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Protective Effect of Turnip Root (*Brassica Rapa*. L) Ethanolic Extract on Early Hepatic Injury in Alloxanized Diabetic Rats

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Abstract: Hepatic insufficiency is one of the most important consequences of diabetes mellitus. The aim of present study was to assess the protective effect of turnip root ethanolic extract (TREE) on early hepatic injuries in alloxan-induced diabetic rats. Eighty male Wistar rats were randomly allocated into 4 equal groups including: 1- normal control, 2- normal treated with TREE, 3- diabetic control, and 4- diabetic treated with TREE. Diabetes was made with a single injection of alloxan (120 mg/kg i.p.). TREE treatment groups received TREE (200 mg/kg) daily for 8 weeks through the gavage. At the end of experiment, levels of functional liver markers (AST, ALT and LDH), TB, Alb and TP were assessed in the serum. Product of lipid peroxidation (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR) activity were also assayed in liver homogenates. Finally, the biochemical findings were matched with histopathological verification. Statistically, the quantitative data obtained, compared among the groups by one-way analysis of variance followed by Tukey post-test. Statistical significance was considered as $p<0.05$. In the diabetic rats, TREE significantly decreased the levels of serum biomarkers of hepatic injury. Furthermore, TREE significantly decreased the lipid peroxidation and elevated the decreased levels of antioxidant enzymes in diabetic rats. Histopathologically, the changes were in agreement with biochemical findings. It seems that TREE has protective effect on early diabetic hepatopathy in the rats with experimentally induced diabetes.

Key words: *Brassica Rapa*. L, Alloxan, Diabetes mellitus, Hepatic injury, Rat.

INTRODUCTION

Diabetes mellitus is a metabolic disturbance that involves human beings and has high prevalence (4-5 percent) throughout the world (WHO, 1980). This endocrine disorder results from abnormal metabolism of carbohydrate, fat and protein and causes the increase of blood glucose level. Hepatic and renal failures are main causes of death in diabetic patients (Pickup and William, 1997). Evidences suggest that oxidative stress and free radicals are important agents in sufferance to diabetes mellitus pathogenesis (Cerielo *et al*., 1997; Kaneto *et al*., 2007 and Bulter *et al*., 2000). The role of free radicals in tissue injuries has been approved in the rat with streptozotocin induced diabetes (Murugana and Pari, 2006). Liver is one of the most important organs that maintain blood glucose level in normal limit thus enhancement of blood sugar yield to imbalance of oxidation-reduction reactions in hepatocytes, so that, hyperglycemia through increasing in AGEs (advanced glycation end products) facilities free radicals production via disturbance in ROS production (reactive oxygen species) such as SOD and CAT (Cameron *et al*., 2005; Kalia *et al*., 2004 and Jandeleit-Dahm *et al*., 2005). Hence, it reveals that diabetic hepatic injuries results from several agents and is not controllable only via inhibition of hyperglycemia (Liu *et al*., 2008). Namely, although in early stages of diabetes, hepatic injuries are induced via hyperglycemia but its progress in latter stages is not related to hyperglycemia (Vestra and Fioretto, 2003). Therefore, monitoring of blood glucose levels solely is not sufficient in delaying of diabetes complications. Alive cells by several mechanisms protect themselves against free radical damages. Meanwhile, SOD, GPX, CAT and GR have substantial role. According to free radicals role in diabetes mellitus, one of the important aspects in treatment is decreasing free radicals (Bulter *et al*., 2000). Meanwhile, during several

