Asian Journal of Biological and Life Sciences | Original Research

Effect of aqueous and ethanolic pomegranate peel extract on the diethylnitrosoamine induced changes on lipid profile and testicular function of albino rats

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Submitted : 15.06.2014 Accepted : 03.12.2014 Published : 31.12.2014

Abstract

Medicinal plants have played an important role in the pharmacology and medicine. Pomegranate (*Punica granatum* L) an ancient fruit, has been used to investigate its anti-carcinogenic effect on the hormonal, lipid profile and histology of testis in Diethylnitrosoamine (DEN) induced albino rats. Group I served as normal control. Group II and Group III were given low and high dose DEN for 40 days. Group IV and Group V were treated with low and high dose DEN + aqueous pomegranate peel extract. Group VI and Group VII were treated with low and high dose DEN + ethanolic pomegranate peel extract. Finally animals were sacrificed, blood collected and assayed for hormone and testis histology. The level of LH, FSH were significantly increased in all the DEN treated pomegranate supplemented groups. The level of prolactin was significantly increased in both the low and high dose DEN induced groups. A slight increase in the level of testosterone observed only in the low dose DEN supplemented group. Except for the high dose DEN treatment, the other groups showed a significant decrease in cortisol level. While cholesterol was significantly increased in both the supplemented groups. The level of triglyceride was significantly increased in all the treatment groups. The level of HDL was significantly reduced in both the DEN treated groups. Low and high dose DEN treated testis showed slight distruption and complete disorganization of basement membrane and seminiferous tubules. Supplementation with APPE and EPPE showed the preservation of the architecture of seminiferous tubule.

Key words : Pomegranate peel, Den, Testis function and Histology, Lipid profile

INTRODUCTION

Herbal and natural products represent one of the most common forms of complementary and alternative medicines. Many natural product extracts have been found to have pharmacological effects [1].Traditionally various plants are being used for treatment of cancer. Today, it is estimated that about 80 percent of the world population relies on botanical preparations as medicine to meet their health needs [2]. *Punica granatum*, which belongs to the family of Punicaceae, is commonly known as pomegranate or "punica apple". *Punica granatum* has been used extensively in a traditional medicine in many countries for the treatment of dysentery, diarrhea, helminthiasis, acidosis, hemorrhage and respiratory disorders. In addition, *P.granatum* is reported to have antioxidant, anti-atherosclerotic, antibacterial and antiviral properties.

MATERIALS AND METHODS

Selection of the animal model

Albino rats, weighing about 130-250 grams were selected. The rats were procured and acclimatized to our laboratory conditions for two weeks. The animals were housed in a well ventilated, temperature and humidity controlled animal house, with a light schedule of fourteen hours and ten hours darkness. They were fed with standard diet and drinking water was made available *ad libitum.*

Preparation of pomegranate peel extracts

Fresh pomegranates (*Punica granatum*) were obtained from local departmental store. The peels of the pomegranate were separated and washed with distilled water. To obtain the aqueous extract of *Punica granatum* peels, 150 gm of the air dried peels were powdered in an electric grinder to a fine powder and passed through a 24 mesh sieve and stored in plastic bags. 50 gm of powdered sample was mixed with 500 ml of distilled water for 15 minutes with continuous stirring. The resultant solution was filtered through a filter paper, and stored as a stock solution for experimentation as aqueous pomegranate peel extract (APPE).

To prepare ethanol extract of pomegranate peel, fresh fruits were peeled and washed with distilled water. 150gm of peels were weighed, and powdered in an electric grinder to a fine powder and passed through a 24 mesh sieve and stored in plastic bags. 50 gm of powdered sample was taken and extract separated for 8 hours using Soxhlet apparatus with 200 ml ethanol (99.9 %). The extract was concentrated under room temperature by evaporation and stored at 4˚c until used, designated as ethanolic extract of pomegranate peel (EPPE).

Diethylnitrosoamine (DEN)

Diethylnitrosoamine was purchased from Sigma Chemical Co. (Bangalore). DEN was dissolved in saline and injected twice a week as intra peritoneal injection at low dose of 0.5 mg/100g BW/day and high dose of 1.0 mg/100g BW/day for 40 days to initiate carcinogenesis.

Experimental design

Healthy male albino rats were divided in to 7 groups of 5 animals and received the following regimen of treatments.

Group I (Control) - Animals received normal saline 1ml/100gm BW/day for 40 days and used as control.

Group II (LD) - Animals were injected DEN 0.5mg/100gm BW twice a week for 40 days intraperitoneally.

