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ROLE OF PUMPKIN SEEDS ON HEPATORENAL DYSFUNCTION INDUCED BY AZATHIOPRINE IN ADULT MALE ALBINO RATS

By

Aziza K. Omer, Gehan A. Youssef, Asmaa F. Yousf and Azza H. Abd El-Wahab

Physiology Department, Faculty of Medicine (Girls), Al-Azhar University

ABSTRACT

Background: Pumpkin contains several phyto-constituents belonging to the categories of alkaloids, flavonoids, and palmitic, oleic and linoleic acids. Various important medicinal properties including anti-diabetic, antioxidant, anti-carcinogenic, anti-inflammatory and others have been well documented. Azathioprine (AZA), known as Imuran is an *immunosuppressive drug*. It is widely used in many diseases. A major drawback is the occurrence of side-effects, especially hepatorenal dysfunction.

Objective: Assessing the association between the daily oral dose of pumpkin supplementation as a strategy for amelioration of the side-effects of AZA on liver functions, renal functions and some inflammatory markers and tumour necrosis factor-α.

Material and Methods: Twenty four adult male albino rats, weighing 150–180 g, were used. They were divided into four equal groups**: Group I** (control), **group II** treated with (4 ml/kg. b. wt.) of pumpkin seeds oil per day for four weeks by oral gavage, **group III** were injected intraperitoneally with AZA (10mg/kg b. wt.) 3 times with an interval of 48 h in between, and **group IV** was treated by oral gavages with 4 ml/kg. b. wt of pumpkin seeds oil for ten consecutive days followed by AZA treatment i.p. at dose 10 mg/kg b. wt. 3 times with an interval of 48 h in between, after which pumpkin seeds oil administration alone was continued for another 2 weeks. Serum ALT, AST, ALP, total bilirubin, urea, creatinine, CRP and TNF-α were detected.

Results: Pumpkin seeds oil supplementation with AZA treatment significantly decreased the levels of serum liver transaminases (ALT and AST), ALP and total bilirubin in addition to serum urea, creatinine as well as CRP and TNF-α compared to AZA group.

Conclusions: Pumpkin seeds oil has the ability to ameliorate the biochemical pathways of the side-effects of AZA on liver and kidney.

Key words: Azathioprine, Pumpkin seed oil, anti-inflammatory activity, antioxidant.

INTRODUCTION

The azathioprine is effective immuno-suppressant and anti-cancer agent and is prescribed increasingly to treat inflam-matory diseases **(Hawwa *et al.*, 2008**). AZA is also used in the therapy of organ transplant patients to prevent rejection following transplantation (**Bendre *et al.*, 2005)**. However, AZA use has been complicated by a high incidence of serious adverse drug reactions including hepatotoxicity and elevation of reactive oxygen species leading to mitochondrial injury and cell death due to necrosis **(Shanmugarajan and Devaki, 2008).**

Pumpkin seeds oil is rich in many antioxidants and beneficial nutritional supplements such as essential fatty acid-omega 6, omega 9, vitamin A and vitamin E, squalene, carotenoids, tocopherols, phytoestrogenes, phytosterols, polyphenols, hydrocarbon, triterpenoids and selemium **(Al-Okbi *et al.*, 2014-b).** Pumpkin seeds oil includes fatty acids:palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) **(Abouseif, 2014).**

MATERIAL AND METHODS

Azathioprine (Imuran), from Novartis Company, was dissolved in normal saline (0.9% NaCl) and injected i.p. with a volume of 0.1 ml, in a dose equivalent to human dose (10 mg/kg b. wt.) according to Paget's formula (Paget, 1964). Each dose was injected 3 times with an interval of 48 h **(Bendre et al., 2005)**.

Pumpkin seeds oil (PSO), from Emtenan Markets, was applied to the animal by oral gavages with a dose of 4 ml/ Kg b. wt. /day (Eraslan et al., 2013).

The present study was carried out in animal house of faculty of Medicine for Girls, Al-Azhar university on twenty four adult male albino rats weighing 150–180 g. Rats were caged in 40x60x40 cm cages (three rats per cage). They were housed in at room temperature range of 25 ± 5 °C, regular light/ dark cycle, and fed chow and water.

Rats were divided into 4 equal groups:

- **Group I:** Control group.
- **Group II:** Rats was treated orally with 4 ml/kg b. wt. of PSO per day for four weeks. Pumpkin seeds oil was applied to the animal by oral gavages.
- **Group III:** Rats was injected i.p. with AZA (10mg/kg b. wt.) 3 times with an interval of 48 h in between.
- **Group IV:** Rats was treated by oral gavages with 4 ml/kg. b. wt of PSO for ten consecutive days, then AZA treatment was started concomitantly with PSO i.p. at a dose of 10 mg/kg b. wt. 3 times with an interval of 48 h in between, after which PSO administration alone was continued for another 2 weeks.