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studies, the effect of antioxidants in prevention or reduction of forced injuries by free radicals in diabetes has been appraised (El-Bassiouni *et al*., 2005 and Peerapatdit *et al*., 2006). Thus, a good drug must be have both antioxidant and blood glucose depreciatory properties (Ramesh and Pugalendi, 2006). Synthetic drugs not only are unable to controlling of forced damages (Liu *et al*., 2008) even, causes several side complications (Akhtar and Iqbal, 1991 and Holman and Turner, 1991) also use of these drugs in pregnancy period is dangerous (Larner, 1985). Nowadays, plants have important position among new pharmaceutical agents (Luo *et al*., 1998). Use of Medicinal Plants in diabetes treatment is referred to many years ago namely before 1550 BC (Kesari *et al*., 2005). Several plants from all over the world have been introduced to diabetic patients (Gupta *et al*., 2005; Ivorra *et al*., 1989; Kesari *et al*., 2005 and Marles and Farnsworth, 1995). Of these, only some of them were approved scientifically and according to WHO recommendation, many researches in this field is necessary (WHO, 1980). Of these plants were used in traditional medicine. Of plants are used in these cases can be refer to the turnip roots. *Brassicacea* family in all over the world is widely cultured and is used. *Brassica Rapa* species has important varieties such as turnip (Sasaki and Takahashi, 2002). Turnip has active biological compounds such as flavonoids (isorhamnetin, kaempferol and quercetin glycosides), phenylpropanoids derivatives (Romani *et al*., 2006), indole alkaloids and sterol glucosides (Schonhof *et al*., 2004). Flavonoids have important effects on diabetic patients. For example, isorhamnetin has inhibitory effect on aldose reductase, which has substantial role in complications of diabetes (Lim *et al*., 2006). Kaempferol has hypoglycemic effect on diabetic rats and was increased glucose absorption in rat's muscles (Jorge *et al*., 2004). In other research observed that quercetin causes decrease in blood sugar and increase in plasma insulin levels in diabetic rats due to streptozotocin (Vessal *et al*., 2003). Jung *et al*. (2008) showed that TREE through increase in glucose and fat metabolism has antidiabetic effect in diabetes mellitus type 2. Most importantly, flavonoids and hydroxycinnamic acid derivatives, which are found frequently in turnip root, have direct and potent antioxidant and free radical removal effects (Bennett *et al*., 2006). According to diverse turnip phytochemical effects, it is assumed that its root extract can be reducing hepatic complication of diabetes. However, there isn't any study about TREE effect on diabetic patients, current study were done on protective effect of TREE on early hepatic injury in alloxanized diabetic rats.

MATERIALS AND METHODS

Experiment plan:

This experimental study was carried out in Islamic Azad University Research Center and all procedures and works on animals was conducted under Animal Rights Monitoring Committee of Islamic Azad University Research Center. For prevention of bias in this study, all stages were done as double blind. So that, anyone such as laboratory and treatment man noticed from allocated and control groups. In this study, 80 male Wistar rats with mean of 200±20 g body weight and 10 weeks of age, which all are provided from Animal breeding ground of Islamic Azad university, Tabriz branch, were used. Rats were divided into 4 groups and each group contains 20 rats. The groups includes: 1- normal control, 2- normal treated with TREE, 3- diabetic control, and 4- diabetic treated with TREE.

Management and nourishing situation for all groups were considered identical with 12/12 h light/dark cycle at 21 ± 2°C. Food and water were provided ad libitum. After 15 h fasting, rats were intraperitoneally treated daily with alloxan monohydrate (Sigma chemicals, U.S.A.) at a dose of 120 mg/kg body weight (bw), freshly dissolved in distilled water (5%). Blood sugar in range of 120-250 mg/dl considered as diabetic (Gupta *et al*., 2005). Glucose measurement kit (produced by Ziest chem. Co., Iran) was used to evaluation of blood sugar. Turnip root after preparation was approved by pharmacognosy department of Islamic Azad University. Fresh roots washed with water and after slices were extracted for three times. Achieved solution was filtrated and then was dried under vacuum by rotary evaporator. Dried extract until application was kept in the refrigerator. Before initiation of study, blood sugar levels in all groups after 12 hours fasting were measured through blood sampling from behind the eyeball Sinus. TREE at dose of 200 mg/kg in 10 ml/kg normal saline (Kim *et al*., 2006) for eight consecutive weeks were gavaged to groups 2 and 4. Simultaneously, to groups 1 and 3 similar volume of normal saline were gavaged.

Biochemical Factors Evaluation:

After 8 weeks treatment, after 12 hours of fasting, for measurement of blood sugar levels and biochemical factors of liver function index, blood sampling from behind the eyeball Sinus were taken and serum of blood samples were isolated via centrifuging in 2500 round per second for 15 minutes at 30°C. Sera were used for measurement of alanine aminotransferase, aspartate aminotransferase (Reitman and Frankel, 1957), lactate

dehydrogenase (Martinek, 1972), albumin, total protein (Lowry *et al*., 1951) and total bilirubin (Malloy and Evelyn, 1937).

