AMERICAN JOURNAL OF RESEARCH IN MEDICAL SCIENCES, 2018
VOL 3, NO. 2, PAGE 48–53
10.5455/ajrms.20180107060815

eJManager

ORIGINAL ARTICLE

Open Access

Evaluation of hepatoprotective activity of *Allium sativum* ethanolic extract in thioacetamide-induced hepatotoxicity in albino Wistar rats

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ABSTRACT

Aim: The present study was designed to evaluate the hepatoprotective effect of ethanolic extract of *Allium sativum* (EEAS) on thioacetamide (TAA)-induced hepatotoxicity in rats.

Methods: Male Wistar rats were administered 200 and 400 mg/kg/p.o. of EEAS for 21 days and simultaneously administered TAA 50 mg/kg/s.c. 1 hour after the respective assigned treatments for every 72 hours. At the end of experimental methods, all the animals were sacrificed by cervical decapitation. Blood samples were collected. Serum was separated and analyzed for various parameters like aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin using commercially available test kits.

Results: Results indicate that EEAS administration blunt TAA-induced increase in activities of different marker enzymes of hepatocellular injury, *viz*., AST, ALT, ALP, and total bilirubin significantly ($p < 0.001$), suggesting that EEAS possibly has a protective influence against TAA-induced hepatocellular injury and degenerative changes.

ARTICLE HISTORY

Received January 07, 2018
Accepted July 15, 2018
Published July 24, 2018

KEYWORDS

Allium sativum; thioacetamide; hepatoprotective activity

Introduction

The liver is the largest glandular organ in the body and performs multiple critical functions to keep the body free from toxins and harmful substances. The liver synthesizes, concentrates, and secretes bile acids and excretes other toxicants, such as bilirubin [1]. In addition to producing bile, the liver also plays a number of important roles, *viz*., it detoxifies the blood to get rid of harmful substances such as alcohol and drugs, stores some vitamins and iron, stores the sugar glucose, converts stored sugar to functional sugar when the body's sugar (glucose) levels fall below normal, breaks down hemoglobin as well as insulin and other hormones, converts ammonia to urea, which is vital in metabolism, and destroys old red blood cells [2]. Hepatotoxicity implies chemical-driven liver damage that causes acute and chronic liver diseases. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market [3]. Hepatotoxicity and drug-induced liver injury also account for a substantial number of compound failures, highlighting the need for drug screening assays, such as stem cell-derived hepatocyte-like cells, that are capable of detecting toxicity early in the drug development process [4,5].

Garlic (*Allium sativum*) is used worldwide as a food additive, spice, and medicine [6]. *Allium sativum*, a member of the Liliaceae family, is a common food for flavor and spice and it is one of the herbs most commonly used in modern folkloric medicine. Garlic was an important medicine to the ancient Egyptians as listed in the medical text Codex Ebers (ca. 1550 BC) especially for the working class involved in heavy labor because it was an effective

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remedy for many ailments such as heart problems, headache, bites, worms, and tumors. *Allium sativum* also reported for its several pharmacological activities such as anti-diabetic and hypolipidemic activity [7], anticancer activity [8], antioxidant activity [9], antihypertensive activity [10], *in vitro* anti-fungal activity [11], and antiplatelet activity [12]. The present study was designed to evaluate the hepatoprotective effect of aqueous extract of *A. sativum* on thioacetamide (TAA)-induced hepatotoxicity in rats.

Methods

Collection and authentication of plant material

The *A. sativum* plant material (raw bulblets) was collected from the local market of Hyderabad, Telangana and was authenticated by Ms. D. Kavitha, Asst. Professor, Department of Botany, Osmania University, Hyderabad.

Preparation of plant extract

The raw bulblets of the *A. sativum* were cleaned, sliced, crushed into small pieces, and dried and then, the dried pieces were pulverized into coarse powder. The coarse powder was prepared by mechanical grinding. The powdered material (100 g) was subjected to continuous hot extraction in a Soxhlet apparatus at a temperature of 50°C–60°C by using ethanol as a solvent. The solvent was evaporated at room temperature to obtain a viscous mass. It was filtered, and the solvent was removed. The dried molten mass was brown in color and was stored in a desiccator until use.

