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Chemical and Biological Studies on Pumpkin seeds Against Benign Prostatic Hyperplasia Induced Testosterone in Rats

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Abstract: In recent years, pumpkin seeds have sparked much interest as a healthy natural sweetener. This study aims to assess the protective effect of pumpkin seeds with two doses (10% and 20%/kg/diet) in testosterone induced benign prostatic hyperplasia in male rats. Thirty rats, divided into five groups (n=6); one served as normal control group (-ve), (+ve), Harnal (3 g/kg/ diet), pumpkin seeds 10 & 20% groups, in order to induce benign prostatic hyperplasia rats were injected by testosterone (5 mg/kg b.w) daily. The results showed the presence of phenolic acids such as salycilic ellagic, pyrogallol, benzoic, hespertin, chlorogenic acid, caffeine acid, vanillin, coumarin and e-vanillic in pumpkin seeds. The results indicated that LH, FSH and total protein, MDA levels were increased in positive control. In addition, the level of prostate volume, prostate weight, prostate weight index and testosterone levels, decrease were reversed with pumpkin seeds groups at levels (20 & 10). An increase in of SOD and TCA activities in treatment with pumpkin seeds were observed in benign prostatic hyperplasia rats. The findings of this study suggest that treatment with pumpkin seeds ameliorates metabolic derangement by enhancing the antioxidant defense against testosterone -induced benign prostatic hyperplasia in rats, the antioxidant activities may be related to their phenolic compounds.

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1. Introduction

Pumpkin *(Cucurbita SP.)* is a pepo type fruit-berries with a hard, thick rind -in the Cucurbitaceae family that includes cucumbers and squash *(***Gossell-Williams** ***et al.,*** **2006***). Pumpkins are traditionally defined as round in shape and orange in color, and like other winter squash, have long vines and a flowering stage.* Pumpkin is sometimes known as the seed or the pulp of the zucchini or the pulp proteins and minerals, such as iron and calcium. It also contains plant seeds on manganese, protein and magnesium, which is stored with you anywhere. It does not need to be cooled for preservation **(Adams** ***et al.,*** **2017).**

Pumpkin plant is a plant species of cucumber, including cucumber, cantaloupe and zucchini. Egypt and the United States are among the most cultivated countries. This plant has a great nutritional value. It contains vitamins A and B, tyrosine, pyurzine, and leucine. Its calories are about 26. Carbohydrates are found in 6.5% and the proteins are found in 1A and rid of that type of plants of cholesterol permanently, which makes it has many therapeutic and health benefits. Pumpkin seed oil contains phenolic compounds, tocopherol, b-carotene, unsaturated fatty acids and sterols **(Stevenson** ***et al.,*** **2007)**. Pumpkin seeds are a great source of magnesium, and magnesium helps to maintain energy, it also helps regulate blood pressure, supports the function of muscles and nerves, and helps maintain the stability of blood sugar levels. Pumpkin seed oil significantly reduced symptoms of bladder hyperactivity, such as incontinence. Reports claim consumption of pumpkin seed oil can help in the prevention and therapy of cardiovascular disease, prostate cancer (benign prostatic hyperplasia-BPH), dysuria, urinary tract infections, and digestive problems **(Makni** ***et al.,*** **2012 and Abou Seif, 2018).**

The prostate gland is a common medical condition as men age **(De Nunzio** ***et al.,*** **2016).** Inflammation of the prostate gland can lead to uncomfortable urinary symptoms, such as obstruction of urine flow outside the bladder. It can also cause bladder, urinary or kidney problems. Studies have suggested that diabetes, heart disease, obesity and the use of beta receptors may increase the risk of benign prostatic hyperplasia **(Jeon** ***et al.,*** **2017)**. In that study we isolated naturally derived phenolic and flavonoid compounds with pumpkin seeds and studied that effect on antioxidant activity. So it can be useful in managing benign prostatic hyperplasia on experimental rats.

2. Materials and Methods:-

Materials:-

Pumpkin *(Cucurbita*) seeds were purchased from local market of different areas in Kuwait. Twenty male albino rats of Sparague Dawley strain, (weight 170 ±5 g) were obtained from the National Research Centre, Cairo, Egypt. Harnal ©: Tamsulosin 0.4 mg Modified Release Capsules. Saja -Saudi Arabian Japanese Pharmaceutical CO.

Methods:-

Chemical analysis:

Pumpkin seeds were analyzed for the moisture, fat, protein, ash, and fiber contents were determined as described in (**AOAC, 2000**). The carbohydrates as nitrogen free extract (NFE) calculated. The extract was subjected to high performance thin layer chromatography (HPTLC) examinations (**Meena *et al.*, 2010).**

Diet composition and animal groups:

Stander diet (Negative): prepared according to **(Reeves *et al.*, 1993).** The vitamin and mineral mixture prepared according to **(Campbell, 1963).** 30 mature rats were divided into 5 groups of 5 rats each and fed on diets for thirty five days as follows:

Group (1): Negative control group (-ve) fed on basal diet.