Group III (HD) - Animals were injected DEN 1.0mg/100gm BW twice a week for 40 days intraperitoneally.

Group IV (LDPA) - Animals were injected DEN 0.5mg/100g BW/day twice a week intraperitoneally and given aqueous extract of pomegranate peel orally (0.2ml/100g BW/day) for 40 days.

Group V (HDPA) - Animals were injected DEN 1.0mg/100g BW/day twice a week intraperitoneally and given aqueous extract of pomegranate peel orally at (0.2ml/100g BW/day) for 40 days.

Group VI (HDPE) - Animals were injected DEN (0.5mg/100g BW/day) twice a week intraperitoneally and given ethanolic extract of pomegranate peel orally at (0.2ml/100g BW/day) for 40 days.

Group VII (HDPE) - Animals were injected DEN (1.0mg/100g BW) twice a week intraperitoneally and given ethanolic extract of pomegranate peel orally at (0.2ml/100g BW/day) for 40 days.

All treatments were given between 9:30 to 10:00 hours in the morning. At the end of the treatment protocol, animals were anesthetized with ether and sacrificed by decapitation. Blood was collected in EDTA uncoated tubes, serum separated and stored properly until analysis of serum hormones. All animals were dissected and their testis were rapidly excised, washed with saline, blotted with a piece of filter paper and weighed. A bit of tissue from the testis was fixed in Bouin's fluid and used for histological studies.

Biochemical analysis

- Assay of Follicle stimulating hormone (FSH) was done by the method of Midgley and Jaffe., 1971[3].
- Assay of Luteinizing hormone (LH) was done by the method of Singh et al., 1984[4].
- Assay of Testosterone hormone concentration in serum based on the method of Tietz., 1995[5].
- Assay of Cortisol was done by the method of Gowenlock A. H., 1986[6].
- Assay of Prolactin was done by the Sandwhich method.
- Estimation of Cholesterol was determined by Tietz., 1963[7].
- Estimation of Triglyceride was determined by Tietz., 1963[7].
- Histological studies were bone by the method of Bancroft and Stevens., 1997[8].

Statistical analysis

Results obtained were tabulated. Statistical analysis was carried out using Dunnetts "t" test. Any significant variation between the control and treated groups were recorded.

RESULTS

Effect on body weight

An increase in body weight was observed on treatment with low dose of DEN, but high dose brought about a significant decrease in body weight. Supplementation with APPE and EPPE pomegranate peel extracts to low dose groups seems to bring about a significant increase in body weight, while the effect of the extracts on high dose groups was not significant.

Effect on testicular weight

On comparison with control, the weight of testis was significantly increased in the low dose DEN treated group and APPE and EPPE supplemented high dose DEN groups only. The increase in testicular weights were not significant in low dose supplementation groups.

Effect on testicular histology (Plate 1)

The Testis of control group showed normal architecture. Seminiferous tubules with active spermatogenesis evident. The low dose DEN treatment brought about slight disruption of the seminal tubular architecture with accumulation of cell debris in the interstitial spaces due to disruption of basement membrane and occurrence of spermatozoa in the lumen of the tubule. But high dose DEN treatment caused a complete disorganization of the seminiferous tubule with increased disruption of basement membrane. Internal derangement of spermatogonic cells can be observed.

Supplementation with APPE to low dose and high dose groups are seen to induce the complete internal disruption of spermatogonial cells. Even though outer structure is maintained, loss of spermatozoa and increase in interstitial space also observed. Supplementation of DEN induced changes with EPPE

Table 1: Effect of aqueous and ethanolic pomegranate peel extracts on the body weight of diethylnitrosamine induced albino rats.

BODY WEIGHT	CONTROL	LD	HD	LDPA	HDPA	LDPE	HDPE
INITIAL	120±9.082	170±3.162	209±15.280	87±2.549	116±3.674	96±4.0	149±12.186
FINAL	138±11.683	187±3.741	178±16.324	129±1.870	127±2.0	126±2.915	142±10.319

Values are expressed as Mean ± S.E.M of five rats.
C Control, LD - Diethylnitrosamine (Low dose), HD - Diethylnitrosamine (High dose), LDPA - Diethylnitrosamine (Low dose) + Aqueous Pomegranate Peel Extract, HDPA- Diethylnitrosamine (High dose) + Aqueous Pomegranate Peel extract, LDPE- Diethylnitrosamine (Low dose) + Ethanolic Pomegranate Peel Extract, HDPE - Diethylnitrosamine (High dose) + Ethanolic Pomegranate Peel Extract.

is seen to be more beneficial, due to preservation of testicular architecture. But slight internal disorganization of the spermatogonial cells as well as loss of spermatocytes and spermatids with vacuolization of tubules observed.

