

**Biochemical and histopathological changes in male albino rats
treated with overdose of an aqueous extract of pomegranate
(*Punica granatum L.*) pericarps**

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Abstract

Pomegranate has been cultivated since ancient times. All parts of this plant were used to treat various ailments.

It mentioned for three times in the Holy Quran under the name of Rumman in Surah (Al-Anaam) verse 99 , Surah (Al-Anaam) verse 141 and Surah (Al-Rahman)verse 68.

In Surah (Al-Anaam) verse 141." It is He who produce the gardens with trellises and without ,and dates and tilth with produce of all kinds and olives and pomegranates ,similar (in kind) and different (in variety) :eat of their fruit in their season ,but the dues that are proper on the day that the harvest is gathered .But waste not by excess. For Allah loves not the wasters".

This investigation aims to study the effect of repeated over doses of an aqueous extract of pomegranate (*Punica granatum L.*) pericarps in male albino rats for a month.

Oral administration of repeated over doses (1.3g/k) of an aqueous extract of pomegranate (*Punica granatum L.*) pericarps in male albino rats for month displayed significant increase in serum aspartate aminotransferase (AST), alanin aminotransferase (ALT),alkaline phosphatase and liver revealed congestion of central vein ,dilatation of sinusoids , vacuolization and (ALT) ballooning also a lot of pyknotic nuclei were detected with many necrotic areas of hepatocytes. Inflammatory cells were detected inbetween hepatocytes, dilatation of the portal tract and fibrosis noticed around it. Elevation of serum urea , creatinine ,dilatation and severe congestion of blood vessels , most glomeruli were congested ,dilatated, and some appeared degenerated. Diffuse extravagations of red blood cells between the degenerated renal tubules were noticed .Augmentation of serum total cholesterol , decreased serum triglycerides and formation of medial calcinosis in aorta. Reduction in serum testosterone level, some seminiferous tubules appeared normal but the others appeared affected.

All things must be taken in moderation because repeated high doses of an aqueous extract of pomegranate pericarps will be toxic.

Introduction

Pomegranate (*Punica granatum L.*), belongs to punicaceae family. Flavonoid-rich poly phenol fractions from pomegranate inducing chemicals with a potentially lower toxicology profile than other therapies. All parts of this plant were used to treat various ailments. The extract of root bark has been reported to exert some sugar lowering action in animals (Carraz, *et al* ,1978),the extract of stem bark is used as anthelmintic, green leaves are made as a paste and applied in conjunctivitis (Satyavati *et al* ,1978), flowers used as anthelmintic, (Singhal ,1983), as

hypoglycaemic agent (Jafri *et al*, 2000), peels displayed ,haemostatic, antidiarrhoeal, antifertility (Duck *et al*, 2002), adjunctive periodontal (Sastravaha *et al*, 2003) ,as an antifungal agent against candidosis associated with denture stomatitis (Vasconcelos *et al* , 2003),as a strong therapy for leukemia cells (Kawaii and Lansky, 2004), seed extract showed antidiarrhoeal activity (Das *et al*, 1999), seed displayed hypoglycaemic activity (Das *et al*, 2001)and fruit extract possesses antioxidant activity (Noda *et al*, 2002), improves a depressive state and bone

properties in menopausal syndrome model (Mori-Okamoto *et al* , 2004), reduced common carotid intima-media thickness, blood pressure and LDL oxidation (Aviram *et al*, 2004) and inhibit skin tumorigenesis in mice (Afaq *et al*, 2004).

In Surah (Al-Anaam) verse 141 God ordered us to eat pomegranate and forbidden to eat excess.

Vidal *et al*, 2003 recorded that LD50 of pomegranate extract determined in mice was 731mg/k and they added that toxic effects of *Punica granatum* fruit extract occurred at higher doses than those effective in the models for the treatment of respiratory diseases in Cuban folk medicine.

All things must be taken in moderation.

This investigation aims to study the effect of repeated over doses of an aqueous extract of pomegranate (*Punica granatum* L.) pericarps in male albino rats for month.

Material and methods

Plant material

200ml boiling distilled water was added to 5gm powder pomegranate peel, left it for 10 minutes and filtered .The filtrate was dried at 40-45°C in the incubator .