Preliminary phytochemical analysis

The ethanolic extract of *A. sativum* (EEAS) was subjected to preliminary phytochemical analysis and EEAS showed that the plant contains flavanoids, terpenes, steroids, glycosides tannins and the absence of proteins, sterols, phenols, glycosides, saponins, and terpenes. The extract was suspended in distilled water using 1% sodium carboxymethyl cellulose (SCMC) as a suspending agent for oral administration to animals.

Experimental animal

A total of 30 male Wistar strain rats weighing 150–220 g, were procured from the Sainath Agencies [Reg.no. 282/99/ Committee for the Purpose of Control And Supervision of Experiments on Animals (CPCSEA)] Hyderabad, Telangana and were housed at CPCSEA approved animal house of School of Pharmacy, Nalla Narasimha Reddy Education Society's Group of Institutions, Hyderabad. The animals were kept in polypropylene cages (six in each cage) under standard laboratory condition (12 hours light and 12 hours dark cycle) and had free access to commercial pellet diet (Hindustan Lever Ltd., Bombay, India) with water *ad libitum*. The animal house temperature was maintained at 25°C ± 2°C with relative humidity at (50% ± 15%). The study was approved by the Institutional Animal Ethical Committee of Nalla Narasimha Reddy School of Pharmacy (002/ Institutional Animal Ethics Committee (IAEC)/ NNRG/2017). Ethical norms were strictly followed during all experiments.

Hepatoprotective activity

Wistar rats were divided into five groups, each group consisting of six animals. Group I served as control group received the 1% SCMC (2 ml/kg). Group II served as disease control received TAA 50 mg/kg/s.c., for every 72 hours for 21 days. Group III received standard drug (silymarin 50 mg/kg p.o.) for 21 days and simultaneously administered TAA 50 mg/kg/s.c. 1 hour after the respective assigned treatments for every 72 hours. Groups IV and V treated with EEAS 200 mg/kg/p.o., 400 mg/kg/p.o. for 21 days and simultaneously administered TAA 50 mg/kg/s.c. for every 72 hours (Table 1). At the end of the experimental period, all the animals were sacrificed by cervical decapitation. Blood samples were collected and allowed to clot. Serum was separated by centrifuging at 2,500 rpm for 15 minutes and analyzed for various biochemical parameters [13].

Assessment of liver function

Biochemical parameters such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin were analyzed using commercially available test kits (Robonik India Pvt. Ltd.). The liver was removed, weighed, and morphological changes were observed. A portion of the liver was fixed in 10% formalin for histopathological studies.

Table 1. Grouping of animals.

S. no	Group	Treatment	Dose and route
1	I	Control (vehicle)	Feed and water *ad libitum*
2	II	Disease control (TAA)	50 mg/kg s.c.
3	III	TAA followed by silymarin	50 mg/kg p.o.
4	IV	TAA followed by EEAS	200 mg/kg p.o.
5	V	TAA followed by EEAS	400 mg/kg p.o.

Aspartate transaminase

AST levels in serum were estimated using glutamate oxaloacetate transaminase (GOT)/AST test kit using the International Federation of Clinical Chemistry (IFCC) method without pyridoxal phosphate. GOT also known as Aspartate aminotransferase is a transaminase, GOT catalyzes the transfer of the amino group of L-aspartate to α-ketoglutarate to give L-glutamate. GOT is widely distributed in the body, but the highest levels are found in heart, liver, skeletal muscles, and kidneys. Damages to cells of these tissues induce GOT activity increase in serum. The activity was measured at 340 nm [14–16].

$$\text{L-Aspartate} + \alpha\text{-ketogluutarate} \xrightarrow{\text{GOT}} \text{Oxaloacetate} + \text{L-Glutamate}$$

$$\text{Oxaloacetate} + \text{NAD} + \text{H}^{+} \xrightarrow{\text{MDH}} \text{L-Malate} + \text{NAD}^{+}$$

