BIOCHEMICAL EFFECTS OF PROPOLIS AND BEE POLLEN IN EXPERIMENTALLY – INDUCED HYPERAMMONEMIA IN RATS

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A B S T R A C T

The main objective of this is study to investigate the biochemical effects of propolis and pollen grains as natural antioxidants on thioacetamide (TAA) at a dose of (150 mg/kg/bw single dose intraperitonelly (i.p.) induced hyperammonemia in rats. One hundred male albino rats were divided into 5 group (20 each). Group (1) act as a control group (2) injected with TAA at dose 150 mg/kg/bw i.p.) act as control hyperammonemia group (3) injected with TAA at a dose 150 mg/kg/bw i.p.) and treated with propolis at a dose 300 mg/kg/bw, group (4) injected with TAA at a dose 150 mg/kg/bw i.p.) and treated with bee pollen grains at a dose 25 g/kg/bw and group (5) injected with TAA at a dose 150 mg/kg/bw i.p.) and treated with both propolis and pollen grains with the same dose for two months. Serum was separated twice after 30 and 60 days of treatment all serum was collected for estimation of Aspartate aminotransferase (SGOT), Alanine aminotransferase (SGPT), Alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT), albumin, total protein, urea, creatinine, uric acid, gamm amino butyric acid (GABA), cholinesterase , N-acetyl glutamate synthase (NAGS), nitric oxide (NO) and plasma ammonia in control and propolis and pollen treated rats against TAA- induced hyperammonemia in rats. liver, brain and kidney were collected for determination superoxide dismutase (SOD), catalase (CAT) and L-malonialdehyde (L-MDA), results revealed a significant decreased in serum SGOT, SGPT, ALP, GGT, urea, creatinine, uric acid, gamm amino butyric acid (GABA), cholinesterase , nitric oxide (NO), plasma ammonia, L-MDA in tissues and also marked significantly increased in albumin, total protein, N-acetyl glutamate synthase (NAGS) and CAT, SOD in liver , kidney and brain tissues. The behavioral biochemical results indicated the effect of pollen grains and propolis against TAA- induced hyperammonemia in rats.

Keywords: Thioacetamide, Propolis, Oxidants, Antioxidants. Bee pollen.

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1. INTRODUCTION

Hyperammonemia is a metabolic disturbance characterized by an excess of ammonia in the blood that may lead to encephalopathy and death (Agarwal et al., 2005). Thioacetamide (TAA) is one of several agents that produce structural and functional changes, not only in liver, but also in other tissues as kidneys, thymus, spleen, intestine, brain and lungs (Hanaa, 2007). TAA is widely used in industry and is known to be one of the most potent hepatotoxicants in experimental animals (Durzong et al., 2012). TAA is metabolized to thioacetamide-S-oxide by cytochrome P450 enzymes system in liver, thioacetamide-S-oxide is responsible for the change in cell permeability, increase in intracellular Ca^{++} concentration, increase in nuclear volume enlargement of nucleoli and inhibition of mitochondrial activity which lead to cell death (Dhorajiya et al., 2012). In recent years, there has been renewed interest in the treatment against different diseases using herbal drugs as they are

generally non-toxic and world health organization has also recommended the evolution of the effeteness of plants in condition where we lack safe modern drugs (Ayynar et al., 2008). Propolis (bee glue) is known as a resinous dark-colored material which is collected by honeybees from the buds of living plants mixed with bee wax and salivary secretions. Crude extracts of propolis contains amino acids, phenolic acids, phenolic acids esters, flavonoids, cinnamic acid, terpenes and caffeic acid, and its compositions alter resulting from variation in geographical and botanical origin (Russo et al., 2002). Propolis became a part of folk medicine and its biological effects, including anti-inflammatory, antiviral, antibacterial, anti microbial, antioxidative, anti-ulcer and anti-tumor activities, immune-stimulatory and carcinostatic activities, the broad spectrum of activity of propolis was mainly attributed to the large number of flavonoids. Bee Pollen is the male gametophyte of flowers (Campos et al., 2008) Bee pollen is an apicultural product, made up of natural flower pollen mixed with nectar and bee secretions and it is rich in sugars, proteins, lipids, vitamins and flavonoids (3-5% dry weight) , commercially traded bee pollen is mainly collected by the honey bee (Apis mellifera L). Bee pollen is used as the main source of other important nutrients, including proteins, minerals and lipids (Almaraz et al., 2007) in general and after intense research on this subject, recent reviews indicate that bee pollen is usually composed of 13-55% total carbohydrates, 0.3-20% dietary fiber, pectin, 1-13% lipids (with a good ratio of unsaturated/saturated fatty acids, including α-linolenic acid) , 10-40% protein, 2-6% ash , accompanied by a variety of secondary plant products, such as flavonoids, carotenoids and terpens in addition it should be enhanced that pollen contains important minerals as Zn, Cu and Fe ,several vitamins: pro-vitamin A, Vitamin E, niacin, thiamine, folic acid and biotin (Campos et al., 2010).

2. MATERIALS AND METHODS

One hundred white male albino rats of 8-10 week old and weighting 150-180 gm were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment, the animals were fed on constant ration and water was supplied ad-libitum.

1-Induction of hyperammonemia

Hyperammonemia was induced by injecting the rats intraperitoneally (i.p.) with a single dose of TAA at a dose 150 mg/kg/b.w (Hanaa M. S. 2007 and Baskaran et al.,2010). TAA purchased from El-Gomhouria CO. for trading chemicals, medicines and medical appliances, Amerya, Cairo, Egypt.

2-Preparation of propolis

Propolis was administered orally to rats at a dose of 300 mg/kg/b.w daily for 60 days, propolis dissolved in warmly distilled water and shaken at room temperature (Cunha et al., 2004). Propolis (purity-99%) was purchased from Faculty of Agriculture Benha university.