Animals

14 adult male albino rats weighing 120-150g were obtained from breeding in animal lab. histology department in NODCAR. The animals were housed under good hygienic condition, diet and water excess

Experimental design

The animals were divided into two groups. Group I, served as control consisted of six rats, group II consisted of 8 animals was given pomegranate aqueous extract (1.3g/k. p.o.) in a dose 3 fold the therapeutic dose (Paget and Barnes,1964)

Blood sampling

After 30 days from pomegranate aqueous extract administration, blood samples were collected from retro-orbital vein in the two groups. The blood was

allowed to collect at room temperature and serum obtained after centrifugation was used for determination serum aspartate aminotransferase , alanine aminotransferase (Reitman and Frankle,1957) alkaline phosphatase (German Society for clinical chemistry,1972) ,urea (Tabacco *et al*, 1979), creatinine (Bartles *et al*,1971) ,total cholesterol (Allain *et al.*, 1974), Triglycerides (Bucolo and David,1973) testosterone was estimated by Kit obtained from IMX Abbott Labs, IL/USA.

Fresh liver, kidney, aorta samples were collected in formalin 10%. Testes were collected in Bouin' fluid. All samples were stained with H&E.

Statistical analysis

All data obtained were analyzed using student's t-test according to Sendecor and Coebram (1969).

Results and Discussion

Pomegranate juice contains, a wide of polyphenolic compounds including ellagic , gallic ,anthocyanins and tannins especially punicalagin, which is a power antioxidant ,pomegranate showed antioxidant activity three times higher than those of green tea (Schubert *et al*,1998).Green tea possessed hepatoprotective activity at the therapeutic dose (Peirce,1999), if one consumed the equivalent of 65 g tea leaves /day for 5 years would exhibit liver dysfunction , much astringent tannins ,which can damage the liver and intestine with prolonged use (Pedersen,1998). Duck *et al*,2002 recommended that tannins should call (poly phenols)useful antioxidant good guys instead of hepatotoxic bad guys, so all things must be taken in moderation.

The Pomegranate rind contains tannins, anthocyanins, flavonoids ,pectins (Nozire and Serpil, 1993), ellagitannins (punicalin , punicalagin , granatin, gallagylidilactone, casurinin,), pedunculagin, tellimagrandin ,corilagin (Satomi *et al*, 1993), ellagic tannins, gallic, ellagic acids , ursolic acid (Ben-Nasr *et al*,1996)and catechin (Chidambara *et al*, 2004) three estrogen

compounds luteolin, quercetin and kaempferol (Van-Elswijk *et al*, 2004).

Pomegranate is known to contain estrogens (estradiol, estrone, estriol) and shows estrogenic activities in mice (Mori-Okamoto *et al*, 2004).

It means that pomegranate rind extract contains poly phenolic compounds which behaves like estrogens (Kummer *et al*, 2001) and also, it contains estrogens.

In this investigation, the repeated overdose administration of pomegranate extract pericarps for month in male albino rats led to significant increase in (AST), (ALT), ALP (table 1) and liver revealed congestion of central vein, dilatation of sinusoids (fig.1), vacuolization and ballooning also a lot of pyknotic nuclei were detected with many necrotic areas of hepatocytes (fig.2). Inflammatory cells were detected in between hepatocytes (fig.2), dilatation of portal tract and fibrosis around it, dilated endothelium with the disappearance of their nuclei, debris of a lot of hepatocytes were observed (d), a large necrotic area could be detected (n) (figs 4 & 4a) compared with control (fig.1) this result was in accordance with conclusion of (Pedersen, 1998) much tannins with prolonged use can damage the liver. Elevation in serum urea and creatinine (table 1) may be due to liver dysfunction (Lanter, 1975), and impairment in kidney glomeruli and tubules concerning

kidney structure dilatation and severe congestion of blood vessels, some nuclei of convoluted tubules appeared faintly stained and their cells appeared moderately affected, some glomeruli appeared lobulated and atrophied (fig.11), most glomeruli were congested, dilated (figs 6 & 7), some appeared degenerated (fig.9, 11) and some appeared compact (figs. 6). Diffuse extravagations of red blood cells between the degenerated renal tubules were noticed (fig.10) compared to control (fig.5). Augmentation of serum total cholesterol, decreased serum triglycerides (table 1) and formation of medial calcinosis in aorta with irregularity of tunica intima (figs. 13, 13a) compared to control (fig.11) Reduction in serum testosterone level (table 1) attributed to regulation for testosterone synthesis may be affected by phytoestrogen rich diet and decreased testosterone levels (Weber *et al*, 2001) and or administration of high estrogens doses affect germ cells via a testosterone deficiency (Kaneto *et al*, 1999), some seminiferous tubules appeared normal but the others contained less spermatogenic activity with reduction in sperm number and Leydig cells (figs. 15 & 15a) compared to control (fig.14).