Measurement of Antioxidant Activity:

Simultaneously all rats euthanized via cervical dislocation. Rat's Liver were removed immediately and then washed in saline normal and homogenate 10% and supplied in 1.15% w/v of potassium chloride. Homogenate were centrifuged in 7000 round per second for 10 minutes at $4^{\circ C}$ and then upper solution were used for measurement of lipid peroxidation via measurement of malondialdehyde, SOD, CAT, GPX and GR. Malondialdehyde were measured as a scale of lipid peroxidation in template of TBARS (Thiobarbituric acid reacting substances) and via Esterbauer and Cheesman methods, TBARS value was expressed as nanomole per milligram of protein (Esterbauer and Cheesman, 1990). SOD activity was measured by Nishikimi method (Rao and Yagi, 1972) and was modified by Kakkar method (Kakkar *et al*., 1984). Each unit of SOD activity was determined as required enzyme concentration for prohibition of creation color at 1 minute, under study conditions. CAT activity was measured by Claiborne method (Claiborne, 1985) and based on hydrogen peroxide breakdown. GPX activity was measured by Rotruck method (Rotruck *et al*., 1973) and was expressed as micromole of GSSG /minute/milligram of protein, based on blew reaction:

$2H_2O+GSSG \rightarrow H_2O_2+2GSH$

GR activity was measured by Mohandas method (Mohandas *et al*., 1984), based on blew reaction:

$NADPH+H^{+}+GSSG \rightarrow NADP^{+}+2GSH$

Microscopic studies:

Remained rat's liver was placed in formalin 10% for fixing. From left diaphragmatic lobe, histological sections with consecutive slices and with 5µ thickness and of each 10 slices, 1 section and totally of each sample 3 sections with H&E staining were supplied (Lee and Luna, 1968). Inflammation rate in portal area, hepatocytes necrosis and inflammatory cells infiltration as semi quantitative scale and double blind as rendered by Frei *et al*. (1984) were evaluated. Injury severity was ranked from 0 to 4 (0: without injury, 1: minimum injury, 2: mild injury, 3: moderate injury, 4: sever injury) (Frei *et al*., 1984). All grades were designated with ×100 magnifications and in five microscopic fields of each section by chance via light microscope, NIKON ECLIPSE E200 produced by Japan.

Statistical analyzing:

We used of SPSS software ver. 13 to analyzing the data. Data were assayed by ANOVA and TUKEY test and $p<0.05$ considered as statistical significance.

RESULTS AND DISCUSSION

In group 3, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase serum levels and total serum bilirubin have significant increase ($p<0.001$) and total protein and serum albumin have significant decrease than group 1($p<0.001$ and $p<0.032$ respectively). In group 4, increase in total serum bilirubin and decrease in total protein and serum albumin were prevented by TREE so that there was not calculated any significant difference among this group and normal control. In group 2, TREE did not make a significant difference on liver injury indexes (table 1). In group 3, GSH, SOD, CAT, GPX and GR significantly decreased ($p<0.001$) and vice versa, malondialdehyde values significantly was increased ($p<0.001$) than group 1. In group 4, increase in malondialdehyde and decrease in GSH, SOD, CAT, GPX and GR are prevented by TREE so that there was not calculated any significant differences among this group and normal control. In group 2, TREE did not make a significant difference on lipid peroxidation of hepatocytes and antioxidant enzymes activity (table 2). Also in this group, 8 weeks treatment by TREE was significantly decreased blood sugar levels ($p=0.01$). But in group 4, 8 weeks treatment by TREE was significantly decreased blood sugar to normal levels ($p=0.03$) (table 3). Pathologically, liver histologic structure was normal and healthy in normal control group (Fig. 1, A). In group 2 also there was no pathological change so that hepatic lobular structure seemed quite normal (Fig. 1, B). In group 3, hepatocytes necrosis concomitant with inflammatory cells infiltration around the central vein and sever inflammation in portal area and also scattered necrotic foci in different portions of hepatic lobules were seen (Fig. 2). Finally, in group 4, TREE prevented the pathological changes and only partial hyperemia and degenerative changes were observed in centrilobular portions (Fig. 3). Quantitative microscopic results of experimental rats are presented in table 4.

