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Protective role of Pomegranate juice Against Hepatotoxicity and Nephrotoxicity Induced by Haloperidol Drug in Male White Rats.

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Abstract

The aim of this study is to investigate the protective effect of Pomegranate juice against haloperidol – induced hepatotoxicity and nephrotoxicity in male albino rats. In this study we used 30 male white rats and divided them into 3 equal groups. Each experimental group consisted of 10 animals as fallows: **Group1:** Control group: They were given only normal saline for 30 days. **Group2:** haloperidol: animals of this group were given haloperidol orally by gavage at a dose level of 1 mg / kg body weight, every day for 30 days. **Group3:** haloperidol + pomegranate juice (n=10): animals of this group were given haloperidol (1 mg / kg body weight) plus pomegranate juice (2 ml/kg body weight) by gavage, every day for 30 days.

After 30 days, biochemical analysis were conducted to evaluate hepatotoxicity and nephrotoxicity. Serum levels of ALT, AST, ALP, total bilirubin, albumin, total protein, urea, and creatinine were measured. Animals treated with haloperidol alone showed a significant increase in serum levels of of ALT, AST, ALP, total bilirubin, creatinine and urea and significant decrease in serum levels of albumin and total protein compared with control group.

Results of this study showed an improvement in the biochemical parameters of rats treated with combination of pomegranate juice with haloperidol (group3) compared with group2, and these parameters are near the in group1. Therefore pomegranate juice may protect against haloperidol induced hepatotoxicity and nephrotoxicity.

Introduction:

Haloperidol (H) is a neuroleptic drug. It is broadly used for the treatment of schizophrenia, delusions and hallucinations. haloperidol belongs to the butyrophenone series of neuroleptic compounds (1). Haloperidol is a high-affinity dopamine antagonist , Its major site of action is the blockage of D2 receptors, and it has some effect on serotonin receptors (5HT) and (α**1)** receptors (2) . One of the major side effects of haloperidol therapy is the development of extrapyramidal symptoms (EPS) such as tremor, rigidity, akathesia, acute dystonia, neuroleptic malignant syndrome, and late onset tardive dyskinesia (3,4). Experimental studies on rats found that administration of haloperidol leads to the occurrence of oxidative stress by increase MDA and ROS generation and decrease GSH level (5).

Pomegranate (Punica granatum L.) is a juicely fruit, is widely used in the folk medicine of many cultures because of its has antioxidant, antiviral, antidiabetic, antidiarrheal, anti-cancer, antimicrobial and antiinflammatory activities(6), due to its high content of antioxidant of polyphenolic class which includes tannins, flavonoids and anthocynins (7).

Pomegranate juice (PJ) is a good source of pectin, sugars (fructose and glucose), organic acids (oxalic, ascorbic, citric, and tartaric acids), minerals (calcium, iron, and potassium), polyphenolic flavonoids and Vitamins (6). The polyphenols in pomegranate juice including anthocyanins, cstechins ,ellagic tannins, gallic and ellagic acids (8). Pomegranate juice has potent antioxidative activities against lipid peroxidation, and increased serum total antioxidant status due to its polyphenols (9).

The aim of this study was to investigate the protective effects of pomegranate juice against

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haloperidol induced hepatotoxicity and nephrotoxicity in male albino rats.

Materials and Methods

Haloperidol

It was given as orally at dose of 1 mg/kg body weight (10), P.O. A 5 mg tablet (HALDOL-5, SOBHAN PHARM. CO. Iran. B.No. 0063000RLS) was dissolved in distilled water and the dose was given to the rats once daily through stomach tube .

Pomegranate juice preparation

The fresh pomegranate fruits were washed and manually peeled, without separating the seeds. Juice was obtained using a commercial blender, filtered and immediately diluted with distilled water to volume of 1:3 and stored at -20ºC (11) .

Experimental animals:

30 adult male albino rats of the Wistar strain were used in this study, 5–6 months of age and 200–250g body weight. Animals were housed in cages with standard conditions under controlled temperature (24-26 'C) and lighting (12 hours light/12 hours dark). Water and food were available *ad labium*. Rats were acclimatized to the laboratory conditions for seven days prior to the start of experiments.

After the period of acclimation, the animals were randomly divided into three groups. Each experimental group consisted of 10 animals as follows:

Group1: Control group (n=10): They were given only normal saline for 30 days.

Group2: haloperidol (n=10):Animals of this group were given haloperidol orally by gavage at a dose level of 1mg/kg body weight, every day for 30 days.

Group3: haloperidol + pomegranate juice (n=10): Animals of this group were given haloperidol (1mg/kg body weight) plus pomegranate juice (2 ml/kg body weight) by gavage, every day for 30 days.

At the end of the experiments, all animals were sacrificed by ether administration, the blood samples were collected directly by heart puncture technique and left to clot then centrifuged. After centrifugation at 3000 rpm for 15 minutes and then serum samples were separated and stored at -20 ºC .

Biochemical analysis:

The activities of the ALT, AST, ALP, total bilirubin, albumin, total protein, creatinine and urea were determined in serum using commercial kits from Bio Merieux, France.

Statistical analysis:

ANOVA analysis and LSD test were used according to (SPSS version 18) program to find the means for all treatments (12).