Effect on hormonal profile

Follicle stimulating hormone (FSH)

While the level of FSH hormone was observed to be significantly increased in the all the low dose DEN treated pomegranate peel extracts supplemented groups ,high dose DEN treatment as well as pomegranate peel extracts supplemented groups, expressed a significant decrease in the level of FSH.

Luteinizing hormone (LH)

All the treatment groups expressed significant increase in the level of LH hormone.

Prolactin

The level of prolactin was significantly increased in the DEN treated low dose and high dose groups of the extract supplemented groups, the aqueous pomegranate peel extract supplemented groups expressed lesser increase in the level of prolactin than the ethanolic extract supplemented groups.

Testosterone

The level of testosterone was increased in all the treatment groups except for the high dose DEN treated groups, with a significant increase observed in low dose DEN treated APPE supplemented group.

Cortisol

All the treated and extract supplemented groups showed a significant decrease in cortisol levels when compared to control level.

Effect on lipid profile (Table 4)

Cholesterol

A significant increase in the cholesterol level can be observed in the aqueous and ethanolic pomegranate peel extract supplemented groups only.

Table 2: Effect of aqueous and ethanolic pomegranate peel extracts on the testicular weight of diethylnitrosamine induced albino rats.

ORGAN	CONTROL	LD	HD	LDPA	HDPA	LDPE	HDPE
TESTIS	1.24±0.100	2.31*±0.063	1.58*±0.056	1.43=0.107	1.76*±0.150	1.41±0.056	1.64*±0.136

Values are expressed as Mean ± S.E.M of five rats.
C Control, LD - Diethylnitrosamine (Low dose), HD - Diethylnitrosamine (High dose), LDPA - Diethylnitrosamine (Low dose) + Aqueous Pomegranate Peel Extract, HDPA- Diethylnitrosamine (High dose) + Aqueous Pomegranate Peel extract, LDPE- Diethylnitrosamine (Low dose) + Ethanolic Pomegranate Peel Extract, HDPE - Diethylnitrosamine (High dose) + Ethanolic Pomegranate Peel Extract.

Table 3: Effect of aqueous and ethanolic pomegranate peel extracts on the hormonal profile of diethylnitrosamine induced albino rats.

PARAMETER	CONTROL	LD	HD	LDPA	HDPA	LDPE	HDPE
FSH	0.55±0.003	0.346*±0.003	0.138*±0.001	0.74*a±0.003	0.448*c±0.002	0.64*b±0.0042	0.026*d±1.87
LH	0.038±2.2	0.18*±0.04	0.08*±2.5	0.124*a±2.48	0.258*c±0.004	0.054±0.07	0.124*d±2.48
PROLACTIN	1.194±0.014	1.228±0.007	1.508*±0.009	0.256*a±0.0026	0.028*c±3.2	0.434*b±0.007	0.764*d±0.139
TESTOSTERONE	0.132±0.003	0.252*±0.001	0.156±0.002	1.046*a±0.02	0.25±0.185	0.248*b±0.004	0.212d±0.045
CORTISOL	3.58±0.21	2.48*±0.127	3.6±0.325	1.0a±0.016	1.18*c±0.014	1.121*b±0.011	2.5*d±0.128

Values are expressed as Mean ± S.E.M of five rats.
C Control, LD - Diethylnitrosamine (Low dose), HD - Diethylnitrosamine (High dose), LDPA - Diethylnitrosamine (Low dose) + Aqueous Pomegranate Peel Extract, HDPA- Diethylnitrosamine (High dose) + Aqueous Pomegranate Peel extract, LDPE- Diethylnitrosamine (Low dose)+ Ethanolic Pomegranate Peel Extract, HDPE - Diethylnitrosamine (High dose) + Ethanolic Pomegranate Peel Extract.

Table 4: Effect of aqueous and ethanolic pomegranate peel extracts on the lipid profile of diethylnitrosamine induced albino rats.