Group (2): Positive control group (+ve) fed on basal diet and subcutaneous injection by testosterone (5 mg/kg) daily for 28 days were induced prostatic hyperplasia in rats according to **(Veeresh Babu *et al.*, 2010)**

Group (3): the same composition as positive diet, in addition harnal at doses over 3 g/kg/ diet according to **(Muramatsu *et al.*, 1998)**.

Group 4 & 5: the same composition as positive diet, treated with 10% & 20% pumpkin seeds. During the experiment period, the quantities of diet, which were consumed and / or wasted, were recorded every day. In addition, rat's weight was recorded weekly, to determine food intake and body weight gain percent according to **(Chapman *et al.*, 1950).**

Biochemical analysis of serum:

Serum testosterone concentration was determined using Radioimmunoassay (RIA) method which is intended for the quantitative determination of total testosterone in the serum. The method is based on the competitive binding principal according to (**Wilke and Utley, 1987**). Levels of FSH and LH hormones in the serum were determined by an enzyme-linked immunosorbent assay (ELISA) using specific commercial kits as described by **(Ballester, et al., 2004).** The supernatant was collected and used for determination of the activity of tissue antioxidant enzymes. Activities of antioxidant enzymes superoxide dismutase (SOD), total antioxidants capacity (TAC), and malondialdehyde (MDA) were determined according to (**Nishikimi, et al., 1972, Cao et al., 1993 and Oshawa, et al., 1979),** respectively. Prostate weight to body weight ratio were calculated by dividing prostate weight with that of animal body weight for the individual study group animal. Prostate glands were dissected and homogenates were made in phosphate buffer solution (0.01 M sodium phosphate buffer, pH 7.4, containing 0.14 M NaCl) at ml volume/g gland wet weight ratio of 4:1. Homogenates were centrifuged at 13,000×*g* for 20 min and supernatant collected **(Shin *et al.*, 2012).** Supernatant was used as source of proteins and concentration was determined by modified biuret end point assay method.

Statistical analysis:

Results were expressed as the mean ± SD. Data were statistically analyzed for variance using one-way analysis of variance (ANOVA) according to SAS, according to **Snedecor and Cochran (1967)**

3. Results and Discussion

Proximate composition of pumpkin seeds (dry basis %)

As seen in Table (1) moisture, ether extract, protein, ash, crude fiber and nitrogen free extract were recorded that (9.80, 29.86, 24.67, 3.55, 4.92 and 27.11%) in pumpkin seeds. **Hashemi (2013)** recorded that wheat flour were characterized by a significantly higher level of dietary carbohydrates, fat and protein, in comparison to pumpkin seeds which in line with our results. **Seyedmajidi *et al.*, (2014) and Hanaa and Rehab (2017)** decided that pumpkin seed contains 32.95, 30. 93, 11.78 and 3.92 % of protein, fat, ash and crude fiber contents respectively on dry weight basis.

Table (1): Proximate composition (dry basis %) of pumpkin seeds

Types Parameter	Pumpkin seeds
Moisture %	9.80
Ether extract%	29.86
Protein %	24.76
Ash %	3.55
Crude Fiber %	4.92
Nitrogen free extract%	27.11

Values are the means of 3 independent determinations.

The concentration of levels of phenolic and flavonoid compounds of pumpkin seeds

Data tabulated in Table (2) show that pumpkin seeds were more in higher phenolic and flavonoid such as salycilic ellagic, pyrogallol, benzoic and hespertin. While, that pumpkin seeds were lower in some phenolic and flavonoid such as chlorogenic acid, caffeine acid, vanillin, coumarin and e-vanillic. **Seyedmajidi *et al.*, (2014)** studied that recorded of tannins, resins, saponins, carbohydrates terpenes and flavonoids in pumpkin seeds. Pumpkin seeds were a rich source of phenolic phytochemicals be forced antioxidant activity. The tannins are the main contributors to the α-glucosidase inhibitory activities of pumpkin seeds may be important for activity.

