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THE EFFECTS OF DIETARY EGYPTIAN PROPOLIS AND BEE POLLEN SUPPLEMENTATION AGAINST TOXICITY OF SODIUM FLUORIDE IN RATS

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SUMMARY

Propolis and bee pollen are substances produced by honey bees. Its components are strong antioxidants and free radical scavengers. The present study aimed to study the protective effects of propolis and bee pollen supplementation against toxicity of sodium fluoride (F) in rats. Rats were divided into six groups each 10 rats and treated for 6 weeks: group 1 as control group; group 2 fed on standard diet with F; groups (3-6) fed on standard diet with F and supplemented with different concentration of propolis or bee pollen at 0.1%, 0.2% and 1%, 2% respectively. After the end of experimental period, the rats were sacrified and biochemical analyses were carried out. The results showed that the administration of fluoride alone caused a significant increase of malondialdehyde (MDA) level and a significant decrease of antioxidant system as measured by erythrocyte superoxide dismutase (SOD) activity and reduced glutathione (GSH) levels in blood and brain. Also F caused a significant increase in alkaline phosphatase (ALP) activity, urea, creatinine, sodium and potassium levels.Moreover, a significant decrease in total protein, calcium, magnesium and phosphorus levels as compared to control group ($P < 0.05$) was recorded. The administration of propolis or bee pollen with F led to a significant decrease in MDA level and a significant increase in SOD activity, GSH levels in blood and brain.As well as significant decrease in ALP activity, urea, creatinine, sodium and potassium levels in serum was also observed. The propolis or bee pollen supplementation enhanced total protein, calcium, magnesium and phosphorus levels in serum as compared to F group alone.

In conclusion; supplementation of natural antioxidants (propolis or bee

pollen) during Fluoride administration, facilitates reduction of the toxic effects and enhanced both the antioxidant system, as well as the levels of minerals in the serum.

Keywords:

Propolis, bee pollen, sodium fluoride, rats, antioxidant system, minerals.

INTRODUCTION

Fluoride (F) is a highly electronegative anion with cumulative toxic effects, from prolonged ingestion that can lead to the pathogenesis known as fluorosis which is a condition especially persistent in the 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 then distributes 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 is 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 flavonoids, phenolic and various aromatic compounds. Propolis also contains many of B-complex vitamins, important mineral and trace elements, caffeic acid phenethyl ester (CAPE) which is an active component of propolis exhibits antioxidant properties.

Nowadays propolis is used in many medical formulae and food supplements for improving health, preventing and treating infections, inflammatory diseases and effects of toxic substances (Attalla and Owayss, 2008).

Bee pollen is rich in carotenoids, flovonoids 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 consistent (Campos et al., 2003). The antioxidant activity of flavanoids 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 therefore to evaluate the protective effects of dietary Egyptian propolis and bee pollen supplementation against toxicity of sodium fluoride in rats.

MATERIAL AND METHODS

1. Materials

Sodium fluoride was purchased from El Gomhoriee Chemical Co. The sodium fluoride was added to the standard

diet at 1 g/kg diet to induce toxicity (Bellack and Schoube, 1968).

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

2. Experimental animals:

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

3. Diet:

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

Table (1): Composition of the standard diet.

Ingredient	g/kg
Protein (Casein)	140
Corn starch	610
Sucrose	100
Soy been oil	50
Fiber	50
Mineral mix.	35
Vitamin mix.	10
L. cystine	1.8
Choline bitartrate	2.5
Tert-butylhydroquinone	0.08

4. Experimental design:

Rats were fed on the standard diet and then they were divided into 6 groups (10 rats in each group):

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

Group (2): Rats fed on standard diet supplemented with sodium fluoride (F) alone 1 g/kg diet to induce F toxicity.

Group (3): Rats fed on standard diet supplemented with F and propolis powder in diet 0.1% (Haro et al., 2000).

Group (4): Rats fed on standard diet supplemented with F and propolis powder in diet 0.2%.

Group (5): Rats fed on standard diet supplemented with F and bee pollen in diet 1% (Haro et al., 2000).

Group (6): Rats fed on standard diet supplemented with F and bee pollen in diet 2%.

5. Biochemical analysis:

After the end of the experimental period (42 days), all rats were fasted overnight and sacrificed. Brain sample and two blood samples were collected from each animal, with and without anticoagulant for 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 mesured in brain according to Satoh (1978).

Erythrocyte superoxide dismutase (SOD) activity was determined in accordance with the method described by Sun et al. (1988). Alkaline phosphatase (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.