PARAMETER	CONTROL	LD	HD	LDPA	HDPA	LDPE	HDPE
CHOLESTEROL	70.36±0.14	63.67±0.13	71.74±1.66	72.28*±0.037	93.68^{*c}±1.47	82.54^{*b}±3.11	90.7^{*d}±1.56
TRIGLYCERIDES	48±1.46	62.06*±0.003	71.3*±0.6	66.52*±0.85	53.06^{c}±2.26	82.02^{*b}±0.82	83.1^{*d}±1.67
HDL	28.42±2.212	15.87*±0.628	13.56*±0.583	29.78^{a}±1.052	30.56^{*c}±1.773	30.16^{b}±0.873	25.1^{*d}±0.59

Values are expressed as Mean ± S.E.M of five rats.
C Control, LD - Diethylnitrosamine (Low dose), HD - Diethylnitrosamine (High dose), LDPA - Diethylnitrosamine (Low dose) + Aqueous Pomegranate Peel Extract, HDPA- Diethylnitrosamine (High dose)+ Aqueous Pomegranate Peel extract, LDPE- Diethylnitrosamine (Low dose)+ Ethanolic Pomegranate Peel Extract, HDPE - Diethylnitrosamine (High dose) + Ethanolic Pomegranate Peel Extract.

Tryglyceride

The level of triglycerides can be seen to be significantly increased in all treatment groups, when compared to the control group.

HDL

The level of HDL was significantly reduced in the DEN alone treated low dose and high dose group, while slight changes were observed in the pomegranate extract supplemented groups.

DISCUSSION

Food intake is influenced not only by nutritional status, but also by diverse environmental factors. Reduction in food intake on cinnamon extract treatment has been observed [9] thus leading to decrease in body weight. The increase in the body weight on low dose DEN treatment may be due to initial response to cell injury in the form of inflammation. The decrease in body weight on high dose DEN treatment may be an expression of the severe adverse effect as a result of collective tissue damage in the body, as well as due to reduction in food take. Effectiveness of APPE and EPPE supplementation is more expressed in low dose DEN treatment. *Piper nigum* treatment had no effect on body weight [10].

Regarding testicular weight the structural and functional integrity of reproductive tissues depends on the circulating androgen and small change in androgen content may result in reduction in weight of the reproductive organs. A significant reduction in testis weight was observed in rats, treated with alcoholic extract of *Sapindus emarginctus , Terminaria belerica, Cumim cyminnum* and *Allium cepa* individually [11]. In the present study, significant increase in low dose DEN treated and high dose DEN treated APPE and EPPE supplemented groups is in correlation with body weight increase fungicide was seen to have antiandrogenic effect on spermatogenesis in rat testis [12].

Mancozeb caused histopathalogical changes in gonads of male rats after chronic exposure, which include significant increase in testis and decrease in epididymal weight, degeneration in seminiferous and epithelial tubules with loss of sperm Vinclozolin [13]. Treating animals with metalaxyl caused significant decrease in diameters and germinal epithelial heights of the seminiferous tubules. Co-administration of aqueous extract of ginger improved the histological as well as the histochemical alterations induced by metalaxyl [14].

The degenerative changes in seminiferous tubules in the form of damage to germinal epithelium, degeneration of spermatozoa, highly reduced in intersititium can be observed on low dose treatment, while the disruptive effect of DEN, which is severe at high dose. But APPE and EPPE supplementation do not seen to very much effective at the mentioned dose and duration.

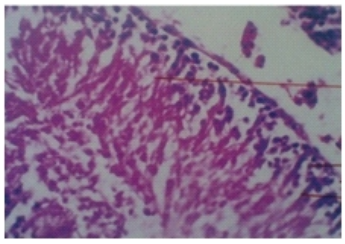
Plant extract as well as other chemical agents cause hormonal imbalance as alkaloids and flavanoids reduce plasma concentrations of LH, estradiol and FSH [15]. Prolactin (PRL) secretion is controlled by the hypothalamus primarily through the release of prolactin inhibiting factor and prolactin releasing factor. Thyrotrophin releasing hormones to stimulate PRL secretion. In the present study the level of prolactin was elevated only in the high and low dose DEN treated groups, while the levels where reduced significantly in all the pomegranate extract supplemented groups.

The observed high levels of prolactin on DEN treatment might be due to the transient infertility conferred by DEN or may be due to primary hyphothyroidism due to increase excretion of TRH [16]. In the present study the level of LH was increased in all the treatment groups, where that of FSH was increased only in low dose DEN treated pomegranate peel extract supplemented groups. But FSH level was decreased in high dose DEN treated APPE supplemented group. The testosterone levels were also increased APPE and EPPE administrated groups as well as low dose DEN treated and supplemented groups. The primary hormonal control on spermatogenesis increases the actions of FSH and testosterone on sertoli cells. FSH act on sertoli cells by increasing the levels of protein synthesis. Testosterone is secreted by Leydig cells and controlled principally by LH. Majority of testosterone is bound to sex hormone binding globulin, but it also exists loosely bound to albumin and in free state [17].

