Journal of Environmental Biology **January 2008,** 29(1) 73-77 (2008)

Protective effects of *Peganum harmala* extracts on thiourea-induced diseases in adult male rat

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(Received: March 26, 2006 ; Revised received: April 23, 2007 ; Accepted: July 10, 2007)

Abstract: *Cancers and hepatoprotective prevention using traditional medicines have attracted increasing interest. The aim of our study was to characterize the putative protective effects of ethanol and chloroform extracts of Peganum harmala on thiourea-induced diseases in adult male rat. We seek to determine the effects of these plant extracts on body weight, thyroid and endocrine cancer parameters. In addition the putative hepatoprotective effect was checked by the determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and the bilirubin level in the blood. Our data show that ethanol and chloroform extracts of Peganum harmala protected the animal against the carcinogenic effects induced by thiourea since neuron-specific enolase (NSE) and thyroglobulin (TG) levels were back to the normal range. In addition, the observed-hepatocytotoxicity after thiourea treatment was greatly reduced (AST and ALT activities were respectively 270 IU/l and 60 IU/l and in the same order of magnitude as in the untreated rats) as well as the bilirubin levels (6µmol/l) especially for animals receiving the choroform preparation. Therefore we may suggest that extracts of Peganum harmala are efficient to reduce the toxicity induced by thiourea in male rat as far as the above parameters are concerned.*

Key words: *Rat, Peganum harmala, Thiourea, Cancer, Hepatotoxicity*
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Introduction

Among the leading causes of death in the world are liver diseases and cancers. Epidemiological studies have strongly suggested that diet plays an important role in the prevention of chronic diseases (Bauman, 2004; Parillo and Riccardi, 2004). Polyphenolics commonly found in fruits, vegetables and grains, provide chemoprotective effects to neutralize oxidative stress in the body leading to maintain balance between oxidants and antioxidants and therefore they help to improve human health (Adom and Liu, 2002; Wu *et al.*, 2003). An imbalance caused by an excess of oxidants leads to oxidative stress, resulting in damage to DNA and protein and an increase risk of degenerative diseases such as cancer (Farombi *et al.*, 2004; Moller and Loft, 2004; van Meeteren *et al.*, 2004) and liver diseases (Jourdana *et al.*, 2004). Indeed injury to the liver induced by hepatotoxic agents induces serious damages (Shahani, 1999) leading to reduced elimination of both capacity-limited and flow-limited drugs (Wynne *et al.*, 1989), aging and death. Consumption of fruits and vegetables has been associated with reduced risks of coronary heart disease (Srinath and Katan, 2004), of chronic obstructive pulmonary disease (Liu *et al.*, 2004) and with a protective effect against different types of cancer, including breast and ovarian cancers (Khanzode *et al.*, 2004), and colon cancer (McCullough *et al.*, 2003). The liver is a very important target, which has a great capacity to remove toxic substances and to synthesize vital molecules; therefore, any damage to the liver inflicted by hepatotoxic agents would be very deleterious.

Peganum harmala L. (Zygophyllaceae) was first found in dry area of central Asia and southern USA (Sobhani *et al.*, 2000; Lamchouri *et al.*, 2000). The plant has a wide spectrum of pharmacological actions as for example monoamine oxidase inhibition (Adell *et al.*, 1996), binding to benzodiazepin receptors (Baum *et al.*, 1996) and antioxidative action (Tse *et al.*, 1991). Moreover *Peganum harmala* was shown to be concerned on cardiovascular actions (Aarons *et al.*, 1977), and DNA topoisomerase inhibition in cancerous cell-lines (Yamada *et al.*, 2006) but has never been studied in animal model. Therefore our aim was to analyze the biological activity of ethanol and chloroform extracts of *Peganum Harmala* seeds on body weight, endocrine and thyroid cancer parameters and to seek for some hepatoprotective role in rats treated with thiourea, a chemical good used in industry and known by its carcinogenic and hepatocytotoxic effects (Rob *et al.,* 2004; Chhabra *et al.*, 1992).