All things must be taken in moderation because repeated high doses of an aqueous extract of pomegranate pericarps for month displayed toxic effects.

Table(1): showing the effect of treatment with overdose(1.3g/kg)of an aqueous extract of pomegranate epricarps on some liver function tests, kidney function tests ,cholesterol ,triglycerides and testosterone for month in male albino rats.

Parameters \ Groups	control	Treated with overdose
AST (U/L)	38.7 ±1.9	46.33↑* ±2.02
ALT (U/L)	22 ±1.76	28↑* ±1.55
ALP (U/L)	83 ±2.21	94↑* ±3.07
Urea mg/dl	21.33 ±1.31	16.6↓* ±0.98
Creatinine mg/dl	0.8 ±0.98	0.35↓* ±0.17
Cholesterol mg/dl	130 ±2.71	145↑** ±4.12
Triglycerides mg/dl	56.4 ±3.11	46.47↓* ±2
Testosterone ng/ml	1.8 ±0.05	0.8↓* ±0.032

Number of rats in control groups =6

Number of rats in treated group =8

* P<0.05

**P<0.01

Legend of figures

Fig.1 : Control rat liver showing the normal histological structure H&E x200

Fig.2: Liver of rat treated with (1.3g/k) aqueous extract of P.granatum for month showing severe congestion of central vein , dilatation of sinusoids and vacuolization of hepatocytes and Inflammatory cells were detected inbetween hepatocytes H&E x400

Fig.3:Liver of rat treated with (1.3g/k) aqueous extract of P.granatum for month vacuolization and ballooning also a lot of pyknotic nuclei were detected with many necrotic areas of hepatocytes. H&E x400

Fig.4: Liver of rat treated with (1.3g/k) aqueous extract of P.granatum for month showing dilatation of portal tract and fibrosis around it H&E x 250

Fig.4a: Liver of rat treated with (1.3g/k) aqueous extract of P. granatum for month showing dilatation of portal tract and fibrosis was noticed around it, dilated endothelium with the disappearance of their nuclei,debris of a lot of hepatocytes were observed (d),a large necrotic area could be detected(n) H&E x 500

Fig.5: kidney of control rat H&E x 250

Fig.6: kidney of of rat treated with (1.3g/k) aqueous extract of P. granatum for month showing glomeruli were congested and compact H&E x 500

Fig.7 : kidney of rat treated with (1.3g/k) aqueous extract of P. granatum for month showing swelling of glomeruli and degenerated tubules H&E x 400

Fig.8 : kidney of of rat treated with (1.3g/k) aqueous extract of P. granatum for month

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showing dilatation and severe congestion of blood vessels,

Fig.9 :some nuclei of convoluted tubules appeared faintly stained and their cells appeared moderately affected, some glomeruli appeared lobulated and atrophied H&E x250

kidney of of rat treated with (1.3g/k) aqueous extract of P. granatum for month showing degenerated glomerulus H&Ex250

Fig.10: kidney of rat treated with (1.3g/k) aqueous extract of P. granatum for month showing diffuse extravagations of red blood cells between the degenerated renal tubules H&E x 500

Fig.11: Aorta of control rat H&E x250

Fig.13: Aorta of rat treated with (1.3g/k) aqueous extract of P. granatum for month showing formation of medial calcinosis in