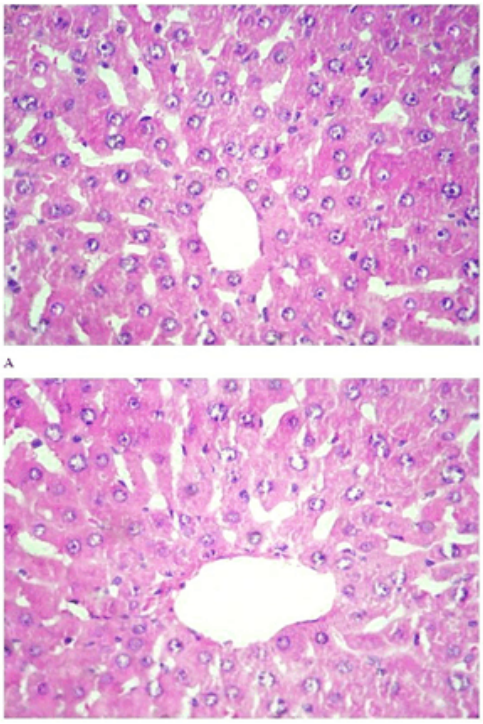
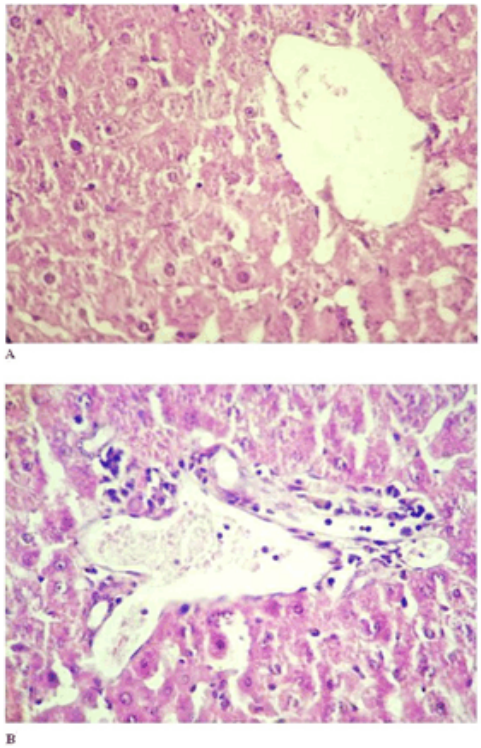


Fig. 1: A, Microscopic view from one of the normal control rat's liver. It has normal structure (H&E, /400). B, Microscopic appearance from one of the group 2 rats' liver. Hepatic structure seems normal and has no pathological changes (H&E, /400).



Fgi. 2: A, Photomicrograph from one of the group 3 rats' liver. Centrilobular hepatic cell necrosis and destruction of central vein wall is seen (H&E, /400). B, Another representative section from one of the group 3 rats' liver. Inflammatory cells infiltration in portal area and hepatocytes necrosis in periportal region is prominent (H&E, /400).

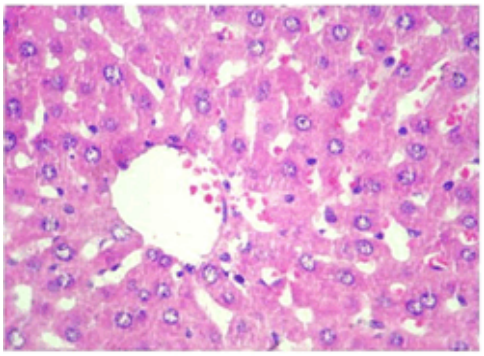


Fig. 3: Microscopic view from one of the group 4 rat's liver. Mild central vein and sinusoidal hyperemia is obvious.

Table 1: Effect of TREE on serum biochemical parameters in hepatic injuries of diabetic rats.