Results and Discussion

Our study reported that haloperidol administration at a dose of mg/kg/day for 30 days leads to a significant increase in serum levels of liver function tests consist of ALT, AST, ALP and total bilirubin, and significant decrease in serum levels of albumin and total protein compared with control group.

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Transaminases are the most sensitive indicators of liver cell damage and toxicity because they are present inside the cells and are released into the circulation after cellular damage (13), so elevation of levels of the hepatic enzymes and bilirubin in the serum are the indicators for hepatocellular diseases (14).

haloperidol administration would increase hepatic toxicity, and the elevation of serum AST and AST levels as a result of increased release of the enzyme from damaged hepatocytes into the blood stream (15). Liver dysfunction and toxicity induced by haloperidol administration it may be due to a generation of free radicals (5), these radicals can bind to organic compounds such as lipids, nucleic acids and proteins, thus leads to lipid peroxidation and damage in hepatocytes (16), and subsequently, elevate concentration of serum AST and AST levels and bilirubin.

Haloperidol induced hepatic toxicity mediated through formation of lipid peroxidation leads to increase of oxidants, and decreases in hepatic antioxidants, especially GSH (15). GSH is the major antioxidative tripeptide, which plays an important role in the detoxification of toxicants and metabolism of nutrients (17).

Experimental studies on rats found that administration of haloperidol leads to production of oxidative stress as demonstrated by decrease GSH level and increase MDA and ROS generation (5). Balijepalli *et al* had found that haloperidol was the most potent inhibitor of mitochondrial ubiquinone oxido-reductase (complex 1) of the electron transport chain by the production of toxic metabolites (Pyridinium metabolites RHPP+) that resulting in the formation of free radicals (18).

Albumin and total protein was synthesized in the endoplasmic reticulum of hepatocytes, may be decreased hepatic production due to hepatocellular dysfunction (19) by haloperidol toxicity, that resulting in the a significant decrease in serum levels of albumin and total protein.

The results of this study showed significant increase ($p<0.05$) in the serum levels of both creatinine and urea of rats treated with haloperidol (group 2) compared to the corresponding levels in the control group, that means giving of haloperidol caused renal dysfunction.

Urea and creatinine are metabolic waste products of protein metabolism that are freely excreted in the urine via glomerular filtration in the kidneys (20), therefore such increase of serum creatinine and urea as reported in this study confirm an indication of functional damage of the kidney.

The basis of haloperidol toxicity is the generation of free radicals and formation of reactive oxygen species that resulting in the formation of lipid peroxides, with further damage to the membrane, cellular protein, alter cellular function and leading to induce oxidative stress (5).

When ROS are generated as a consequence to different cell components injury as DNA, RNA, proteins and lipids is possibly involved in the pathophysiology of renal diseases, renal failure, renal interstitial fibrosis and nephropathy (21). Uyanik *et al* had found that there is a significant relationship between haloperidol treatment and structural changes in kidneys such as tubular deformations, dilatation of the renal tubules, and glomerular basal membrane thickening. Thus, these structural abnormalities may cause renal injury (22).

Results of this study showed an improvement in the serum biochemical parameters levels of rats treated with combination of pomegranate juice with haloperidol (group3) compared with group2, and these levels are near the levels in group1, attributed to the free-radical scavenging properties of the pomegranate juice. These results are in agreement with results of other study which showed that Pomegranate juice has protective effect against the damage in liver and kidney cells from oxidative stress induced by lead in rats (23). The antioxidant effects of Pomegranate juice was attributed to its wide range of polyphenols, including anthocyanins, ellagitannins, and organic acid such as ascorbic acid and enzymes (glucose oxidase, catalase and peroxidase) which responsible for protective and antioxidative effects and reduces oxidative stress (24), this effect may lead to repairing damage in tissue and cells, and respectively, improvement in the serum biochemical parameters levels.

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Table (1). The Effect of pomegranate juice on the serum biochemical parameters levels of rats treated with haloperidol.

Groups Parameters	G1	G2	G3
ALT (U/L)	30.3 ± 0.31 b	65.4 ± 0.44 a	31.2 ± 0.21 b
AST (U/L)	42.5 ± 0.9 b	75.2 ± 0.8 a	43.1 ± 0.1 b
ALP (U/L)	92.6 ± 0.05 b	143 ± 0.02 a	94.2 ± 0.07 b
Bilirubin (µmol /L)	3.9 ± 0.3 b	14.5 ± 0.6 a	4.7 ± 0.8 b
Albumin (g/L)	35.8 ± 0.11 a	20.7 ± 0.15 b	34.1 ± 0.21 a
Total protein (g/L)	66.2 ± 0.03 a	41.5 ± 0.02 b	65.7 ± 0.07 a
Creatinine (µmol/L)	45.8 ± 0.9 b	69.9 ± 0.5 a	47.1 ± 0.6 b
Urea (µmol/L)	6.3 ± 0.4 b	16 ± 0.2 a	7.2 ± 0.1 b

values represent means ± SE.
Similar Letters represent no significant differences between the groups at ($P<0.05$).
Different letters represent significant differences between the groups at ($P<0.05$).

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