At the end of the experiment, animals were fasted for 12 hours before collection of blood samples. Blood was collected from retro–orbital sinus by heparinized capillary tubes under ether anesthesia **(Simmons and Brick, 1970)**. The blood samples were collected in centrifuge tubes and allowed to clot for an hour at room temperature, and then centrifuged at 3000 rpm for 15 minutes. Sera were separated and stored at-20° C (using Co REVCO refrigerator).

The separated sera were analyzed for estimation of:

- Liver transaminases **(**AST and ALT- **Young, 1995**).
- Alkaline phosphatase (ALP- **Young et al., 1972**).
- Serum urea and creatinine **(Schirmeister et al., 1964 and Jung et al. (1975)**.
- CRP **(Hirschfield and Pepys, 2003)**.
- TNF-α was estimated by ELISA **(Englmann et al., 1990)**.

Statistical Analysis of results was done using statistic package for social science version 12 (SPSS, 12) for windows. Results were expressed as Mean ± Standard deviation (SD) and statistically analyzed using one-way analysis of variance (ANOVA) for a completely

randomized design and Tukey multiple comparison tests. The level of significance was taken at P value of <0.05.

RESULTS

Azathioprine administration resulted in deterioration of both liver and renal functions as well as enhancement of the inflammatory state as manifested by the significant elevation of in serum ALT, AST, ALP, bilirubin, urea, creatinine, CRP and TNF-α when compared to control group. Pumpkin administration alone did not cause any significant change in all the tested parameters when compared to control group. Pumpkin administration to AZA- treated rats resulted in improvement in liver and kidney functions and in the inflammatory state disturbed by AZA. There was a significant decrease in serum liver functions (serum ALT, AST, ALP and bilirubin) and renal functions (serum urea and creatinine) as well as the inflammatory markers (CRP and TNF-α) when compared to AZA group. Moreover, pumpkin administration to AZA treated rats could return serum creatinine, CRP and TNF-α to normal as there was a non significant difference if compared to pumpkin group (Table 1).

Table (1): The effect of Pumpkin seeds oil treatment on serum ALT, AST, ALP, bilirubin, urea, creatinine, CRP and TNF- α of azathioprine administered rats.

Groups / Parameters	Group I (Control group)	Group II (Pumpkin Group)	Group III (AZA Group)	Group IV (AZA+pampikan)	ANOVA	
	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	F	P- value
Serum ALT (U/L)	**20.25±1.05**	**22.01±1.65**	**119.1 [a]±11.71**	**44.8 [b,c] ±6.75**	8.2	**0.002**
Serum AST (U/L)	**20.88±2.97**	**23.3±2.37**	**287.4 [a] ±56.46**	**123.3 [b,c] ±11.35**	17.06	**0.000**
Serum ALP (U/L)	**73.01±6.67**	**74.92±6.66**	**249.07 [a] ±38.7**	**146.28 [b,c]±6.68**	8.8	**0.001**
Bilirubin (mg/dl)	**0.02±0.02**	**0.05±0.04**	**1.77 [a] ±0.57**	**0.08 [b,c] ±0.02**	8.2	**0.002**
Serum Urea (mg/dl)	**26.2 ± 2.09**	**25.8 ± 2.7**	**62.4 [a] ± 4.5**	**52.6 [b,c] ± 2.7**	8.2	**0.002**
Serum Creatinine (mg/dl)	**0.45 ± 0.06**	**0.52 ± 0.07**	**1.15 [a] ± 0.3**	**0.52 [b] ± 0.13**	9.7	0.001
CRP (µg/L)	**0.49±0.3**	**0.55±0.23**	**1.6 [a] ±0.22**	**0.48 [b] ± 0.24**	6.9	0.004
TNF- α (Pg/ml)	**1.78±0.5**	**1.4±0.44**	**2.21 [a] ±0.37**	**1.5 [b]±0.43**	7.09	0.003

a: significant values versus control group. b: significant values versus AZA group.
c: significant values versus pumpkin group.