Materials and Methods

Animals and treatments: Adult male Wistar rats aged of two months (105 ± 2 g body weight), were bred in the animal house of the general pharmacy (Sfax, Tunisie). All animals were kept in well-ventilated cages with a light-dark cycle of 12 hr, temperature of 24 ± 4°C; diet and water were provided *ad libitum*. The animals were starved 24 hr before treatment. Induction of cancers and hepatotoxicity were obtained by gavage methods with thiourea at a dose of 0.3 mg/day/Kg body weight. Rats were divided into four groups (6 animals /group) : negative control (T-) rats drink water *ad libitum*; positif

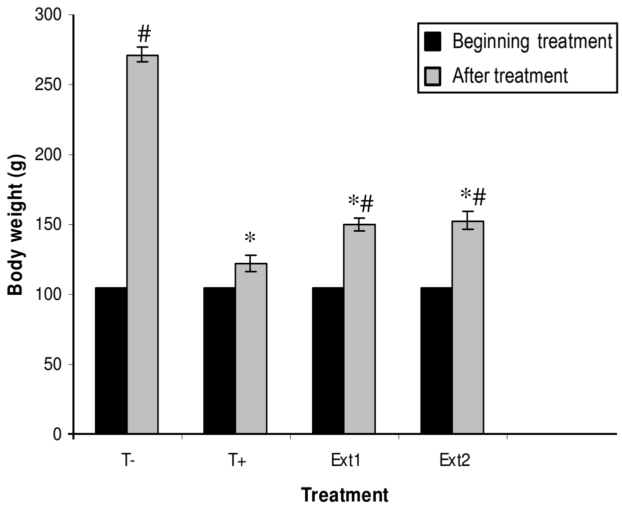

Fig. - 1: Body weights of rats after 78 days of treatment in control negative group (T-), thiourea positive group (T+), thiourea-treated rats and feeded with *Peganum harmala* either as ethanol extract (Ext. 1) or chloroform extract (Ext. 2), Values are given as means ± standard deviation (n=6 rats per group) * = compared to T (-) group, # = compared to T (+) group

control (T+) rats were given thiourea, and the two other groups received thiourea and *Peganum harmala* included in food at a concentration of 2% either as ethanol extract (Ext 1) or chloroform extract (Ext 2) as described by Debersaca *et al.* (2001). After 78 days of treatment, the animals were weighed, decapitated and the arterio-venous blood was collected, then centrifuged at 1000 g, 4°C. The tumor parameters of the neuroendocrine system (NSE) and thyroid (TG), the hepatic enzyme activities (AST and ALT) and the bilirubin levels were determined in the serum.

Preparation of ethanol and chloroform extracts: The air-dried and finely ground samples of *Peganum harmala* were extracted by using a protocol published by Itharat *et al.* (2004) and modified in our laboratory. Briefly, 100 g of plant samples were extracted in a Soxhlet either with 1liter ethanol (Ext 1) or 1liter chloroform (Ext 2) at 50°C for 30 min. After filtration on a Whatman paper and evaporation at 50°C in a Soxhlet apparatus, the extract was kept at 4°C. Everyday, the extract was mixed with the food according the method described by Debersaca *et al.* (2001).

Tumor parameters :

Neuron-specific enolase (NSE) level: The neuron-specific enolase is a glycolytic enzyme recognized as a valuable tumor marker for cancers of neuroendocrine type such as neuroblastoma (Viallard *et al.*, 1988), melanoma (Lorenz *et al.*, 1989) or seminoma (Fossa *et al.*, 1992). NSE was further shown to be released into the cerebrospinal fluid and blood as a result of cerebral injury (Martens *et al.*, 1998). The determinations of NSE levels were performed using a commercial kit (Elisa NSE kit -CIS Biointernational, Gif sur Yvette, France).

Thyroglobulin (TG) level: The TG assay has been recommended as an important marker for thyroid cancer (KePing *et al.*, 1995). The serum thyroglobulin level was evaluated using a specific kit (Elecsys®TG, Roche Diagnoses, France).

Liver cytotoxicity parameters :

Bilirubin level: Bilirubin results mainly from the hepatic catabolism of cytochromes and from the stem red cells destruction in the spinal cord. The bilirubin reacts directly with the sulphonic acid diazotized in an acid plug to form the azobilirubine coloured in red (Molly and Evelyn, 1937).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities: The determination of the ALT activity was realized by photometry in presence of an optimized concentration of substrate according to the method described by Karmen (1955). Similarly the determination of AST was performed by photometry following the technique described by Wroblewski and Ladue (1956).