3-Preparation of pollen grains

Pollen grains was administered orally to rats at a dose of 25g/kg/b.w daily for 60 days pollen grains dissolved in warmly distilled water and shaken at room temperature (Güldeniz et al., 2007). Pollen grains (purity-99%) was purchased from Faculty of Agriculture Benha university.

4- Experimental design

Animals were randomly divided into five main groups placed in individual cages and classified as follow:

Group 1: Control group: 20 rats administered constant ration and water was supplied ad-libitum for 60 days.

Group 2: Hyperammonemic group as positive control: 20 rats injected intraperitonelly (i.p) with a single dose of TAA at a dose 150 mg/kg/bw.

Group 3: Propolis treated group: 20 rats were injected intraperitonelly (i.p) with a single dose of TAA at a dose 150 mg/kg/bw, and treated with propolis at a dose of 300mg/kg/b.w orally for 60 days.

Group 4: Pollen grains treated group: 20 rats were injected intraperitonelly (i.p) with a single dose of TAA at a dose 150 mg/kg/bw), and treated with bee pollen at a dose of 25g/kg/b.w orally for 60 days.

Group 5: Propolis and pollen grains treated group: 20 rats were injected intraperitonelly with a single dose of TAA at dose 150 mg/kg/bw and treated with both propolis and pollen grain with the same dose orally for 60 days.

5- Sampling

A- Blood samples :

Blood samples were collected from the retro-orbital venous plexus by heparinized capillary tubes after overnight fasting from all animals (control and experimental groups). 1ml blood sample was collected on Ethylene diamine tetra acetic acid (EDTA) as anticoagulant for plasma separation for estimation of ammonia level in blood according to the method described by (Neely and Phillipson, 1988). Clear serum were separated by centrifugation at 3500 r.p.m for 15 minutes and then collected in Eppendrpfs tubes using automatic micropipettes. ALT, AST was measured according to the method of Murray, 1984 and Mohammed, 2012), ALP according to method of Rosalki et al., 1993), GGT according to method of Beleta and Gella, (1990), nitric oxide (NO), by Montgomery and Dymock (1961), serum were kept in deep freezer at (-20) for analysis of the following biochemical parameters: Albumin was measured according to the method of Doumas et al. (1997), total protein according to method of Kaplan and Szalbo (1983), urea according to method of Kaplan (1984), uric acid according to method of Fossati et al. (1980), creatinine according to method of Fabiny (1971).

B-Tissue samples

Rats of each group were sacrificed by decapitation; the liver and kidney were rapidly excised gently, rinsed with ice-cold isotonic saline, cleared off blood, photographed and immediately into ice-cold isotonic saline again, then blotted between 2 filter papers for subsequent biochemical analyses: Catalase activity was measured according to method of Sinha (1972), SOD acivity according to method of Nishikimi et al. (1972) and L-MDA concentration at liver, kidney and brain according to method of Mesbah et al. (2004). 6-Statistical analysis. The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan' s multiple test. All analyses were performed using the statistical package for social science (SPSS, 13.0 software). Values at 0.05 were considered to be significant.

3. RESULTS

The obtained data in Table (1) revealed that rats injected with TAA showed a significant increases in plasma ammonia, serum SGOT, SGPT, ALP, GGT, urea, creatinine, uric acid,, and nitric oxide (NO) and significantly decreased albumin, total protein, when compared with control. Treatment with propolis and bee pollen showed a significant decreases in plasma ammonia, serum SGOT, SGPT, ALP, GGT, urea, creatinine, uric acid, (GABA), cholinesterase and nitric oxide (NO), significantly increased in albumin, N-acetyl glutamate synthase and total protein in comparison with hyperammonemic group. The obtained data in Table (2) revealed that rats injected with TAA showed a significant increase in L-MDA concentration and significant decrease in SOD and CAT in liver, kidney and brain tissues when compared with control group. Treatment with propolis, bee pollen grain revealed a significant decrease in L-MDA concentration and significant increase SOD and CAT activities when compared with hyperammonemic rats.

Table (1) The effects of propolis and bee pollen grains treatment on plasma ammonia, serum SGOT, SGPT, ALP, GGT, urea, creatinine, uric acid, nitric oxide (NO), NAGS, GABA, cholinesterase, albumin and total protein on experimentally TAA- induced hyperammonemia in rats after 30 day.

4.

Animal groups parameter	Ammo-nia (µg/dl)	SGOT U/L	SGPT U/L	ALP U/L	GGT U/L	Urea Mg/dl	Uric acid Mg/dl	Creatinine Mg/dl	NAGS (pg/ml)	Cholinesterase (Mmol/l)	GABA (ng/ml	NO Mmol g/l	T.p. g/dl	Albumin g/dl
Control	55.85 ±1.02^{cA}	141.00 ±3.98b	46.25 ±0.97aA	108.25 ±4.09aA	24.90 ±1.34aA	38.63 ±0.88cB	4.16 ±0.23Ca	0.86 ±0.04aA	74.23 ±1.54bA	832.85 ±7.48^{cA}	5.00 ±0.17^{cA}	28.83 ±1.59^{aA}	6.95 ±0.16Ca	4.64 ±0.11bA
TAA	94.08 ±3.42^{dA}	177.88 ±3.2^{cA}	99.25 ±4.57^{dA}	152.00 ±3.74^{dA}	39.48 ±1.58^{cA}	62.93 ±3.36^{eA}	6.03 ±0.07^{eA}	3.04 ±0.36^{bA}	56.71 ±0.66^{aA}	952.14 ±31.42^{dA}	7.58 ±0.31^{dA}	79.04 ±2.74^{eA}	4.63 ±0.18^{Ab}	3.38 ±0.11^{aB}
Bee pollen+TAA	60.7 ±2.13^{abA}	160.88 ±4.46^{bB}	69.00 ±3.17^{cB}	133.25 ±2.78^{cB}	32.35 ±0.47^{bA}	53.50 ±1.24^{dB}	5.33 ±0.05^{dB}	0.92 ±0.06^{aB}	72.05 ±0.62^{bA}	742.69 ±10.25^{bB}	4.55 ±0.14^{cB}	68.03 ±1.48^{dB}	6.08 ±0.15^{Ba}	6.46 ±0.17^{cA}
propolis + TAA	57.78 ±1.05^{aA}	154.13 ±3.41^{aB}	67.88 ±1.28^{cB}	118.25 ±1.93^{bB}	27.25 ±1.56^{aB}	42.63 ±0.75^{bB}	5.30 ±0.15^{bB}	0.90 ±0.10^{aB}	74.70 ±0.66^{bcA}	693.07 ±6.26^{aB}	3.90 ±0.07^{bB}	59.18 ±1.26^{cB}	8.20 ±0.15^{dA}	7.41 ±0.11^{dA}
Bee pollen & propolis + TAA	62.72 ±0.64^{bB}	145.88 ±2.05^{aB}	56.75 ±0.78^{bB}	105.75 ±2.17^{aB}	27.25 ±0.85^{aB}	40.00 ±1.58^{aB}	5.83 ±0.05^{aB}	0.77 ±0.07^{aB}	76.68 ±0.65^{cA}	672.9 3±23.47^{aB}	2.50 ±0.11^{Ab}	49.2 ±0.58^{bB}	10.13 ±0.09^{eA}	8.43 ±0.11^{eA}