6. Statistical analysis:

Results are expressed as mean ± 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 (Levesque, 2007) SPSS Chicago / L, USA.

RESULTS

From the results shown in Table (2), it was evident that fluoride administration alone in group (2) caused a significant increase of MDA level in the brain and significant decrease in GSH levels in blood and brain, erythrocyte SOD activity as compared to control group ($P < 0.05$). However, the administration of propolis or bee pollen with fluoride significantly decrease the MDA level in the brain and also significantly increase GSH levels in the blood and brain, erythrocyte SOD activity as compared to F group ($P < 0.05$) and were nearly to control group.

Table (2): Effects of propolis and bee pollen supplementation on lipid peroxide as (MDA) and antioxidant system in rats (mean ± SD).

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

n=10 rats each group.
Values in the same column with different superscripts vary significantly ($P < 0.05$).

Table (3) shows that there was a significant increase in ALP activity, urea and creatinine, and a significant decrease in total protein in F group as compared to control group ($P < 0.05$). However, propolis or pee pollen improved the liver and kidney function which manifested by decrease the ALP activity, urea and creatinine and increase the total protein in treated groups as compared to F group and were nearly to the control group.

Table (3): Effects of propolis and bee pollen supplementation on serum ALP activity, total protein, urea and creatinine in rats (mean ± SD).

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

n=10 rats each group.
Values in the same column with different superscripts vary significantly ($P < 0.05$).

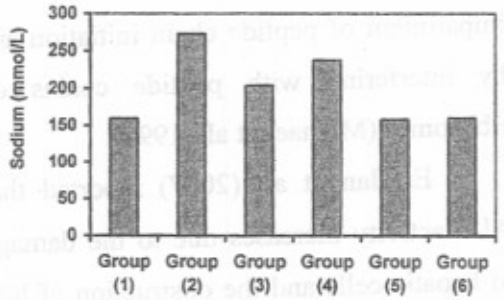
Table (4) and Figs. (1-5) show the levels of serum cations in control group (G 1), fluoride group (G 2) and treated group (G 3 - G 6).

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 groups as compared to F group ($P < 0.05$) and were nearly to the control group.

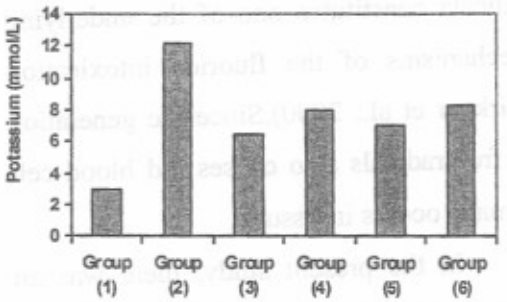
Table (4): Effects of propolis and bee pollen supplementation on serum cations in rats (mean ± SD).

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

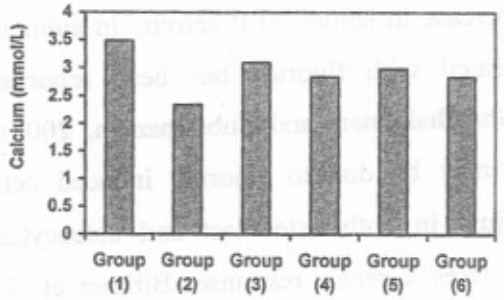
n=10 rats each group.
Values in the same column with different superscripts vary significantly ($P < 0.05$).



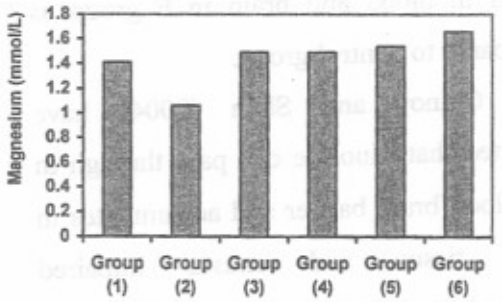
Fig, (1): Effects of propolis and bee pollen on serum sodium in rats.



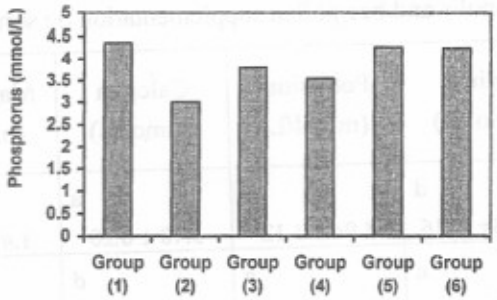
Fig, (2): Effects of propolis and bee pollen on serum potassium in rats.