Statistical analysis: The data are presented as means ± SEM. The comparisons of data were carried out between the control group (T-), thiourea-treated rats (group T+) and rats receiving at the same time thiourea and *Peganum harmala* ethanol extract (Ext 1) or thiourea and *Peganum harmala* extract (Ext 2) in food. The statistical evaluation of the data was achieved by using the Student's t-test. A difference was considered significant at $p \leq 0.05$ (SigmaStat for Windows, Version 3.1; SPSS Inc. Chicago, Illinois, USA).

Results and Discussion

Body weights (Fig. 1): From these data a clear negative effect of thiourea on body growth was observed. Compared to the control rats (T-), a 55% ($p<0.05$) decrease of body weight in the rats feeded with thiourea (T+) was recorded; for the rats receiving the plant extracts the diminutions were significantly less important: 44 and 43%, respectively for Ext 1 and Ext 2. In fact the body weight of thiourea treated-rats feeded with either Ext 1 or Ext 2 was 23 and 25% higher, respectively than in control treated rats (T+).

Cancers parameters (Fig. 2A, 2B) :

NSE (marker of neuroendocrine cancers): In the control group (T-), the basal level of NSE was 3.2 ng/ml but in the treated rats (T+) receiving thiourea, the value was increased more than 4 fold ($p<0.01$). In rats simultaneously treated with thiourea and the extract of plant, a highly significant decrease of the NSE levels of 350% (Ext 1) and 300% (Ext 2) compared to the group thiourea-treated group (T+) was observed. In addition if one compared to the negative controls (T-) the adverse effects of thiourea on NSE levels were not significant for the rats exposed to Ext 1 but slighltly increased in the Ext 2 group (51%).

TG (marker of thyroid cancer): Compared to the negative control group (T-) in the treated rats (T+) a significant increase 44% ($p<0.05$) of TG level was recorded. Conversely whatever the plant extract, a