SE: Standard error of mean

a, b & c: There is no significant difference (*P< 0.05)* between any two means, within the same column have the same superscript letter.

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Table (2) The effects of propolis and bee pollen grains treatment on plasma ammonia, serum SGOT, SGPT, ALP, GGT, urea, creatinine, uric acid, nitric oxide (NO), NAGS, GABA, cholinesterase, albumin and total protein on experimentally TAA induced hyperammonemia experimentally in rats after 60 day.

Animal groups parameters	Ammonia (µg/d1)	SGOT U/L	SGPT U/L	ALP U/L	GGT U/L	Urea Mg/dl	Uric acid Mg/dl	Creatinine Mg/dl	NAGS pg/ml	Cholinesterase Mmol/l	GABA (ng/ml	NO Mmol g/l	T.p. g/dl	Albumin g/dl
Control	64.92 ±5.59^{cB}	137.88 ±2.77^{Ba}	45.5 ±0.65^{aA}	106.75 ±3.75^{bA}	25.63 ±0.75^{bA}	12.33 ±0.27^{aA}	4.03 ±0.19^{dA}	0.85 ±0.03^{bA}	74.23 ±2.13bA	855.45 ±15.36^{cA}	5.20 ±0.52^{d}	28.19 ±2.73^{aA}	7.20 ±0.29^{bA}	4.58 ±0.09^{bA}
TAA	94.4 ±2.91^{dA}	183.25 ±3.49^{cB}	101.88 ±4.1^{dA}	155.25 ±4.15^{cA}	46.5 ±1.84^{cB}	62.65 ±2.00^{dA}	6.40 ±0.15^{eB}	4.43 ±0.11^{cB}	55.96±1.1 1^{Aa}	1038.16 ±36.88^{dB}	9.00±0.0 9^{eB}	79.39 ±2.22^{eA}	3.18 ±0.13^{aA}	2.18 ±0.15^{aA}
Bee pollen + TAA	62.54 ±9.21^{bB}	133.63 ±5.14^{bA}	62.63 ±4.9^{cA}	111.75 ±1.55^{Ba}	29.68 ±4.53^{bA}	39.50 ±0.65^{cA}	3.43 ±0.23^{cA}	0.71 ±0.04^{abA}	74.10±0.5 4^{bB}	526.03 ±9.87^{bA}	3.50±0.1 5^{cA}	61.48 ±0.39^{dA}	7.68 ±0.13^{cB}	7.43 ±0.15^{cB}
Propolis + TAA	56.51 ±0.57^{aA}	114.00 ±1.22^{aA}	54.38 ±3.89^{bA}	106.75 ±1.11^{Ba}	16.93 ±1.12^{aA}	19.38 ±0.90^{bA}	2.38 ±0.18^{bA}	0.41 ±0.03^{aA}	76.70±0.6 6^{bB}	479.62 ±4.66^{aA}	3.00±0.1 7^{bA}	52.68 ±0.47^{cA}	9.83 ±0.13^{dB}	8.43 ±0.15^{dB}
Bee pollen & propolis TAA	60.23 ±3.86^{abA}	106.38 ±3.33^{aA}	46.38 ±1.52^{aA}	85.25 ±8.67^{Aa}	14.13 ±1.03^{aA}	16.00 ±1.58^{aA}	1.70 ±0.17^{aA}	0.47 ±0.02^{aA}	84.33±2.0 1^{cB}	541.47 ±17.49^{bA}	1.43±0.1 7^{aA}	43.83 ±0.81^{bA}	12.33 ±0.27^{eB}	9.60 ±0.15^{eB}

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Table (3) the effects of propolis and bee pollen grains treatment on L-MDA (nmol/gm. Tissue), SOD (U/g. tissue) and CAT (K/g. tissue) activities in liver, kidney and brain on experimentally TAA induced hyperammonemia in rats after 30 day.