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



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



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

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 constitutes one of the underlying mechanisms of the fluoride intoxication (Birkner et al., 2000).Since the generation of free radicals also causes red blood cell damage occurs in tissues.

In the present study, there was an increase in the level of MDA in brain and decrease of SOD activity and glutathione levels in blood and brain in F group as compared to control group.

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

In the present study, the obtained results revealed that a 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) reported that ALP activity increases due to the damage of hepatic cells and the obstruction of bile ducts. ALP is the marker enzyme of fluoride toxicosis and bone pathology. The 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 an 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 a significant increase in the serum potassium and sodium levels, calcium, magnesium and phosphorus decreased significantly as compared to control group. The results are similar to Chinoy et al. (1993) who have reported that demonstrated rats fed with sodium fluoride, it may lead to alteration in aderneal 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, supplementation of propolis or bee pollen to rats with F toxicity led to a significant decrease in MDA level in brain and a significant increase in antioxidant system as SOD activity and GSH levels. The propolis or bee pollen significantly 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 are 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 the highest content than other 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 a 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 and its components, are strong antioxidants and free radical scavengers. Ameliorated the liver, kidney and brain from toxicity with sodium fluoride as well as enhanced the levels of minerals in serum.

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تأثير المتناول الغذائي من صمغ العسل وحبوب اللقاح ضد سمية فلوريد الصوديوم في الجرذان

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صمغ العسل وحبوب اللقاح مواد من منتجات عسل النحل وهي مواد مضادات أكسدة قوية وتخلص الجسم من الشقوق الحرة .

تهدف الدراسة الحالية إلى معرفة التأثير الوقائي ضد السمية المحدثة بواسطة فلوريد الصوديوم في الجرذان . قسمت الجرذان إلى ست مجموعات كل مجموعة تحوى عشرة حيوانات ، المجموعة الأولى تناولت الغذاء المتوازن بدون أي إضافات ، المجموعة الثانية تناولت الغذاء المتوازن بالإضافة إلى فلوريد الصوديوم ١ جم/كيلو جم ، المجموعات من ٣-٦ تناولت غذاء متوازن بالإضافة إلى فلوريد الصوديوم وتم تدعيمها بصمغ العسل وحبوب اللقاح بتركيزات ٠.١% ، ٠.٢% ، ١% ، ٢% على الترتيب و التوالي . بعد انتهاء فترة التجربة (٦ أسابيع) تم تشريح الجرذان وكانت النتائج كالتالي: المجموعات التي تناولت فلوريد الصوديوم بمفرده بدون أي إضافات ظهرت بها ارتفاع معنوي في مستوى المالون داي ألدهيد بينما ظهر انخفاض معنوي في مستوى مضادات الأكسدة بالجسم وهي تتمثل في انخفاض قيم الجلوتاثيون المختزل وكذلك انخفاض نشاط إنزيم السوبر أوكسيد ديسميوتيز في كل من الدم والمخ .

أيضاً تناول فلوريد الصوديوم بمفرده أدى إلى ارتفاع في نشاط الفوسفاتيز القاعدي ومستوى اليوريا ، الكرياتينين ، الصوديوم والبوتاسيوم كما تسبب في انخفاض مستوى البروتين الكلي ، الكالسيوم ، الماغنسيوم والفوسفور مقارنة بالمجموعة الضابطة (الكنترول) . بينما إعطاء صمغ العسل وحبوب اللقاح كمكمل غذائي أدى إلى تقليل تأثير السمية المحدثة بواسطة فلوريد الصوديوم وظهر في ارتفاع نشاط السوبر أوكسيد ديسميوتيز ومستوى الجلوتاثيون المختزل في الدم والمخ . كما أظهر تحسن في مستوى البروتين الكلي ، الكالسيوم ، الماغنسيوم والفوسفور وحدوث انخفاض في مستوى الصوديوم والبوتاسيوم.

وخلص هذا البحث إلى أن تناول صمغ العسل بتركيز ٠.١% و ٠.٢% وحبوب اللقاح بتركيز ١% و ٢% كمكمل غذائي إلى الوجبات يؤدي إلى تقوية الجهاز المناعي ويقلل من تأثير السموم كما يرفع مستوى الكالسيوم والفوسفور ويحسن مستوى البروتين في الجسم ويرفع مستوى مضادات الأكسدة في الجسم لكونهم مضادات أكسدة طبيعية .