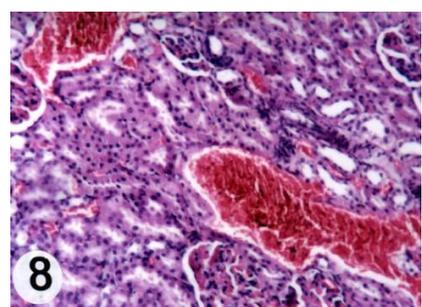
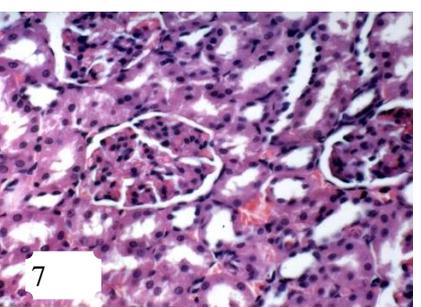
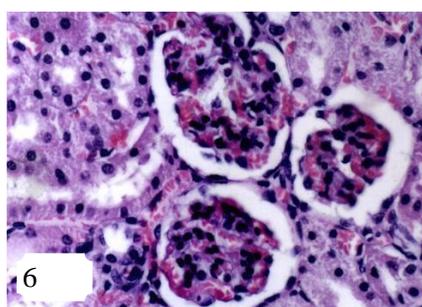
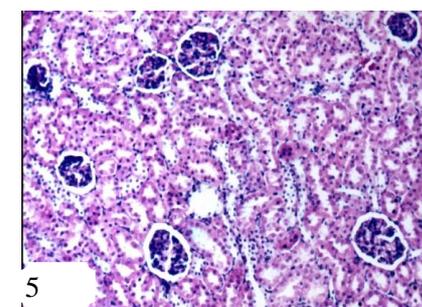
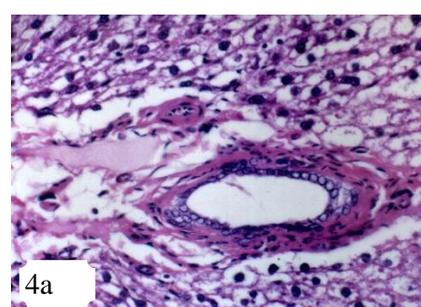
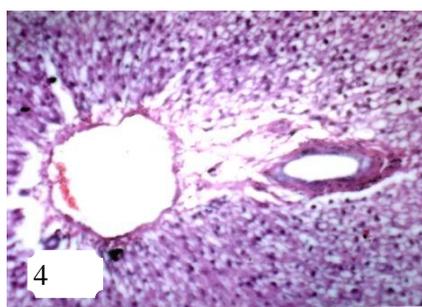
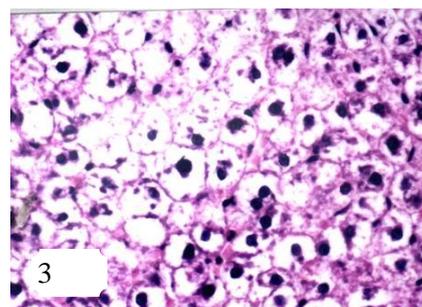
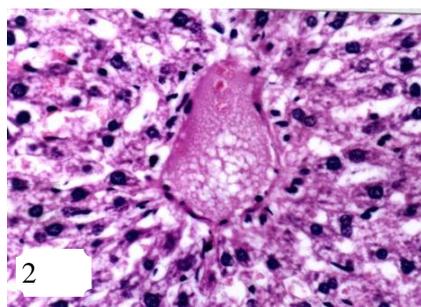
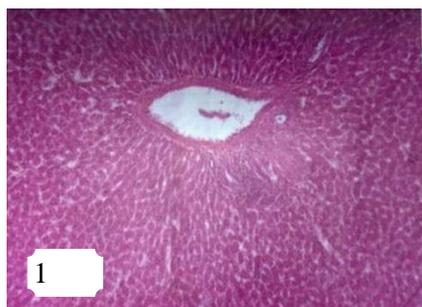
aorta with irregularity of tunica intima H&E x100