Animal groups parameters	L-MDA Liver	L-MDA Kidney	L-MDA brain	CAT liver	CAT kidney	CAT brain	SOD liver	SOD kidney	SOD brain
Control	28.68 ±0.75^{aA}	35.37 ±1.44^{aA}	27.74 ±0.78^{aA}	56.74 ±0.40^{dA}	56.17 ±1.16^{eA}	53.87 ±0.48^{dA}	37.23 ±1.61^{dA}	34.65 ±2.14c	43.28 ±1.14^{dA}
TAA	64.08 ±1.81^{eA}	69.82 ±5.34^{dA}	60.97 ±3.09^{dA}	26.10 ±0.53^{aA}	34.81 ±0.73^{aA}	33.33 ±0.42^{aA}	19.50 ±0.31^{aB}	19.17 ±1.85^{aA}	16.98 ±2.01^{aA}
Bee pollen + TAA	56.17 ±0.75^{dB}	53.08 ±0.39^{cA}	49.2 ±0.55^{cA}	34.21 ±0.99^{bA}	38.49 ±0.39^{bA}	40.05 ±0.78^{bA}	23.10 ±1.25^{bA}	31.35 ±0.51^{bA}	23.60 ±1.70^{bA}
Propolis+ TAA	46.7 ±1.19^{cB}	51.78 ±1.37^{bcB}	46.93 ±0.68^{cB}	43.03 ±0.61^{cA}	42.26 ±0.92^{cA}	46.08 ±0.77^{cA}	31.58 ±0.71^{cA}	41.85 ±0.76^{dA}	31.85 ±0.76^{cA}
Bee pollen and propolis+ TAA	40.76 ±3.22^{bB}	48.03 ±0.74^{bB}	39.03 ±1.96^{bB}	53.60 ±0.93^{dA}	52.69 ±0.86^{dA}	56.65 ±0.66^{eA}	43.80 ±1.28^{eA}	52.20 ±0.35^{eA}	43.83 ±1.00^{dA}

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A, B & C: There is no significant difference *(P< 0.05)* between any two means, within the same row have the same superscript letter.

Table (4). The effects of propolis and bee pollen grains treatment on L-MDA (nmol/gm. Tissue), SOD (U/g. tissue) and CAT (K/g. tissue) activities in liver, kidney and brain on experimentally TAA induced hyperammonemia in rats after 60 day.

Animal groups parameters	L-MDA Liver	L-MDA Kidney	L-MDA brain	CAT liver	CAT kidney	CAT brain	SOD liver	SOD kidney	SOD brain
Control	27.37 ±1.30^{aA}	35.29 ±1.66^{aA}	28.77 ±0.79^{aA}	58.73 ±0.39cA	58.15 ±1.16eB	55.08 ±0.89dA	36.29 ±0.63cA	35.4 ±1.98bA	44.2 ±0.70dA
TAA	65.09 ±1.90^{eA}	67.45 ±0.99^{dA}	64.28 ±1.32^{dA}	25.83 ±1.00^{aA}	34.05 ±0.49^{aA}	32.83 ±1.92^{aA}	17.68 ±0.56^{aA}	18.67 ±1.26^{aA}	15.99 ±1.31^{aA}
Bee pollen+ TAA	50.74 ±0.56^{dA}	50.88 ±0.62^{cA}	54.85 ±6.67^{cB}	36.71 ±0.98^{bA}	40.48 ±0.39^{bB}	42.05 ±0.78^{bB}	26.35 ±1.52^{bB}	35.1 ±0.61^{bB}	25.60 ±1.70^{bB}
Propolis+ TAA	42.28 ±1.39^{cA}	46.38 ±1.09^{cA}	41.90 ±0.64^{bA}	55.53 ±9.68^{cB}	44.2 ±0.91^{cA}	48.08 ±0.77^{cB}	34.58 ±0.92^{cB}	44.85 ±0.64^{cB}	34.85 ±0.64^{cB}
Bee pollen & propolis + TAA	33.26 ±0.93^{bA}	40.8 ±0.83^{bA}	32.90 ±0.94^{aA}	55.85 ±0.84^{cA}	52.43 ±2.56^{dA}	58.68 ±0.64^{eB}	45.05 ±1.25^{dA}	54.98 ±0.19^{dB}	46.83 ±1.21^{dB}

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5. DISCUSSION

The obtained data demonstrated in Table (1) revealed that, administration of TAA to normal rats exhibited a significant increase in plasma ammonia level after induction of hyperammonemia when compared with control. These results were similar to that reported by studies of Bruck et al., (2002) who recorded a significant increase in plasma ammonia level in rats treated with TAA. Also, TÚnez et al. (2006) revealed a high degree of hyperammonemia 437.10±15.42 µmol/l in the TAA group versus 75.17±2.05µmol/l in the control group as evident of liver dysfunction. Ammonia is a key factor in the pathogenesis of hepatic encephalopathy, a major complication in acute and chronic liver failure and other hyperammonemic states, such as inborn errors of urea synthesis, during hepatic inadequacy, large quantities of ammonia in the portal blood escapes, the detoxification process and enters systemic circulation. Thus, blood and tissue (brain) ammonia levels are elevated rapidly (Reddy et al., 2004). After TAA injection, the blood ammonia level was increased significantly in comparison with the control groups (Fadillioglu et al., 2010). Administration of propolis or pollen grains and both of propolis and pollen grains to rats injected with TAA exhibited a significant decrease in plasma ammonia concentration in comparison with TAA group as shown in table (1) These results were in accordance with those reported by Radwan et al. (2008) stated that, after propolis and pollen grains treatment, plasma ammonia concentration have shown a marked tendency to normalization compared to TAA treated group, this may be explained that pollen grains and propolis contain natural antioxidants, phenolic compounds and flavonoids have the ability to remove excess ammonia and to offer protection against hyperammonemia (Essa et al., 2006). Fernandes et al. (2010) stated that after propolis and pollen grains treatment, plasma ammonia concentration has shown a marked tendency to normalization when compared to TAA group, maximum reduction in ammonia level with treatment propolis and pollen grains may be due to the significant anti- hyperammonemic activity this is probably indicative of the antioxidant efficacy of the used polyphenolic flavoniod of propolis and pollen grains. Phenolic of propolis are known to have a hepatoprotective function which correlated to the antioxidant activity (Banskota et al., 2001) Propolis counteracts hepatotoxic effects of alcohol liver injury in mice. The obtained data demonstrated in Table (1) revealed that, administration of TAA to normal rats exhibited a significant increase in serum ALT, AST, ALP and GGT level after induction of hyperammonemia when compared with control group. Similarly, Ansil et al. (2011) and Shaker et al. (2011) stated that, TAA administration to normal rats resulted in hyperammonemia which showed a significant increases in serum AST, ALT, ALP and GGT. Similarly, (Eraslan et al., 2007) stated that, TAA administration to normal rats resulted in hyperammonemia and showed a significant increase in serum AST, ALT, ALP and GGT. Interpreted the elevated levels of AST, ALT and ALP as a result of the hepatocytes damage or alterations in the membrane permeability indicating the severity of hepatocellular damage induced by TAA, which is in accordance with previous reports of Sehrawat et al. (2006). When the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into blood stream. Their estimation in the serum is a useful quantitative marker for the extend and type of hepatocellular damage and hyperammonemia (Kumar et al., 2004). Moreover increase in the activities of serum AST and ALT indicated occurrence of hepatic dysfunction. Therefore, the elevation in serum AST and ALT activities may be due mainly to the leakage of these enzymes from the liver cytosol into the blood stream, which reflects the hepatotoxicity and liver damage (Fernandes