DISCUSSION

Treatment with AZA exhibited a significant increase of AST, ALT, ALP, bilirubin, urea and creatinine compared to control group. These results were in agreement with those of **El-Beshbishy *et al.* (2010)** who attributed the occurrence of hepatic enzyme elevation to endogenous protein breakdown due to possible increase in tissue wasting. **Shanmugarajan and Devaki (2008)** noticed an increase in serum levels of AST, ALT, ALP, lactate dehydrogenase (LDH), and gamma glutamyl trans-peptidase (GGT) in serum 24 h after AZA treatment. They described that AZA administered rats displayed declined levels of endogenous antioxidants [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione (GSH)], along with elevated levels of malondialdehyde (MDA)]**.**

The results of the present study also demonstrated that AZA induced deterioration in renal functions in the form of significant increase in serum urea and creatinine. These results were hand in hand with **Shin *et al.* (2006)** who described that AZA could induce acute interstitial nephritis. **Bir *et al.* (2006)** also reported that AZA could be a cause of rapidly progressive renal failure. AZA treatment could induce a variety of DNA modifications such as chromatid damage, DNA strand breaks and DNA–protein cross links. Also, it is required as an active DNA mismatch repair system and, while the effects of purine starvation have been suggested, DNA damage seems to be the main mechanism for the cytotoxic effects of thiopurines **(Karran and Attard, 2008).**

Pumpkin seed oil is suggested to be healthy addition towards human diet and have protential suitability for food and industrial applications because it is being rich in unsaturated fatty acids especially linoleic acid, oleic acid, tocopherols, and with very oxidative stability **(Abouseif, 2014**). PSO is an excellent source of protein, zinc, manganese and phosphorus. It contains a high amount of tryptophan, an essential amino acid involved in the synthesis of key brain chemical serotonin (**Amin *et al.*, 2013 and Galaly *et al.*, 2014).**

The present investigation demonstrated that PSO reduced the toxic effect of AZA on liver male rats, and this might be due to high content of beta-carotene: **Makni *et al.* (2008)** discussed the anti- oxidant and hepatoprotective effect of active groups treated with pumpkin. Beta-carotene had been proved to be a powerful antioxidant and profound protective action against oxidative stress **(Oboh, 2005).**

The pumpkin seeds oil has a powerful antioxidant and protective actions against tissue damage because of the role of antioxidant vitamins like 13- carotene in neutralization of free radicals and overtly aggressive oxygen species **(Nkosi *et al.*, 2006).** Beta-carotene was among the most efficient substance known for quenching the excitation energy of single oxygen, and also for trapping certain organic free radicals. It has a direct inhibitory effect on liver microsomal enzymes **(Ardabili *et al.*, 2011).** Moreover, the experimental study of **Abouseif (2014)** showed that the natural plant components found in pumpkin could improve the liver against

alcohol-induced liver toxicity and oxidative stress.

Renal functions improved according to the present study as serum urea and creatinine both significantly decreased with PSO and AZA- treated group in comparison to AZA group. These results were agreed with **Makni *et al.* (2010)** who found that pumpkin seeds ameliorated the antioxidant enzymes activities in nephropathic kidneys of rats. They also noticed that the histopathological changes were less prominent in pumpkin seeds treated rats than nephropathic kidneys. This improvement in the antioxidant enzyme system caused by pumpkin could be the cause in the manifested improvement of renal functions as observed in the present study.

In the present study, it was noticed that TNF-α and CRP significantly decreased in combined pumpkin and AZA group compared to AZA group. These results were in agreement with **Xanthopoulou *et al.* (2009)** who showed that pumpkin seeds oil has anti-inflammatory activity which depends on their total phenolic content. The anti-inflammatory activity of pumpkin was due to their inhibitory activity against lipid peroxidation catalyzed by lipoxyganase. **Sedigheh *et al.* (2011)** who showed that pumpkin powder (1 and 2 g/kg for 4 weeks) supplementation in male diabetic rate revealed a significant reduction in CRP level. It is speculated that the anti-inflammatory effects of this plant is related to its anti-oxidant compounds such as flavonoids. It is also established that quercetin, from the class of flavonoids, provides protection against free radicals, chelate metal ion transporters, and also inhibit oxidases including lipoxygenase.

Al-Okbi *et al.* (2014-a) demonstrated that PSO produced a significant reduction in plasma level of TNF-α. They suggested that the hypolipidemic, antioxidant and anti-inflammatory effects of pumpkin seed oil in their study are attributed to the presence of its bioactive constituents. **El-Mosallamy *et al.* (2012)** reported that PSO exhibited anti-inflammatory effects as pumpkin seeds are rich in linoleic and oleic unsaturated fatty acids. Linoleic and lionlenic acids compete with arachidonate for oxidative enzymes, thereby reducing the production of arachidonate cyclo-oxygenase products. It has been shown that a diet rich in C-linolenic acid has action similar to no steroidal anti-inflammatory agents in reducing the production of prostaglandin E2 and leukotriene B4 generated during inflammation.