Table (2): The concentration of levels of phenolic and flavonoid compounds of pumpkin seeds

Phenolic and flavonoid compounds	**Total Phenols (ppm)**
Gallic acid	4.02
Pyrogallol	51.11
Chlorogenic acid	2.99
Fedic acid	26.77
Protocatechuic	13.78
Quercetin	15.74
Caffeine	8.87
Hesperidin	34.32
Caffeic acid	2.86
Vanillin	3.50
Hesperetin	46.74
Coumarin	23.29
Salycilic	130.97
Cinnamic	6.80
Ellagic	83.51
e-vanillic	11.19
Ferulic	5.66
Kaempferol	18.70
Benzoic	61.67

Effect of different levels of pumpkin seeds on feed intake, body weight and FRE of the experimental rats groups

As shown in Table (3), it could be observed that group (5) the highest level feed intake compared to other groups. On the body weight (93.44 g) of the same group recorded while, control group, positive group, drug group and group (4) were (101.87, 51.62, 98.44 & 85.41 g) respectively. FER in group (5) was more than group (3) and group (4). These data may be due to the biochemical properties of pumpkin seeds which acts as antioxidant agent and its pharmacodynamics. These results are in harmony with those obtained by **Sunar *et al.* (2009) and Sutyarso *et al.* (2016).**

Effect of different levels of pumpkin seeds on prostate weight, prostate volume, prostate weight index, weight index of the experimental rats groups

The statistical data in Table (6) presented that weight of prostate, prostate volume, prostate weight index and wet weight index of the experimental rats groups recorded significant different at ($p \leq 0.05$). Also as can be seen, in the groups administrated with pumpkin seeds different levels 20% were the best treatment as the weight of prostate were reduced by 38.1% compared with (+ve) untreated group. These results are in pumpkin seeds with those obtained by (**Vnder *et al.*, 2004 and Zak *et al.*, 2009**) who noticed that testosterone may cause reproductive dysfunction therefore, reproductive dysfunction observed in patients. As such, exploration of testosterone in rat's model linked with an increase in prostate size. Furthermore, administration of pumpkin seeds with different doses causes a reduction in prostate volume, prostate weight index and wet weight index of prostate in rats comparing with BPH (+ve) untreated group.

Effect of different levels of pumpkin seeds on serum level of sex hormones of the in rats

Serum levels of sex hormones in experimental rats were shown in Table (8). The testosterone, LH and FSH in rats fed on croissant supplemented maple syrup group (5) with 20% of were found to be (31.74 ± 1.5, 5. 80 ± 1.40 & 163.00 ± 7.20 mg/ml) respectively. These results showed higher than positive group which were 16.22, 1.8 and 95.16 mg/ml respectively. The previous result increased in significantly compared to positive control group and other experimental groups except control group. These results were in agreement with those obtained by (**Mohamed *et al.*, 2016**) who found that the effects of testosterone induction of prostate in rat model causes an increase in testosterone level. The reduction in FSH level that observed after testosterone induction is agreement with reports of **(Crawford *et al.*, 2014)** who reported that dysfunction of (FSH) the follicle-stimulating hormone play an important role in progressive of abnormal growth of prostate in benign prostatic hyperplasia disease. In addition **Zeng *et al.*, (2012)** mentioned that there is a positive correlation between FSH and LH sex hormone levels and aging as well as with international Prostate Symptom Score. On the other hand, group 5 showed higher than group 4 and group 3 (drug) compared to positive control. These results may be due effect of increase in pumpkin seeds and effect of drug in group 3. There has been much research into the health benefits conferred by the phenol compound and total antioxidant found in ***pumpkin seeds***. Scientific studies in vitro have established that compounds in ***pumpkin seeds*** have potent antioxidant, antiviral and antifertility properties and potential for use in treating cancer **(Al-Masri *et al.*, 2015).**

Joanne and Sonia (2016) suggest that glucocorticoids inhibit the release of LH from the gonad tropes. In contrast, high levels of circulating glucocorticoids do not affect or only slightly affect serum levels of FSH. This indicates that glucocorticoids either increase synthesis or block the degradation of FSH in the gonad tropes. The treated with pumpkin seeds; pituitary content of FSH was increased in male rats completely reversed the effects of a gonadotropin-releasing hormone (GnRH) antagonist on pituitary FSH.

Activity of antioxidant enzymes of the experimental rats:

The effect of different levels of maple syrup on superoxide dismutase (SOD), Total antioxidants capacity (TAC) and malondialdehyde (MDA) showed in Table (6). It was found that the rats fed on level at 20% of pumpkin seeds group (5) increased in significantly in the levels of SOD and TAC (23.91±3.61µ/mg and 3.99 ± 0.47 mmol/L) compared to (+ve) group. While the level of MDA recorded the highest value (15.41±2.77µmol/g) for (+ve) group compared to the other rats groups. Pumpkin seeds level of 20% were more in some phenolic and flavonoid these may be caused increase in SOD and TAC. These results were agreement with (**Ruann and Hélia 2015**). These data may be due to the effect is exacerbated by the fact that sodium valproate increases in oxidative stress in the rats. Therefore, a person taking testosterone may suffer a deficiency unless there is a compensatory increase in the intake of these nutrients. The effects of pumpkin seeds are not limited to its anti-inflammatory benefits properties because evidence indicates that it also can lower oxidative damage in rats.