et al., 2010). ALT is a cytosolic enzyme of the hepatocyte and an increase of its activity reflects an increase in plasma membrane permeability, which, in turn is associated with cell death, however, alkaline phosphatase is an ectoenzymes of the hepatocyte activity has been related to damage to the liver cell membrane (Kaplan, 1986). It was reported that, ALP activity increases in case of the damage of hepatic cells and obstruction of bile ducts arising from cellular reproduction (Essa et al., 2006). The obtained data demonstrated in Table (1) revealed that, administration of propolis or pollen grains and both of propolis &polen grains to rats injected with TAA exhibited a significant decrease in serum ALT, AST, ALP, and GGT activities in comparison with TAA induced hyperammonemia group. These results demonstrates that, daily oral administration of propolis and pollen grains for two months resulted in significant reduction of serum ALP, AST, ALT and GGT activities, when compared with control hyperammonemic rats. These results were came in accordance with those reported by Uzbekova et al. (2001) stated that after poroplis treatment ALT and AST activities have showed a marked tendency to normalization compared to CCl_4 treated group. Treatment of propolis significantly reduced the leakage of ALP and AST and ALT, in circulation ($P \leq 0.05$), thereby, confirming its protective effect in chronic injury. Administration of propolis and pollen grains to rats exhibited a significant decrease in serum ALT, AST, ALP, and GGT activities in comparison with TAA induced hyperammonemia group, these results accordance with (Monika, 2011) who recorded a significant decreased of serum ALT, AST, ALP and GGT activities in propolis treated rats, than Ochratoxin A (OA) group. Güldeniz et al. (2007) stated that bee pollen has positive effects on liver and kidney parameters and lead to significant reduction of serum ALP, AST, ALT and GGT activities when compared to control group. activities of ALT, AST and ALP decreased in rats treated with pollen grains compared with CCL4 group, the decrement of these hepatic enzymes may be attributed to the antioxidant properties of pollen grains, it is reported that phenolic compounds can act by scavenging free radicals against oxidative damage, important factor in the hepatoprotective activity of any drug is the ability of its constituents to inhibit the aramatose activity of cytochrome p-450, by their favoring liver regeneration (Gil et al., 2000).

Propolis is interestingly effective in ameliorating acute, subchronic, and chronic injury to liver. It also has wider therapeutic index, and thus it may serve as clinically useful hepatoprotective natural product in future (Bhadauria, 2011). The obtained data demonstrated in Table (1) revealed that, administration of TAA to normal rats exhibited a significant increase in serum urea and uric acid and creatinine concentration, after induction of hyperammonemia when compared with control group. BUN, uric acid and creatinine levels can be useful indicators of renal function. Renal damage can be accompanied by an increase in BUN, uric acid and creatinine indicating reduced urea, uric acid and creatinine clearance (Huang et al., 2011). In addition to the hepatic damage, also presented renal damages that were evidenced by the elevation in serum urea levels, which is considered as significant marker of renal dysfunction (Kumar et al., 2004). Fan et al. (2009) investigated a significant increase in serum urea, uric acid and creatinine concentration after TAA administration. It may be due to dysfunctional and dystrophic changes in the liver and kidney due to severe renal impairments, urea excretion falls and its concentration in serum rises rapidly. These results were similar to the reported studies of Galisteo et al. (2006) recorded that administration of TAA to normal rats produced a significant increase of serum urea, uric acid and creatinine concentration compared to the control normal group. The obtained data demonstrated in Table (1)