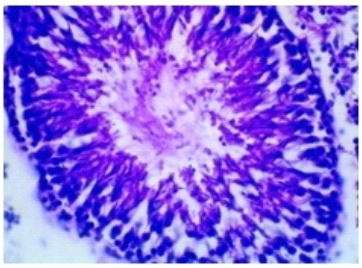
Cortisol is the major glucocorticoid produce and secreted by adrenal cortex. ACTH activates the synthesis and release of cortisol from adrenal cortex. The physiological activity of cortisol depends upon levels of the small fraction of circulating unbound cortisol. Cortisol bound to protein is protected from metabolism by liver [18]. In the present study the level of cortisol seems to be reduced, may be due to the lessening of the activity of adrenal cortex as a result of DEN induction. Perhaps APPE and EPPE

PLATE 1: TESTIS HISTOLOGY

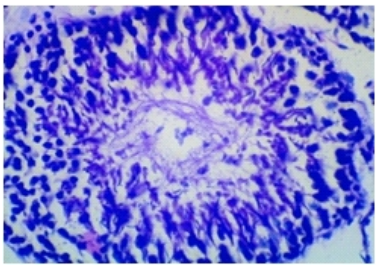
A. Normal Control testis



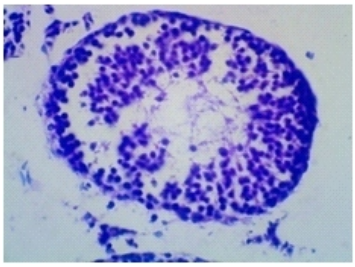
B. Low dose DEN treated Testis



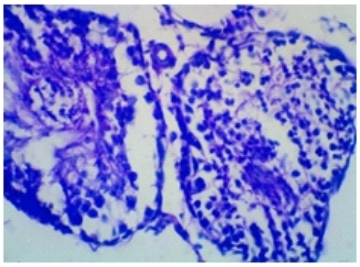
C. High dose DEN treated testis



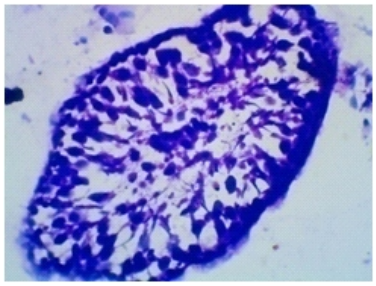
D. Low dose DEN + APPE treated Testis



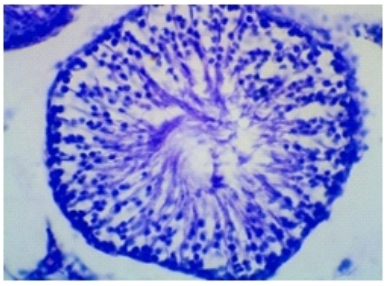
E. High dose DEN + APPE treated Testis



G. Low dose DEN + EPPE treated Testis



H. High dose DEN + EPPE treated Testis



supplementation at the current doses are not effective in amelioneting DEN induction stress.

Significant reduction in serum cholesterol, triglycerides were observed in the study that evaluates the effect of acute and sub acute treatment of *Teucrium polium* decoction [19]. The *Solanum* species reduced plasma total cholesterol, low density lipoprotein, triglyceride and increased high density lipoprotein compared to the test control, thus indicating the possible use of these *Solanum* species in the treatment of diseases associated with hyperlipidemia such as ischaemic heart disease and arteriosclerosis[20]. The dry fruit supplement of *Tetrapleura tetraptera* caused a decrease in the level of total cholesterol, low density lipoproteins, triglycerides and LDL/HDL ratio with increase level of HDL when compared to the control group [21].

The increase in the level of cholesterol and triglycerides in the present study, on APPE and EPPE supplementations and treatment may be an expression of the enhanced nature of activation of lipid synthetic machinery by the extracts. This may in turn, results in increase in androgen synthesis in testis as noticed in the present study.

CONCLUSION

The objective of the present study was to ascertain the efficacy of aqueous and ethanolic extracts of pomegranate peel in alleviation of DEN induced toxicity in the testicular tissue as well as on changes in the lipid and hormonal profile of albino rats. As pomegranate peel contains substantial amounts of

polyphenols such as ellagic acid and gallic acid, these are considered to be useful in the prophylactic action on the oxidative stress arising due to DEN treatment. But the toxicity to testicular tissue and function of hormones and lipid profile does not seem to be fully alleviated in nature, as the testis function was not ably corrected by pomegranate peel extract. Further work is required for the isolation and characterization of individual compounds in peel extract and mechanisms involved to be determined.

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