Alanine transaminase

ALT levels in serum were estimated using an ALT test kit using the IFCC method. Glutamate-pyruvate transaminase (GPT) also known as ALT is a transaminase. GPT catalyzes the transfer of the amino group of L-alanine to α-ketoglutarate to give L-glutamate. The highest levels were found in the liver and the kidneys, and in smaller amounts in heart and skeletal muscle. GPT activity is increased when hepatic cells are damaged (liver cells necroses or injury of any cause). The levels were measured at 340 nm [16–18].

$$\text{L-Alanine} + \alpha\text{-ketogluutarate} \xrightarrow{\text{GPT}} \text{Pyruvate} + \text{L-glutame}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^{+} \xrightarrow{\text{LDH}} \text{L-Lactate} + \text{NAD}^{+}$$

Alkaline phosphatase

ALP levels in serum were estimated using the ALT test kit using the IFCC method. ALP is an enzyme of the hydrolase class of enzymes and acts in an alkaline medium. It is found in high activity on the liver, biliary tract epithelium, and in the bones. Normal levels are age-dependent and increase during bone development. Increased levels are associated mainly with liver and bone diseases, hepatotoxicity caused by drugs are osteomalacia, as well as obstruction. p-nitro phenyl phosphate is converted to nitro phenol and phosphate by ALP. The rate of formation of p-nitro phenol is measured as an increase in absorbance which is proportional to ALP activity in the sample and measured at 405 nm [15,16].

$$\text{p-nitro phenyl phosphate} \xrightarrow{\text{ALP}} \text{p-nitro phenol} + \text{Phosphate}$$

Total bilirubin

Total bilirubin levels were estimated using total bilirubin test kit using a modified Jendrassik-Grof method. Total bilirubin is the sum of unconjugated bilirubin and conjugated fractions. Bilirubin is elevated in the hemolysis or lysis of increased breakdown of red blood cells, hepatitis, and cirrhosis azo abstraction of the biliary tract e.g., gallstone. In the determination of total bilirubin, bilirubin is coupled with diazotized sulphanilic acid in the presence of caffeine benzoate solution to produce azobilirubin which has a maximum absorbance at 546 nm. Direct bilirubin presence of diazotized sulphanilic acid forms a red colored azo compound in an acidic medium which has a maximum absorbance at 546 nm [15,16,19].

Histopathology of liver

Livers of sacrificed animals were identified and carefully dissected out for histopathological studies. After rinsing in normal saline, sections were taken from each harvested liver. The tissue was fixed in 10% formal saline, dehydrated with 100% ethanol solution, and embedded in paraffin. It was then processed into 4–5 m thick sections stained with hematoxylin-eosin and observed under a photomicroscope (magnification power—×40).

Statistical analysis

Graph pad prism software, version 6.0 was used in the statistical analysis of experimental data. The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnet's multiple comparison test. p values $p < 0.0001$, $p < 0.01$, and $p < 0.05$ were considered as significant.

Results

Effect of EEAS on body weights and liver weights against thioacetamide-induced animals

A significant ($p < 0.001$) decrease in body weight was observed in TAA-induced Group II animals (disease control group) when compared with the control group, silymarin (50 mg/kg) treated and EEAS (200 and 400 mg/kg) treated animals showed a significant ($p < 0.01$) dose-dependent maintenance in bodyweight when compared with the control group animals. A significant ($p < 0.001$) increase in the liver weights was observed in disease control group animals when compared with the control group animals whereas animals treated with silymarin and EEAS (200 and 400 mg/kg) showed a significant ($p < 0.001$) protection as compared with the disease control group (Tables 2 and 3).

Table 2. Effect of EEAS on body weights against TAA-induced animals.