revealed that administration of propolis or bee pollen and both of propolis and pollen grains to rats injected with TAA exhibited a significant reduction in serum urea and uric acid and creatinine concentration, in comparison with TAA group. These results indicate hepato-protection induced by propolis this protective effect may be due to the antioxidant effect of propolis which was previously confirmed (Almaraz et al., 2007), Significant reduction of serum urea and creatinine levels was noticed after administration of propolis compare to TAA group, these results may indicate that propolis can attenuate renal damage by decreasing the concentrations of urea and creatinine. It was recently found that feeding mice with bee pbe ollen could protected from the toxic effects of TAA, which is thought to induce oxidative stress this is confirmed by Eraslan, et al., (2008) who reported that pollen grains significantly decreased serum urea, uric acid and creatinine when compared with TAA-treated rats. The obtained data demonstrated in Table (1) revealed that administration of TAA to normal rats exhibited a significant decrease in serum total protein and albumin concentration, after induction of Hyperammonemia when compared with control and treated groups. The reduction of the number and function of mitochondria in hepatocytes of rats with hyperammonia have been considered to cause uncoupling in oxidative phosphorylation leading to accumulation of NADH and lactate and diminished energy synthesis rate. This is also suggested to decrease hepatic protein synthesis; since most of the cell energy is used by the process (Reddy et al., 2004). Stanikova et al. (2010) investigated that TAA also decreased albumin synthesis. This is in agreement with the finding that short-term treatment with thioacetamide decreases protein synthesis. These results came in accordance with Galisteo et al. (2006) recorded that TAA administration to normal rats produced a significant reduction of serum total protein and albumin levels when compared with control normal group. Also, Kishioka et al. (2007) and Sarkar and Sil (2007) found that the level of plasma T.pt and albumin in TAA treated group was significantly lower than that of the control group. (Stankova et al., 2010) reported that these obligate intermediate of TAA binds to proteins with the formation of acetyl-imidolysine derivatives that are partly responsible for TAA-induced hepatotoxic effects and reduction in total protein level. Induction of these effects requires a lower concentration of TAA than the concentration of TAA needed for ROS production, inhibition of mitochondrial respiration. Decreased protein contain of blood serum in hyperammonemia were reported by (Mahbood et al., 2005) indicating elevated lipid peroxidation process and decreased antioxidant defensive system. The obtained data demonstrated in Table (1) revealed that, administration of propolis or, pollen grains and both of propolis and pollen grains to rats injected with TAA exhibited a significant elevation in serum T.pt. and albumin concentration, in comparison with TAA induced hypperammonemia. Zakaria et al. (2009) reported that oral administration of propolis to hyperammonemic rats lead to a significant increase in total protein and Albumin when compare with TAA treated rats. Demasi and Davies (2003) stated that bee pollen has positive effects on liver and kidney parameters and lead to significant increase in total protein and Albumin when compare with TAA treated rats. The effect of propolis is in agreement with other study (Nirala et al., 2008) who stated that propolis significantly improved the total proteins content of the liver and kidney and showed more profound therapeutic effects. Cellular recovery was also evident through the improvement in total proteins and albumin after treatment with propolis. Güldeniz et al. (2007) stated that bee pollen has positive effects on liver and kidney parameters and lead to a significant increase in total protein and Albumin when compare with TAA

treated rats. The obtained data demonstrated in Table (1) revealed that, administration of TAA to normal rats exhibited significant increase in NO level after induction of hyperammonemia when compared with control group. Bruck et al. (2004) who evaluated the effect of TAA on hepatic and NO level and revealed a significant increase in its concentration in TAA treated rats other than control normal group. Moreover, Huang et al. (2007) recorded a significant increase in NO level in TAA treated rats when compared to control rats. NO is a signaling molecule that plays a key role in the pathogenesis of inflammation and it is overproduced in abnormal physiological conditions. Physiological amounts of NO acts on different energy linked and metabolic mitochondrial pathways while relatively higher concentrations of NO deplete cellular GSH by conjugating with NO to form an S-nitroso-glutathione adduct. Gong et al., (2010) reported that NO in TAA- treated wild-type mice was increased compared to control normal mice. Rehman et al.,(2003) have shown that liver failure accompanied with excess ammonia induces nitric oxide synthesis, which leads to enhanced production of nitric oxide, leading to oxidative stress and liver damage. The obtained data demonstrated in Table (1) revealed that, administration of propolis, bee pollen and both of propolis and bee pollen to rats injected with TAA exhibited a significant decrease in brain NO level in comparison with TAA group, Similar results were recorded by Marzouk et al.,(2007) investigated the anti-inflammatory effect of propolis and that propolis has an important role in the inhibition of nitric oxide production anti-inflammatory effects of flavonoids including propolis and bee pollen have been reported in several studies. The obtained data demonstrated in Table (1) revealed that administration of TAA to normal rats exhibited a significant increase in serum cholinesterase after induction of hyperammonemia when compared with control group. Cholinesterase is a family of enzymes that catalyze the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation (Fuortes et al., 1993). (Lionetto et al., 2013) who evaluated the effect of TAA on brain and revealed a significant increase in cholinesterase concentration in TAA treated rats other than control group. (Agarwal et al., 2005) who reported that inhibition of AChE may be a better biomarker for the assessment of neurotoxic effects in the living, toxicants generally elicit their effects by inhibition of acetyl cholinesterase, which lead to accumulation of the neurotransmitter acetylcholine in synapses and in the neuromuscular junction. The obtained data demonstrated in Table (1) revealed that, administration of propolis, bee pollen and both propolis and bee pollen to rats injected with TAA exhibited a significant decrease in cholinesterase, in comparison with TAA group. These results were came in accordance with the recorded data by (monika, 2012) Showed that administration of flavonoids (pollen and propolis) significant decrease cholinesterase activity compared to diseased rats. (El-Masry et al. (2011)Propolis has beneficial effects and could be able to antagonize Pb-induced neurotoxicity, the biological effects exhibited by propolis could be related to an overall effect of the phenolic compounds present in propolis, 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 obtained data demonstrated in Table (1) revealed that administration of TAA to normal rats exhibited significant increase in GABA level after induction of hyperammonemia when compared with control group. These result were similar to the reported studies of (Helewski K et al., 2003) who recorded that administration of TAA to normal rats

produced a significant increase of serum GABA concentration compared to the control group. Excess ammonia may indirectly increase GABA-ergic neuro-transmission and also inhibit the function of CNS, loss of GABA receptors was observed with TAA which probably raise GABA release (Chatauret and Butterworth, 2004).

The obtained data demonstrated in Table (1) revealed that administration of propolis, bee pollen and both of propolis and bee pollen to rats injected with TAA exhibited a significant decrease in GABA, in comparison with TAA group. These results were came in accordance with the recorded data by Gökhan et al. (2009) who recorded a significant decrease in serum GABA concentration in rats treated with flavonoids and CAPE of (propolis and pollen grains) compared to untreated rats. Marzouk et al. (2007) reported that propolis and bee pollen, naturally occurring antioxidant, as a powerful ROS scavenger in rats, they had been shown to have broad biological activities which are principally attributed to the presence of flavonoids (major component: Rutin, quercetin and galangin) and caffeic acid phenethyl ester (CAPE).