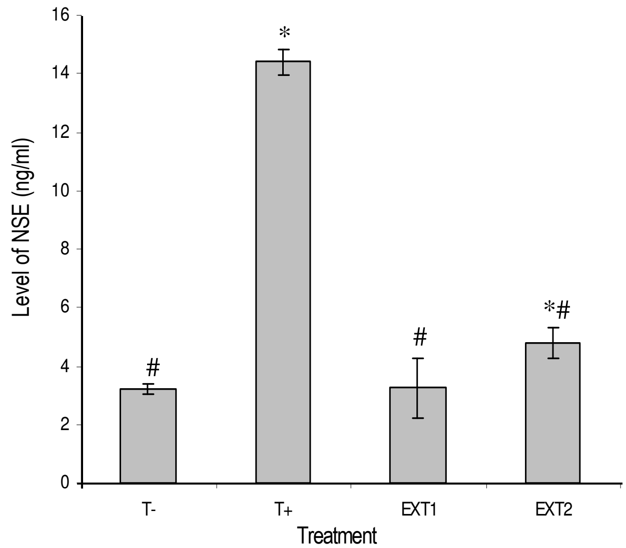

Fig. 2-A: Serum concentrations of NSE after 78 days of treatment

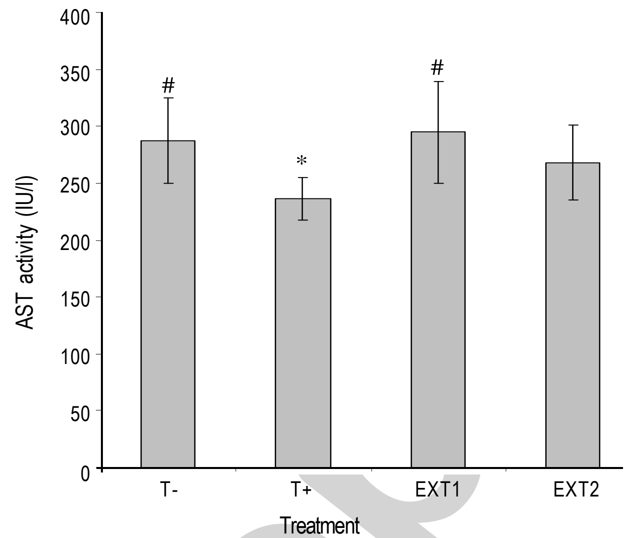

Fig. 3-A: Serum AST activity after 78 days treatment

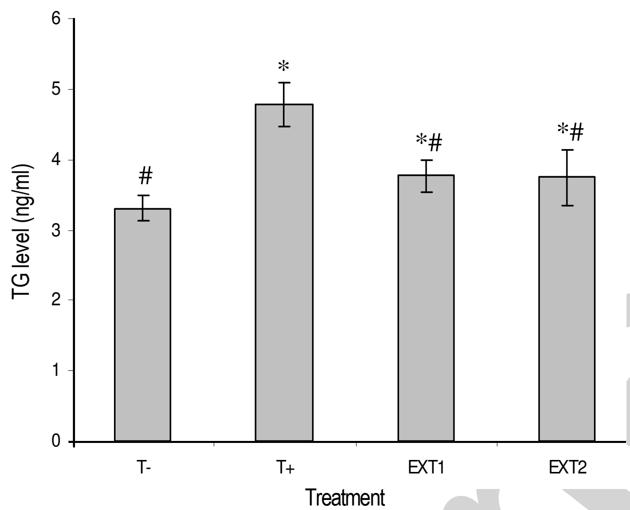

Fig. 2-B: Serum concentrations of TG. Same details as in Fig. 1 legend. Values are given as means ± standard deviation (n=6 rats per group) * = compared to T (-) group, # = compared to T (+) group

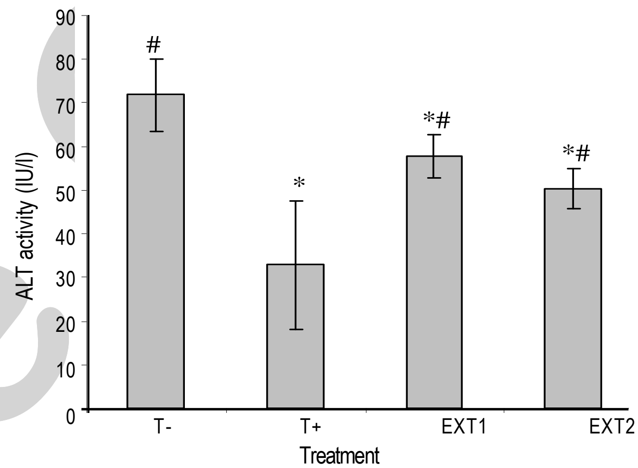

Fig. 3-B: Serum ALT activity. Same details as in Fig. 1 legend. Values are given as means ± standard deviation (n=6 rats per group) * = compared to T (-) group, # = compared to T (+) group

significant reduction (21%) of the blood TG levels was obtained when comapred to thiourea-treated rats. It is noteworthy that the TG augmentations were also significant ($p<0.05$) when compared to the group (T-).

Liver cytotoxicity parameters (Fig. 3A and 3B) :

Aspartate aminotransaminase activity (AST): In the control rats (T -), the mean AST activity was 287 IU/l. For the rats treated by thiourea, one noticed a reduction of 18% of AST. In animals feeded with thiourea and the plant extracts, an increase in the AST activities was observed: 25 and 14%, respectively for Ext 1 and Ext 2 when compared to the (T+) rats. In the plant extract feeded groups the AST activity was of the same magnitude as that of the negative control (T-).

Alanine aminotransaminase activity (ALT): The level of ALT was 71.8 IU/l in the control group and a 54% decrease ($p<0.05$) of ALT was registered in the thiourea-treated rats (T+). In the rats receiving plant extracts the ALT activity was significantly increased of 76% for Ext 1 and of 53% for Ext 2, compared to T+. Nevertheless the ALT levels were still lower (20 and 30%, respectively for Ext 1 and Ext 2) when compared to (T-) rats.

Leves of bilirubin (Fig.4): The bilirubin level was 6.2 µmol/l in the control group and in rats treated by thiourea a significant increase by +48% was observed. For the rats receiving thiourea and plant extracts, the bilirubin level was not modified with Ext 1; conversely a 35% diminution ($p<0.05$) was recorded in presence of Ext 2 when