Table (3): Effect of different levels of pumpkin seeds on body weight, feed intake and FRE of the experimental rats groups

Groups Parameters	Control group	Positive Group	Treated groups		
			Group 3 Drug	**Group 4 (10%)**	**Group 5 (20%)**
Feed intake (g/d)	17.33± 1.21 a	13.55± 1.18c	16.80± 1.61b	16.65± 1.27b	17.15± 1.41a
Body weight (g)	101.87± 8.62 a	51.62± 5.11 d	98.44± 6.61 b	85.41± 7. 31 c	93.44± 8.14 b
FER	0.097± 0.008 a	0.063± 0.006 d	0.085± 0.004 b	0.085± 0.002c	0.091± 0.003 b

Values with the same letters indicate nonsignificant difference and vice versa. Significant at P < 0.05

Table (4): Effect of different levels of pumpkin seeds on prostate weight, prostate volume, prostate weight index, wet weight index of prostate and increase in prostate weight of the experimental rats groups

Groups Parameters	Control Group	Positive Group	Treated groups		
			Group 3 Drug	**Group 4 (10%)**	**Group 5 (20%)**
prostate weight (g)	0.62± 0.03 c	1.05± 0.07 a	0.82± 0.11b	0.78± 0.05 b	0.65± 0.05 b
Prostate volume (cm^2)	0.42± 0.11 c	0.80± 0.12 a	0.55± 0.04b	0.50± 0.12 b	0.55± 0.04 b
prostate weight index (g/g*1000)	2. 64± 0.17 a	4.64± 0.13 d	4.18± 0.21c	3.76± 0.32 b	2.94± 03.12 b
Wet weight index of prostate (mg/100gB.W)	59.24± 6.87 d	173.19± 14.83 a	75.65± 8.005 c	84.044± 9.34 b	72.33± 8.21 b
Increase in prostate weight (%)	___	100	75. 65± 8.005 c	84.044± 9.34 b	82.33± 8.21 b

Values with the same letters indicate nonsignificant difference and vice versa. Significant at P < 0.05

Table (5): Effect of different levels of pumpkin seeds on serum level of sex hormones of the experimental rats groups

Groups Sex hormones	Control Group	Positive Group	Treated groups		
			Group 3 Drug	Group 4 (10%)	Group 5 (20%)
Testosterone (mg/ml)	32.50± 1.5 a	16.22± 1.3 c	27.14± 1.3 b	28.34± 1. 4 b	31.74± 1.5 a
luteinizing (mg/ml)	5.9± 0.4 a	1.8± 0.2 c	4.80± 0.4b	4.96± 0.4b	5. 8± 0.4a
FSH (mg/ml)	165.11± 7.3 a	95.16± 7.2 c	156.09± 6.4 b	159.76± 6.5b	163.00± 7.2 a

Values with the same letters indicate nonsignificant difference and vice versa. Significant at $P < 0.05$ FSH: Follicle stimulating hormone.

Table (6): Effect of different levels of pumpkin seeds on serum activities of antioxidant enzymes of the experimental rats groups

Groups Parameters	Control Group	Positive Group	Na-Valproate		
			Group 3 Drug	Group 4 (10%)	Group 5 (20%)
SOD (µ/mg)	25.26± 7.01a	13.41± 1.21c	22.87± 2.11b	22.96± 3.21b	23.91± 3.61b
TAC mmol/L)(	4.56± 0.33 a	1.03± 0.12 c	3.62± 0.75b	3.84± 0.44b	3.99± 0.47b
MDA (µmol/g)	7.55± 1.44 c	15.41± 2.77a	8.64± 1.42 b	8.56± 1.73b	8.40± 1.44 b

Values with the same letters indicate nonsignificant difference and vice versa. Significant at $P < 0.05$
SOD: Superoxide dismutase. TAC: Total antioxidants capacity. MDA: malondialdehyde

Conclusion

It could be concluded that, the pumpkin seeds at higher concentration 20 % followed by 10 % improved the primarily at the prostate weight, prostate volume, prostate weight index, wet weight index, hormones parameters level and activities of antioxidant enzymes that may be due to effect of testosterone on benign prostatic hyperplasia in male rats. The high amount of phenolic and flavonoid compounds is likely to be responsible for the higher antioxidant activity of the pumpkin seeds. So it can used traditional herbal medicines can be side by side Medicine with Medicines.

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