The obtained data demonstrated in Table (1) revealed that, administration of TAA to normal rats exhibited significant decrease in NAGS level after induction of hyperammonemia when compared with control and treated groups. These result in accordance with studies of (Lionetto et al., 2013) who evaluated the effect of TAA on brain and revealed a significant decrease in NAGS activity in TAA treated rats other than control group. N-Acetyl glutamate which in turn is synthesized from acetyl-CoA and glutamic acid in the reaction catalyzed by N-Acetyl glutamate synthase, commonly called NAGS. N-Acetyl Glutamate is required for the Urea cycle to take place (Helewski K et al., 2003). Gökhan et al. (2009) found that NAGS level, was significantly decreased in all TAA treated rats than control rats. Administration of propolis, bee pollen and both of propolis and bee pollen to rats injected with TAA exhibited a significant increase in NAGS, in comparison with TAA group as shown in table (1). These results were in agreement with (El-Masry et al., (2011) who evaluated the effect both propolis and bee pollen contain bioflovniod as antioxidant on brain and revealed a significant increase in NAGS activity as compared to toxicity group. The effect of propolis on brain cells protect the brain from damage and atrophy of nerve cells because propolis, prevents the brain oxidative stress, increases antioxidative defense of the brain tissue, neutralizes free radicals in the brain repairs the free-radicals induced DNA damage, strengthens the gene that aids transmission of nerve impulses stimulates the DNA replication in the brain (kishioka et al., 2007). Propolis in synergy with the bee pollen increases the blood supply of the brain and facilitates the more rapid recovery of disrupted and lost functions (khayyal et al., 1993). The obtained data demonstrated in Table (2) revealed that, administration of TAA to normal rats exhibited a significant reduction in liver and brain SOD and CAT activities, after induction of hyperammonemia when compared with control group. These results were in accordance with those recorded by Tunez et al. (2006) reported that TAA administration to normal rats led to a marked reduction in CAT and SOD activities in brain, kidney and hepatic homogenates, when compared with normal control rats, studies in animal models of liver failure indicate a higher free radical activity in the liver as shown by the increase in mitochondrial superoxide radical and H_2O_2 and the induction of the microsomal cytochrome P-450. Generation of a large amount of ROS due to TAA can overwhelm the antioxidant defense mechanism and damage cellular ingredients such as lipids, proteins and DNA; this in turn can impair cellular structure and function (Ansil et al., 2011). The intra cellular antioxidant system comprises of different free radical scavenging antioxidant enzymes along with some non enzyme antioxidants like GSH

and other thiols. CAT and SOD constitute the first line of cellular antioxidant defense enzymes. Thus to eliminate free radicals, these cellular antioxidants play an important role and equilibrium exists between these enzymes under normal conditions. When excess free radicals are produced, this equilibrium is lost and consequently oxidative insult is established ((El-Masry et al., 2011). SOD is the only enzymes that disrupts superoxide radicals and are it presents in all cells with high amounts in erythrocytes, it protects the cells against superoxide- and hydrogen peroxide-mediated LPO, decreased SOD activity was observed in TAA group (Monika, 2011). The obtained data demonstrated in Tables (2) revealed that, administration of propolis and bee pollen to rats exhibited a significant increased in liver and brain SOD and CAT activities in comparison with TAA treated rats. Mahmoud (2011) recorded that oral treatment with propolis in hyperammonemic rats significantly increased the levels of the antioxidant parameter SOD and CAT in liver, brain and kidney when compared with ammonium chloride treated rats. Propolis co-administration with cypermethrin induced a significant increase in the mean values of antioxidant enzyme activities (CAT, SOD) as compared with cypermethrin treated group ($P<0.05$). Kanbura et al. (2009) reported a significant increase in the antioxidant enzymes parameters (SOD, CAT in tissue liver, kidney and brain) of animals that were administered bee pollen in association with propetamphos, in comparison to the group that was administered propetamphos alone. SOD and CAT activities were significantly decreased ($P<0.05$) in (4-tertiary-octylphenol) 4-tert-OP group compared to control group. Improvement in biochemical parameters (SOD and CAT), was observed in values pertaining to the group that was administered bee pollen in association with propoxur as compared propoxur group. This effect is considered to be related to the radical scavenging effect of bee pollen. A study in which the detoxifying effect of bee pollen on pesticides and other compounds is investigated by Eraslan et al. (2007).

Gökhan et al. (2008) reported that carbaryl was determined to cause negative changes in most of the oxidative stress markers SOD and CAT investigated, These effects were observed to alleviate with the administration of bee pollen. The obtained data demonstrated in Table (2) revealed that, administration of TAA to normal rats exhibited a significant increase in liver, kidney and brain L-MDA concentration, after induction of hyperammonemia when compared with control group. Tunez et al. (2006) reported that TAA administration to normal rats led to a significant increase in MDA level, in brain and kidney and hepatic homogenates, when compared with normal control rats. Also, Sarkar and Sil (2007) recorded that TAA administration increased liver MDA level which indicates the extent of TAA-induced lipid peroxidation to 160% with respect to the normal cells. Furthermore, Mehmetcik et al. (2008) and Ansil et al. (2011) observed that TAA treatment caused a significant increase in hepatic MDA level, when compared with normal control group. Stankova et al. (2010) reported that cultured rat hepatocytes treated with various concentrations of TAA, showed a significant increase in MDA content, when compared with, control normal group. The obtained data demonstrated in Tables (2) revealed that administration of propolis and pollen grains to rats exhibited a significant decrease in liver, kidney and brain L-MDA concentration in comparison with TAA treated group, 24 hrs after induction of hyperammonemia. MDA is an end product of lipid peroxidation and it is considered a late biomarker of oxidative stress and cellular damage (Abdel-Wahhab et al., 2005). The antioxidant activities of propolis are related to its ability to scavenge singlet oxygen, superoxide anions, proxy radicals, hydroxyl radicals and peroxy nitrite. The primary mechanism of the effect of propolis may involve the scavenging of free radicals