S. no	Groups	Treatment	Dose	Before treatment (g)	After treatment (g)
1	I	Normal	-	215.20 ± 1.23	218.7 ± 2.171
2	II	TAA + vehicle	50 mg/kg/s.c.	215.16 ± 1.28	201.7 ± 7.032***
3	III	Silymarin	50 mg/kg/p.o.	219.12 ± 2.86	218.3 ± 9.18**
4	IV	EEAS	200 mg/kg p.o.	215.26 ± 2.56	211.84 ± 6.24**
5	V	EEAS	400 mg/kg p.o.	219.62± 3.12	217.12 ± 5.57**

Values are expressed as mean ± standard error of the mean of six animals.
Statistical significance test for comparisons was done by one-way ANOVA, followed by "Dunnet's multiple comparison test."
Comparisons were done between (a) Goup I *vs* Group II and (b) Group II *vs* Groups III–V. $^{**}p < 0.01$, $^{***}p < 0.001$, ns = non-significant.

Table 3. Effect of EEAS on liver weights against TAA-induced animals.

S. no	Groups	Treatment	Dose	Liver weights (g)
1	I	Normal	-	6.825 ± 0.066
2	II	TAA + vehicle	50 mg/kg/s.c.	9.633 ± 0.121***
3	III	Silymarin	50 mg/kg/p.o.	6.90 ± 0.061***
4	IV	EEAS	200 mg/kg p.o.	8.99 ± 1.085***
5	V	EEAS	400 mg/kg p.o.	7.55 ± 0.086***

Values are expressed as mean ± standard error of the mean of six animals.
Statistical significance test for comparisons was done by one-way ANOVA, followed by "Dunnet's multiple comparison test."
Comparisons were done between (a) Goup I *vs* Group II and (b) Group II *vs* Groups III–V. $^{**}p < 0.01$, $^{***}p < 0.001$, ns = non-significant.

Effect of EEAS on biochemical parameters against thioacetamide-induced animals

The liver function test was carried out by measuring the serum biochemical parameters and was observed that there is the significant elevation of AST, ALP, ALT, and total bilirubin levels in the disease control group ($p < 0.001$). This is clearly indicating that the incidence of liver damage is due to TAA administration. Administration of EEAS at the doses 200 and 400 mg/kg and standard drug silymarin (50 mg/kg) remarkably prevented TAA-induced hepatic damage in a dose-dependent manner and comparatively significant as control group animals (Table 4).

Histopathological studies

Figure 1 discusses the histological examination of the liver. The control group shows the maintenance of normal lobular architecture and normal histology. In the group treated with TAA, the portal tract showed small aggregates of chronic inflammatory cells. In the group treated with standard drug silymarin, insignificant or no portal inflammation or hepatocellular damage were seen. In the group treated with EEAS 200 and 400 mg/kg, the portal tract showed dose-dependent observations such as a small aggregate of chronic inflammatory cells and moderate portal inflammation, which confirms the severe hepatic damage in the group treated with the TAA and protective effect of the ethanolic extract.

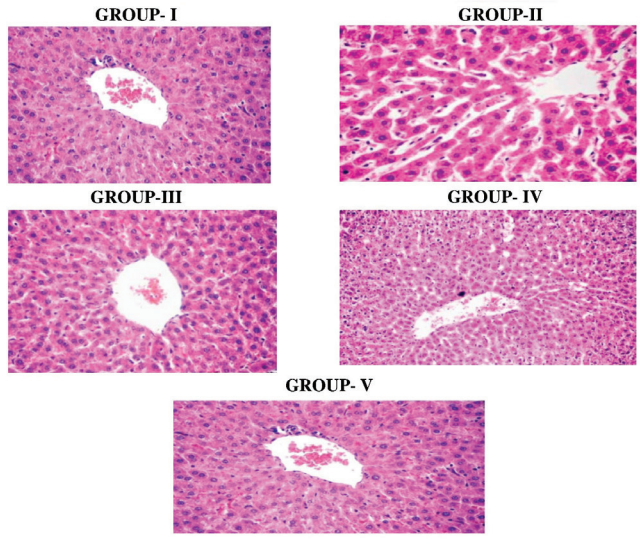

Figure 1. Histopathological studies of Liver.