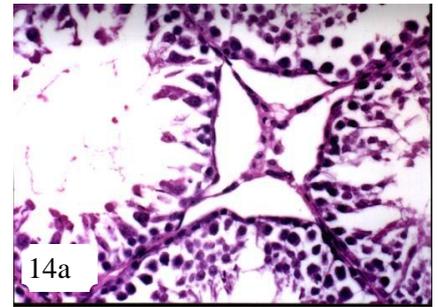
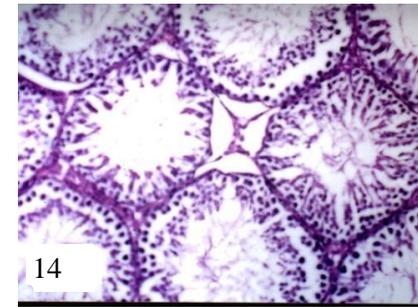
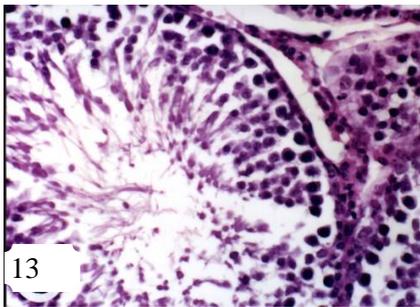
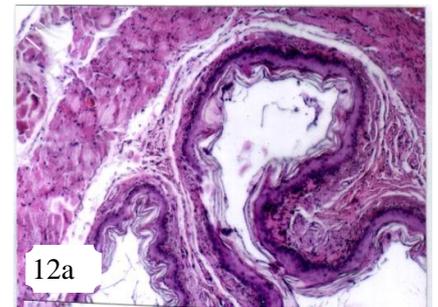
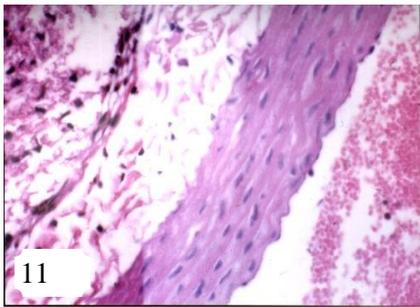
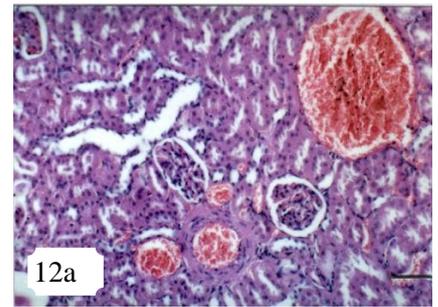
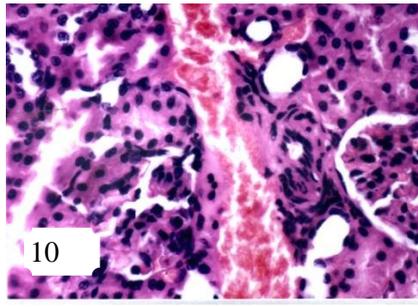
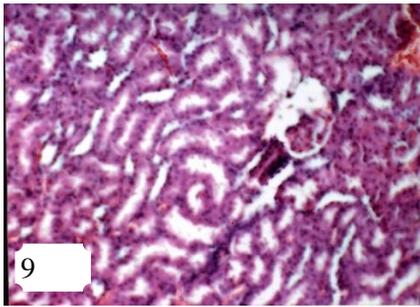
Fig.13a: Aorta of rat treated with (1.3g/k) aqueous extract of P. granatum for month showing formation of medial calcinosis in aorta with irregularity of tunica intima H&E x160

Fig.14: Testis of control rat H&E x 200

Fig.15: Testis of rat treated with (1.3g/k) aqueous extract of P. granatum for month showing some tubules appeared normal and others contained less spermatogenic activity with reduction in sperm number and leyding cells H&Ex250

Fig.15a: Testis of rat treated with (1.3g/k) aqueous extract of P. granatum for month showing some tubules appeared normal and others appeared moderately affected H&Ex500





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دراسات كيميائية حيوية وهستوباثولوجية على التغيرات الحادثة في ذكور الجرذان المهق المعامل بالجرعة الذائده للمستخلص المائى لقشرة ثمرة الرمان

إيناس على مهدى خليل
الهيئة القومية للرقابة والبحوث الدوائية

يزرع الرمان من عصور قد يمه وتستخدم كل أجزاء شجره الرمان فى علاج
أمراض مختلفه .
ويهدف البحث الى دراسة تأثير الجرعه الذائده للمستخلص المائى لقشرة الرمان لمدة شهر
فى ذكور الجرذان
وأظهرت المعامله بالجرعه 1,3 جم /كم من الستخلص المائى لقشرة الرمان لمدة شهر فى
الجرذان ارتفاعا ذو دلالة احصائيه فى مستوى
مصل الأنزيمات الناقله للأمين وفى مستوى مصل الفوسفاتاز القاعدى وأحتقان فى
الوريد المركز بالكبدى وأتساع الجيوب وخلو خلايا الكبد من المحتوى السيتوبلازمى مع تنكز
خلاياه وتحلل الأنويه وظهور خلايا التهابيه بين خلايا الكبد وأتساع فى المنطقه البابيه وظهور
تليف حولها وأنخفاض فى مصل اليوريا والكرياتين ووجد احتقان وتضخم فى معظم كرات
مليجي وبعض منها مضمحل وأتساع وأحتقان الأوعيه الدمويه وتضخم وتحلل لبعض الخلايا
المبطنه لأنابيب الكلى ولوحظ ارتفاع ذو دلالة احصائيه فى مصل الكوليستيرول الكلى ونقص
فى مصل الدهون الثلاثيه وظهر تكلس فنجدار الأورطى الشريانى وأنخفاض مستوى هرمون
التيسترون وموت كثير من الحيوانات المنويه
ويوضح البحث الأعتدال فى تناول أى شئ
ويستنتج من هذا البحث أن الجرعه الذائده من المستخلص المائى لقشرة الرمان قد تسبب
أثار ا جانبيه للتدبيات ولهذا ينصح بالاعتدال فى تناول أى مواد طبيعيه ويحسن اجراء العديد
من التجارب لتقييم الجرعات المعتدله والذائده على تكوين ووظائف الجسم للتدبيات.