that cause lipid peroxidation. The other mechanism may comprise the inhibition of xanthine oxidase, which is known to cause free radicals to be generated (Kanbura et al., 2009). Marzouk et al. (2007) reported a significant decrease in the plasma and tissue (liver, kidney and brain) MDA level after administration propolis. Bee pollen, being an anti-lipoperoxidant agent, inhibits formation of lipid peroxides (Eraslan et al., 2008), it acts by lowering the lipid peroxidation. Scavenging free radicals and its activity is attributed to its structure, the study on the bioflavonoid of bee pollen showed it decreased MDA levels and increased antioxidant enzyme levels in cardiac ischemia reperfusion injury. The effect of bee pollen on liver functions in old rats was studied by Uzbekova et al. (2003), after one month they had a diminution of malondyaldehyde levels and the sulphydryl groups (SH-G) content was normalized at the end of the experiments).

Conclusion and recommendation: So we recommend that administration of diet rich in the natural propolis and bee pollen is very important for protection of different body organs, especially liver, kidney and brain, against oxidative stress or even inflammation or infections. Also, we strongly support the use of propolis and bee pollen as pure active ingredients in pharmacological industry for production of new drugs used as therapeutics for treatment of different liver, kidney and brain affections.

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ملاحظة التأثير الكيميائي الحيوي لمادتي البروبوليس وحبوب اللقاح على ارتفاع أمونيا الدم المحدث تجربيا فى الفئران

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[1] قسم الكيمياء الحيوية-كليه طب بيطري-جامعه بنها. [2] قسم الطب الشرعي والسموم كليه طب بيطري-جامعه بنها. [3] قسم الكيمياء الحيوية -كليه علوم جامعه المنوفية

الملخص العربي

الهدف الاساسى من هذه الدراسة هو توضيح التاثير الكيمائى الحيوى لمادتى البروبوليس وحبوب اللقاح كمضادات للأكسدة طبيعيه فى الفئران المحدث بيها مرض ارتفاع امونيا الدم تجريبيا عن طريق حقن ماده الثيواستاميد جرعه واحده ومقدراها 150ملليجم /كيلو جرام /من وزن الجسم فى الغشاء البروتونى.اجريت هذه الدراسه على عدد (100) من ذكور الفئران البيضاء وقسمت الى 5 مجموعات كل مجموعه اشتملت على (20) فأرا. المجموعه الاولى (المجموعه الضابطه ولم تعطى اى ادوية. المجموعة الثانية: تم حقنهم بجرعه واحده من ماده الثيواستاميد بمقدار 150 ملللى جرام /كيلو جرام فى الغشاء البروتونى وتستخدم كمجموعه ضابطه لارتفاع الامونيا. المجموعة الثالثة: تم حقنهم بجرعه واحده من ماده الثيواستاميد بمقدار 150 ملللى جرام /كيلو جرام فى الغشاء البروتونى ثم تجريعهم بماده البروبوليس يوميا عن طريق الفم بجرعة مقدارها 300 ملللى جرام /كيلو جرام من وزن الجسم. لمجموعه الرابعة: تم حقنهم بجرعه واحده من ماده الثيواستاميد بمقدار 150 ملللى جرام /كيلو جرام فى الغشاء البروتونى ثم تجريعهم بماده حبوب اللقاح يوميا عن طريق الفم بجرعة مقدارها 25جرام /كيلو جرام من وزن الجسم. لمجموعه الخامسة: تم حقنهم بجرعه واحده من ماده الثيواستاميد بمقدار 150 ملللى جرام /كيلو جرام فى الغشاء البروتونى ثم تجريعهم بكلا المادتين الروبوليس وحبوب اللقاح بنفس الجرعة. السيرم تم فصله مرتين، بعد 30يوم والاخر بعد 60 يوم من العلاج.كل عينات الدم استخدمت لتعين الامونيا، الانين أمينوترانسفيريز، أسبرتات أمينوترانسفيريز، الفوسفاتيز القاعدي، جاما جلوتاميل ترانسفيريز، ألبومين، البروتين الكلى، اليوريا، الكولينستريز، جاما امينوباتيريك اسيد، إن-استيل جلوتاميل سينسيز، حمض البوليك، الكرياتنين، أكسيد النيتريك يقاس الدم فى المجموعه الضابطة والمجموعات المعالجة ضد مجموعه ارتفاع امونيا الدم المحدث تجربيا بماده الثيواستاميد. ويتم تجميع الانسجه (الكبد والمخ والكى) لقياس الاكسده الفوقيه للدهون، نشاط إنزيم الكاتاليز ونشاط إنزيم سوبر أكسيد ديسميوتيز.النتائج اوضحت قله علميه فى السيرم ل الانين أمينوترانسفيريز، أسبرتات أمينوترانسفيريز، الفوسفاتيز القاعدى، جاما جلوتاميل ترانسفيريز، اليوريا، حمض البوليك، الكرياتينين، الكولينستريز، جاما امينوباتيريك اسيد، أكسيد النيتريك.والبلازما امونيا والاكسده الفوقيه للدهونفى الانسجه بلاضافه الى زياده واضحه فى البومين، البروتين الكلى، إن- استيل جلوتاميل سينسيز، نشاط أنزيم الكاتاليز وسوبر اكسيد ديسميوتيز فى الكبد، المخ والكلى.وبالتالى السلوك الحيويى للنتائج بينت تأثير كلا من البروبوليس وحبوب اللقاح ضد مرض ارتفاع امونيا الدم المحدث بماده الثيواستاميد فى الفئران.

(مجلة بنها للعلوم الطبية البيطرية: عدد 27(1):8- 24, سبتمبر 2014)