Discussion

Liver is one of the vital organs of the animal body and plays a central role in transforming and clearing the chemicals, but it is susceptible to the toxicity from these agents. Certain medicinal agents, like paracetamol, when taken in overdoses or sometimes even within therapeutic ranges, may damage the liver [13]. Liver diseases which are still a global health problem may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory diseases), and cirrhosis (degenerative disorder resulting in liver fibrosis). Unfortunately, treatments of choice for liver diseases are controversial because the conventional or synthetic drugs for the treatment of these diseases are insufficient and sometimes cause serious side effects

Table 4. Effect of EEAS on ALT levels against TAA-induced animals.

S. no	Groups	Treatment	Dose	ALT (U/l)	AST (U/l)	ALP (U/l)	Total bilirubin (mg%)
1	I	Normal	-	76.050 ± 0.090	147.54 ± 0.32	207.54 ± 1.32	0.28± 0.24
2	II	TAA + vehicle	50 mg/kg/s.c.	245.388 ± 0.2642**	284.963 ± 2.65**	484.963 ± 2.65**	4.76 ± 0.45**
3	III	Silymarin	50 mg/kg/p.o.	114.798 ± 0.131***	172.80 ± 0.32**	199.80 ± 1.32***	0.56 ± 0.36**
4	IV	EEAS	200 mg/kg p.o.	194.512 ± 0.19*	245.267 ± 1.89**	315.267 ± 2.19^{ns}	0.72 ± 0.92**
5	V	EEAS	400 mg/kg p.o.	122.475 ± 0.147**	186.360 ± 0.53***	246.360 ± 2.23***	0.66 ± 0.23**

Values are expressed as mean ± standard error of the mean of six animals.
Statistical significance test for comparisons was done by one-way ANOVA, followed by "Dunnet's multiple comparison test."
Comparisons were done between (a) Goup I *vs* Group II and (b) Group II *vs* Group III–V. $^{**}p < 0.01$, $^{***}p < 0.001$, ns = non-significant.

[20,21]. Since ancient times, mankind has made use of plants in the treatment of various ailments because their toxicity factors appear to have lower side effects [22]. Many of the currently available drugs were derived either directly or indirectly from medicinal plants. Recent interest in natural therapies and alternative medicines has made researchers pay attention to the traditional herbal medicine. In the past decade, attention has been centered on the scientific evaluation of traditional drugs with plant origin for the treatment of various diseases [23]. In this regard, the present study was carried out to find the effect of traditional palatable plant *A. sativum* ethanolic bulb extract against TAA-induced hepatotoxicity in rats. The preliminary phytochemical analysis of EEAS showed that the plant has flavonoids, terpenes, steroids, glycosides tannins and the absence of proteins, sterols, phenols, glycosides, saponins, and terpenes which are used for many disorders including tuberculosis [24]. Injury to hepatocyte and bile duct cells leads to accumulation of bile acid inside the liver, which promotes further liver damage [25]. Administration of TAA at doses of 50 mg/kg/i.p. alternatively for every 72 hours for 21 days results in the hepatic damage in animals [26]. The mechanism behind its toxicity is thought to be associated with its toxic metabolite (s-oxide). It interferes with the movement of RNA from the nucleus to the cytoplasm which may cause membrane injury [13,27]. It reduces the number of viable hepatocytes as well as the rate of oxygen consumption and also decreases the volume of bile and its content, i.e., bile salts, cholic acid, and deoxycholic acid.

Administration of TAA shows significant elevation of serum biochemical parameters like AST, ALP, ALT, and total bilirubin which are primary markers for a liver function test. The elevation of these biomarkers because of systemic damage of the hepatic cells by reactive oxygen species released by the TAA, treatment with EEAS significantly reduces the serum biomarkers dose dependently and the above observations also explain that the EEAS has shown clear protection at the cellular level by preventing inflammation in histological studies. The loss of body weight and liver weight gain is identified by the disease control animals, which indicates that the severity of the hepatic damage and treatment with EEAS regain the hepatic damage significantly. From the above observation, it is concluded that the herbal drug possesses hepatoprotective activity and it has been proven by inhibiting the reactive oxygen species produced by the administration of TAA. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

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