rats.**



**Fig. (3): Effects of propolis and bee pollen on serum calcium in fluorotic rats.**



**Fig. (4): Effects of propolis and bee pollen on serum magnesium in fluorotic rats.**



**Fig, (5): Effects of propolis and bee pollen on serum phosphorus in fluorotic rats.**

#### 4. Discussion

Reactive oxygen species (ROS) play key roles in many physiologic and pathogenic processes. In fact, many ophthalmologic and neurodegenerative diseases seem to be mediated, at least in part, by oxidative stress (Finkel and Halbrook, 2000). The generation of free radicals constitute one of the underlying mechanisms of the fluoride intoxication (Birkner et al., 2000).

In the present study, the elevated level of MDA in brain and reduction of SOD activity and glutathione level in blood and brain in F group as compared to control group. Since the generation of free radicals also causes red blood cell damage occurs in tissues.

Chinoy and Shah (2004) have reported, fluoride can pass through in the blood brain barrier and accumulates in brain tissue and causes impaired antioxidant defense system. Furthermore, the other researchers have obtained similar results Kumari and Rao (1991) have reported an increase in MDA level in chronic fluoride intoxication.

In the present study, the results revealed that decrease in total protein and increase in ALP activity, urea and creatinine in F group as compared to control group. Fluoride is known to inhibit protein synthesis, mainly due to impairment of peptide chain initiation and by interfering with peptide chains on ribosomes (Michael et al., 1996).

Eraslan et al. (2007) have also reported ALP activity increase of the damage of hepatic cells and the obstruction of bile ducts. ALP is the marker enzyme of fluoride toxicosis and bone pathology increase in serum ALP activity in animals treated with fluoride has been reported (Shanthakumari and Subramanian, 2007), it may be due to fluoride induced cell injury in both osteoblast and osteocytes initiates a repair response. Birkner et al. (2000) have also reported increase in the serum urea level of rats

with acute fluoride intoxication. The administration of fluoride suggests failure of excretion in the kidney. In the present study, the F group showed the serum potassium and sodium levels increased significantly, calcium, magnesium and phosphorus decreased significantly as compared to control group. The results are similar to Chinoy et al. (1993) have reported that demonstrated rats with sodium fluoride, it may be due to change to alteration in aderenal function. Fluoride interacts and alters the metabolism of calcium and magnesium, the decrease in serum calcium related to decrease of intestinal absorption of calcium by fluoride (Xin et al., 2006).

Propolis and bee pollen are opicultural products which are composed of nutritionally valuable substances and contain considerable amounts of polyphenol substances which may act as potent antioxidant (Teixeira et al., 2008). Flavonoids and phenolic acids are major classes of polyphenolic compounds, whose structure-antioxidant activity (Gardjeva et al., 2007). Mechanisms of antioxidant action may include suppression of ROS formation, removal or inactivation of oxygen reactive species and up-regulation or protection of antioxidant defenses (Montoro et al., 2005).

The results of the present study revealed that, administration of propolis or bee pollen with F led to significant decrease in MDA level in brain and significant increase in antioxidant system as SOD activity and GSH levels. The propolis or bee pollen significant decrease the ALP activity, urea, creatinine, potassium and sodium levels. Also propolis or bee pollen enhanced total protein, calcium, magnesium and phosphorus levels.

Caffeic acid phenethyl ester (CAPE) is an active component of propolis and has been used in traditional medicine to treat a number of diseases, CAPE treatment have been shown to protect tissues from ROS mediated oxidative stress and reduce lipid peroxidation in ischemia and toxic injuries. The antioxidant activity of CAPE is due to the presence of two hydroxyl groups in its structure (Sud'ina et al., 1993).

Twelve different flavonoids, pinocembrin, acacetin, chrysin, rutin, catechin, naringenin, galangin, luteolin, kaemferol, a pigenin, myricetin and quercetin, two phenolic acids, cinnamic and caffeic acid present in propolis (Volpi, 2004). Propolis contain acid derivatives such as benzoic-4-hydroxy benzoic which improves the digestive utilization of calcium, phosphorus and magnesium (Haro et al., 2000).

Propolis has an anabolic effect and bee pollens are rich in essential amino acids, protein, unsaturated fatty acids and also contains many

vitamins, minerals and trace elements which contribute to the health effects (Campos et al., 2003). Pollen is extremely rich in rutin and may have highest content of any source. Bee pollen has been shown to improve immune system and remove toxins from our bodies (Campos et al., 1997).

The recent investigations indicated that bee pollen contain significant amount of polyphenolic substances, mainly flavonoids. The polyphenols also have metal chelation properties and free radical scavenging activity (Abdella et al., 2009).

The conclusion of the present study suggests that the propolis or bee pollen its components, are strong antioxidants and free radical scavengers. Ameliorated the liver, kidney and brain from toxicity with sodium fluoride and enhanced the levels of minerals in serum.

#### Corresponding author

Fatma A. Khalil

Biochemistry and Nutrition Department, Women's College, Ain Shams University, Cairo, Egypt.

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