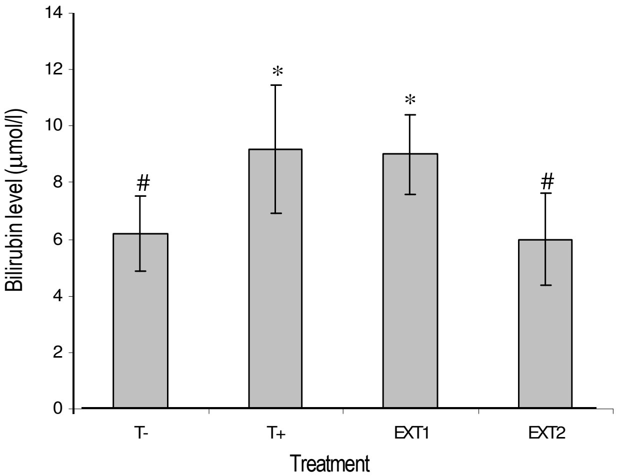

Fig. 4: Serum concentration of bilirubin in rats after 78 days of various treatments as detailed in Fig. 1 legend. Values are given as means ± standard deviation (n=6 rats per group) * = compared to T (-) group, # = compared to T (+) group

compared to T+. In that latest group the bilirubin level was identical to that of control untreated animals.

Several studies have shown that the prolonged administration of thiourea to rats induces thyroid neoplasms and related lesions such as thyroid solid-cell adenomas and either hyperplasic or simple goiter as demonstrated by a higher incidence of follicular cell neoplasms (Frakes, 1988; Weisburger *et al.,* 1981). The thiourea-intoxication is also characterized by cancers and hepatic syndrome of cytolysis (Kim *et al.*, 1999; Takegawa *et al.*, 1997). The toxicity of thiourea depends on its reductive metabolism which generates reactive free radicals, leading to tumors and necrosis (Kim *et al.*, 1999). The reactive free radicals of thiourea induce lipidic peroxidation of membranes, enzymatic inhibition and covalently bind to the cellular macromolecules. The disturbance of cellular calcium homeostasis related to the lipidic peroxidation represents the irreversible stage of the process which leads to the necrosis of hepatocytes by either karyolysis or acidophilic necrosis. A red or yellow liver atrophy is characteristic of liver thiourea intoxication (Krieter *et al.*, 1984).

In medicine, plants are widely used for the treatment of numerous diseases. The extracts from *Peganum harmala* are very potent antitumors in cultured cancer cell lines but have never been studied in animals. The main bioeffective molecules from that plant are beta-carboline alkaloids such as harmaline, harmine and harman specific inhibitors of cyclin-dependent kinases (Owen *et al.*, 2000; Li *et al.*, 1995). Our study was set up to determine if *Peganum harmala* extracts could reverse the carcinogenic effects induced by a thiourea treatment of 78 days in adult rat especially on the neuroendocrine system and on the thyroid tissue in one hand, and on the hepatocytoxicity on the other hand. From the two *Peganum harmala* extracts prepared we have cleary shown a protective role against the adverse effects induced by thiourea *i.e.* a recovery of the liver function, and also a decrease of NSE and TG levels. These observations are likely related to the richness of this plant in beta-carbolines known to exert antitumor activities on cultured cancer cell lines (Sakakibara *et al.*, 2003; Yan *et al.*, 2001). These beta-carbolines are potent and specific inhibitors of cyclin-dependent kinases in cell cultures (Pan *et al.*, 1997; Li *et al.*, 1995).

Concerning the protective effects against the thiourea-induced liver cytotoxicity, both extracts appear efficient by maintaining ALT and AST activities when compared to the thiourea-treated rats (T+); that is probably related to the richness of this plant in substances of phenolic nature which may decrease the free-radical lipid peroxidation level leading to the stabilisation of membrane structures. Moreover an increase of the cytochrome P450 content in the microsomal fraction which induces an enhancement of the detoxicative function of the liver and accelerates elimination of metabolic products originating from thiourea treatment may be evoked and indeed, in that process a protective effect of polyphenols has been reported by Lima *et al.* (2005).

In traditional medicine *Peganum harmala* is used to curate various diseases, such as cancers, cerebral insufficiency and mental pathology. Flavonoids are phenolic compounds found in *Peganum harmala*; these substances in the diet are powerful antioxydant by scavenging the superoxide anion (Husain *et al.*, 1987), singlet oxygen (Torel *et al.*, 1986), lipid peroxy radicals (Hyuncheol *et al.*, 2004) and by stabilizing free radicals involved in oxidative processes through either hydrogenation or complexing with oxidizing species (Ji-Young, 2004). The role of antioxidants in preventing oxygen radical- and hydrogen peroxide-induced cytotoxicity and tissue damage in various human diseases is becoming increasingly recognized especially during aging process (Barouki, 2006). The importance of these plant antioxidants in the maintenance of health and protection against heart diseases and cancers is raising more and more interest among scientists, food manufacturers and consumer trends.

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