Singh *et al.* (2005) and **Calder *et al.* (2009)** attributed the anti-inflammatory effect of PSO to the presence of vitamin E, which is one of the important components of PSO, as it has a potent antioxidant with anti-inflammatory properties. Vitamin E has effects on inflammatory properties. Vitamin E has effects on inflammatory processes due to the antioxidant function of α-tocopherol. α-Tocopherol exerts anti-inflammatory effects through a number of different mechanisms, for example, by decreasing levels of CRP and pro-inflammatory cytokines and by inhibiting the activity of protein kinase C, an important cell-signaling molecule, and other enzymes such as syslooxygenase-2.

Gammone *et al.* (2015) attributed the anti-inflammatory effect of PSO to the presence of beta-carotene which is one of the important components of pumpkin seed oil reducing the expression of IL-1a, VCAM-1 and E-selectin.

CONCLUSION

Pumpkin seeds oil was effective in preventing the toxic effect of AZA as an immune suppressant drugs on liver and kidney of male rats. Much additional studies are needed before the authors might confidently make recommendations regarding dietary pumpkin in the prevention of toxic effect of immune suppressant drugs. Further clinical studies are also required to assess the safety and benefits of pumpkin seeds oil in human beings.

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عزيزة خليل عمر - چيهان أحمد يوسف - أسماء فتحي يوسف- عزة حامد عبد الوهاب

قسم الفسيولوجيا- كلية الطب (بنات)-جامعة الأزهر

خلفية البحث: اليقطين (قرع العسل) يحتوي على العديد من المكونات النباتية، التى تنتمى إلى فئات القلويدات، والفلافونويد، وأحماض النخيلي، الأوليك واللينوليك، والعديد من الخصائص الطبية الهامة بما في ذلك مضادات الأكسدة، ومضاد الالتهابات و دوره في علاج السكرى والحد من السرطان وغيرها موثقة جيدا. والآزوثيوبرين (AZA) المعروف باسم Imuran هو دواء مناعة يستخدم على نطاق واسع في العديد من الأمراض. والعيب الرئيسي هو حدوث الآثار الجانبية، خصوصا الإعتلال الكبدي الكلوي.

الهدف من البحث: معرفة التأثير الوقائي لتناول زيت بذور اليقطين علي وظائف الكبد والكلي في ذكور الجرذان التي تم معالجتها بالآزوثيوبرين المسبب لإختلال وظائف الكبد والكلي وارتفاع في مستوي إنزيمات الكبد واليوريا والكرياتينين بالإضافة إلي بعض دلالات الالتهاب كالبروتين التفاعلي ج وعامل نخر الورم ألفا.

مواد و طرق البحث : شملت الدراسة الحالية 24 من ذكور الجرذان البيضاء البالغة التى يتراوح وزنها بين 150-180 جرام، واستمرت التجربة لمدة 4 أسابيع وتم تقسيمها إلي أربع مجموعات متساوية علي النحو التالي:
المجموعة الأولي (المجموعة الضابطة) تغذت علي غذاء متوازن ولم تخضع لأي معالجات
المجموعة الثانية: مجموعة معالجة بزيت بذور اليقطين - مجموعة تناولت زيت بذور اليقطين (4 ملليلتير؟ كجم) يوميا عن طريق أنبوبة تصل من الفم إلي المعدة.
المجموعة الثالثة: مجموعة معالجة بالآزوثيوبرين داخل الغشاء البريتوني (10 مجم؟ كجم) 3 مرات بين كل جرعة 48 ساعة
المجموعة الرابعة: مجموعة معالجة بزيت بذور اليقطين (4 ملليلتير؟ كجم) يوميا عن طريق أنبوبة تصل من الفم والي المعدة وبعد عشرة أيام يتم حقنها بعقار الآزوثيوبرين داخل الغشاء البريتوني (10 مجم؟ كجم) 3 مرات بين كل جرعة 48 ساعة مع زيت بذر قرع العسل ، ثم يتم تناول زيت بذور اليقطين (4 مليلتير؟ كجم) فقط لمدة أسبوعين حتى نهاية التجربة.

وفي نهاية التجربة تم تخدير الفئران كليا وجمع عينات الدم من الوريد العيني لقياس مستوى إنزيمات الكبد واليوريا والكرياتينين ، وكذلك مستوى البروتين التفاعلي ج وعامل نخر الورم ألفا.

النتائج : تقل مستويات إنزيمات الكبد واليوريا والكرياتينين و مستوى البروتين التفاعلي ج وعامل نخر الورم ألفا في المجموعة المعالجة بزيت بذور اليقطين بالمقارنة بالمجموعة الثالثة المعالجة بالآزوثيوبرين.

الاستنتاج: العلاج بزيت بذور اليقطين يؤدى إلي تحسين التأثير المدمر الناتج عن الحقن بالآزوثيوبرين.