Groups	Treatment	Biochemical parameters					
		Alanine aminotransferase U/L	Aspartate aminotransferase U/L	Lactate dehydrogenase U/L	Total serum bilirubin Mg/dl	Albumin g/dl	Serum total protein g/dl
1	Normal control	75.18±1.42[a]	158.17±5.20 [a]	681.17±20.52 [a]	0.706±0.035 [a]	4.60±0.45 [a]	7.78±0.64 [a]
2	Normal treated with TREE	77.40±2.20 [a]	152.13±4.65 [a]	691.11±23.34 [a]	0.756±0.067 [a]	4.53±0.36 [a]	6.76±0.49 [a]
3	Diabetic control	175.32±3.1 [b]	231.39±6.92 [b]	1110.72±29.35 [b]	1.366±0.070 [b]	2.83±0.33 [b]	4.32±0.56 [b]
4	Diabetic treated with TREE	77.04±1.35 [a]	166.95±4.13 [a]	710.38±21.51 [a]	0.739±0.046 [a]	4.57±0.41 [a]	6.52±0.61 [a]
ANOVA		P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000

Values are mentioned as mean ± standard deviation.
*: indicator of significance differences (p<0.05).

Table 2: Effect of TREE on antioxidative activity in hepatic injuries of diabetic rats.

Groups	Treatment	Biochemical parameters					
		GSH µg/mg protein	MDA nmol/g protein	SOD U/mg protein	CAT U/mg protein	GPXU/mg protein	GR U/mg protein
1	Normal control	9.00±0.59 [a]	3.49±0.12 [a]	16.32±0.82 [a]	69.52±4.51 [a]	12.37±0.85 [a]	113.24±3.71 [a]
2	Normal treated with TREE	8.87±0.71 [a]	3.58±0.14 [a]	14.73±0.63 [a]	64.89±3.37 [a]	12.68±0.73 [a]	110.97±3.51 [a]
3	Diabetic control	4.85±0.52[b]	4.66±0.12 [b]	9.87±0.56 [b]	41.96±2.87 [b]	7.21±0.46 [b]	80.17±1.76 [b]
4	Diabetic treated with TREE	8.51±0.61 [a]	3.40±0.11 [a]	15.11±0.71 [a]	66.32±3.30 [a]	11.95±0.65 [a]	111.54±2.53 [a]
ANOVA		P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000

Values are mentioned as mean ± standard deviation.
*: indicator of significance differences (p<0.05).

Table 3: Effect of TREE on blood glucose level in normal and diabetic rats.

	Group 1	Group 2	Group 3	Group 4	ANOVAGlucose
Day 0	88.7±4.2	86.5±3.4	156.7±5.4[*]	154.3±4.8[*]	P<0.05
End of the study	86.8±3.7	61.3±2.3[*]	154.2±6.1[*]	90.1±3.5	P<0.05

Values are mentioned as mean ± standard deviation.
*: indicator of significance differences (p<0.05).

Table 4: Effect of TREE on hepatic injuries of diabetic rats.

Groups Grades	1	2	3	4
Hyperemia and inflammation in the portal area				
0	20	19	0	12
1	0	1	0	7
2	0	0	0	1
3	0	0	15	0
4	0	0	5	0
Necrosis				
0	20	20	0	15
1	0	0	0	3
2	0	0	3	2
3	0	0	15	0
4	0	0	2	0
Interstitial inflammatory cells infiltration				
0	20	20	0	16
1	0	0	0	3
2	0	0	4	1
3	0	0	14	0
4	0	0	2	0

Discussion and conclusion:

In this study TREE at dose of 200 mg/kg for 8 weeks causes decrease in blood sugar in normal rats and diabetic rats due to alloxan. Hypoglycemic effects of TREE are compatible with Jorge *et al*. (2004); Vessal *et al*. (2003) and Jung *et al*. (2008) researches results. In current study, obtained results of biochemical and Histopathologic assessments are representing liver injuries in diabetic rats due to alloxan. In this study, significant increase in ALT, AST, LDH and bilirubin levels and significant decrease in total protein and serum albumin in alloxanized diabetic rats than to normal control group were observed. Also, oral administration of TREE at dose of 200 mg/kg in 10 ml/kg normal saline for 8 consecutive weeks can change liver function indexes to normal ranges. In the liver evaluation, ALT, AST, LDH were used widely. Necrosis occurrence in the liver causes release of these enzymes to circulation. Enhancement of AST level in serum shows hepatic injuries like in viral hepatitis, infarction and muscular damages. ALT, which mediated converting of alanine to pyruvate and glutamate, is special for liver and is good indicator of hepatic injuries. Increased levels of above enzymes are indicator of cells infiltration and functional disturbance of liver cell membranes (Drotman and Lawhan, 1978). On the other hand, bilirubin, albumin and total protein serum values are associated with hepatic cells function (Muriel *et al*., 1992). Return of above enzymes serum levels to normal range due to TREE received rats may be result from prevention of intracellular enzymes infiltration because of cell membrane stability or regeneration of new cells (Thabrew and Joice, 1987). Effective Control of bilirubin and total protein shows early improvement of functional and secretory mechanism of hepatic cells. In this survey, widely degenerative changes and central lobular necrosis in diabetic rats were observed. With oral administrating of TREE in diabetic rats only mild degenerative changes were observed and did not seen any necrosis impression that shows protective effect of TREE against hepatic complications of diabetes. However, pathologic findings are matched with biochemical results and approved them. In current study it seems that free radicals cause membrane lipid and intra reticulum endoplasmic unsaturated fatty acids peroxidation that yields to production of lipid peroxides (malondialdehyde), loss of cell membrane stability and finally liver injuries. Enhancement of malondialdehyde in diabetic rats is an indicator of peroxidation reactions that causes depression of antioxidants defensive mechanisms. Hence, preventing the production of free radicals cannot be sustained (Naik, 2003). In other words, increased amounts of malondialdehyde in liver is an indicator of lipid peroxidation that yields to hepatic injury and antioxidant defensive mechanism disability in prohibition of excessive free radicals production. SOD, CAT and GPX are antioxidant enzymes which establish a defensive system against ROS (Lil *et al*., 1988). Reduction in SOD activity is a sensitive index of hepatic injuries. SOD eliminates superoxide anion by converting it to hydrogen peroxide and hence reduced its toxic effects (Curtis *et al*., 1972). In current study, SOD amounts in diabetic rats are significantly decreased because of frequently production of superoxide anions. CAT and GPX also in these rats significantly decreased. It seems that inactivation of SOD by increased superoxide anions yields to inactivation of CAT and GPX. In this study, oral consumption of TREE were prevented of decrease in SOD, CAT and GPX that is may be due to TREE active substance which is results in maintenance and remaining of this enzymes. CAT is one of the antioxidants enzymes which is diffused widely in animal tissues and has high activity in liver and RBCs. CAT is protecting tissues from extreme toxic hydroxyl via decomposing of hydrogen peroxide (Chance *et al*., 1952). GR is one of the cytosolic enzymes which are involved in GSSG reduction, as end product of GPX activity on GSH (Naik and Panda, 2008). Use of TREE in diabetic rats reestablished GR activity that involves GSSG usage to make GSH and enhances detoxification of active metabolites by its conjugation with GSH. With attention to mentioned reactions revealed that TREE exert itself protective effects in diabetic rats liver injuries by compensation of antioxidant defensive system activity and removing of free radicals. Current study approves other reports about TREE antioxidant and free radicals removing effects. Franciscoa *et al*., 2009 showed that TREE is full of phenolic antioxidants particularly flavonols and hydroxycinnamic acid (Franciscoa *et al*., 2009). These compounds have strong and direct antioxidant effects and also causes expression of different genes involve in metabolic enzymes encoding which is effective in decrease of incidence of disorders and diseases risks (Bennett *et al*., 2006). In Kim *et al*. (2006) research has been shown that TREE has protective effect against cisplatin by reduction in oxidative stress so that decrease malondialdehyde and increases GSH, SOD, CAT and GPX activity. TREE action mechanism in Kim study is similar with our research results. In Rafatullah *et al*. (2006) study has been shown that TREE prohibits of hepatocytes injuries due to tetrachloride carbon. According their aspects, TREE protective effect is probably via its antioxidant effects (Rafatullah *et al*., 2006). Choi *et al*. (2006) were showed TREE hepatoprotective and antioxidant effects in both in-vivo and in-vitro. In this study oral administration of TREE to rats which their liver was damaged by D-Galactosamine yields to improvement.

Present study showed pharmacologic effect of TREE in liver complications of diabetes. Therefore, it seems that TREE has positive effects in prevention of hepatic injuries due to oxidative stress of diabete and can be recommend in diabetic humans as herbal drug. Thus, determination of different doses of TREE and exact distinguishing of TREE active substance future studies are required.

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