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Hepatoprotective effects of thyme extract in Cisplatin-induced liver toxicity in rabbits

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ASTRACT

The administration of aqueous thyme extract was investigated for its hepatoprotective effect in rabbits with acute liver injury induced by a single i.p. injection of cisplatin (6.5 mg/kg). Aqueous thyme extract treatment (500 mg/kg/day, orally) was applied for 7 consecutive days, starting 4 days before cisplatin administration. Aqueous thyme extract significantly reduced serum levels of liver enzymes and increased serum albumin levels which were altered by cisplatin. Aqueous thyme extract significantly compensated deficits in tissue glutathione level, suppressed lipid peroxidation, and decreased the elevations of serum tumor necrosis factor-alpha resulted from cisplatin administration. Also, histopathological liver tissue damage mediated by cisplatin as well as inflammatory cells infiltration was greatly ameliorated by aqueous thyme extract treatment. It was concluded that aqueous thyme extract represents a potential therapeutic option to protect against acute cisplatin hepatotoxicity commonly encountered in clinical practice.

Keywords: Aqueous Thyme Extract, Cisplatin, Hepatotoxicity.

INTRODUCTION

The liver plays a major role in transforming and clearing chemicals which lead to increase its susceptibility to the toxicity from these agents. Drugs are an important cause of liver injury, more than 900 drugs, toxins, and herbs have been reported to cause hepatic injury [1].

Cisplatin [cis-diamminechloroplatinum (II)] is a potent antineoplastic agent used for the treatment of a wide range of cancers [2, 3]. Nevertheless, this drug has severe toxic effects that interfere with its therapeutic efficacy, namely nephrotoxicity and hepatotoxicity. Although the nephrotoxicity of cisplatin has been recognized as the most important dose-limiting factor, little is known about cisplatin induced liver injury. Hepatotoxicity is not considered as a dose limiting toxicity for cisplatin, but liver toxicity can occur when the antineoplastic drug is administered at high doses [4]. Oxidative stress is one of the most important mechanisms involved in cisplatin induced toxicity. The mitochondrion is the primary target for cisplatin induced oxidative stress, resulting in loss of mitochondrial protein-SH, inhibition of calcium uptake and a reduction in the mitochondrial membrane potential [2].

Thyme (Thymus vulgaris) was belonging to the Lamiacea family an aromatic native herb in the Mediterranean region. Thyme was now widely cultivated as spice, tea and herbal medicine [5, 6]. Thymus vulgaris possess

various beneficial effects, like antiseptic, antimicrobial, bactericidal, anthelmintic and antioxidant properties. Also, it has lately recommended as a natural replacement for synthetic antioxidant [7]. Moreover, thyme was enhanced blood circulation and functions as an exciting stimulant for the entire circulatory system. It is also effective in the treatment of depression and mood changes [8]. The therapeutic potential of thymus vulgaris rests on its contents of flavonoids, thymol, carvacrol, eugenol and aliphatic phenols in addition to luteolin and saponins [9, 10].

This study was designed to evaluate whether the hepatotoxic effect caused by administration of cisplatin could be prevented by treatment with thyme extract.

MATERIALS AND METHODS

Chemicals

Cisplatin intra venous solution (50mg/ 100ml) was obtained from Ebewe, Austria. Reagent kits for assay of transaminases were purchased from BioMerieux, France. ALP assay kit was purchased from Biolabo, France. Total bilirubin assay kit was obtained from Randox, United Kingdom. Reagent elisa kits for determination of tissue malondialdehyde (MDA), reduced glutathione (GSH) and tumor necrosis factor alpha (TNF-α) were purchased from Cusabio, China. The work was done in accordance with the method prescribed in each diagnostic kit.

Thyme aqueous extraction

Dried leaves of thyme (Thymus vulgaris) were purchased from local market in Baghdad-Iraq and were identified by the National Iraqi Institute for Herbs. The leaves of thyme were grounded into powder using an electrical grinder. One hundred gram of the fine-powder were subjected to extraction with 200 ml of boiling distilled water in a covered flask and left for 30 minutes. After that, the extract was cooled and filtered by means of Whatman No.1 filter paper to remove the particulate material then the filtrate was dried in a vacuum. The required doses then weighted and reconstituted in 5 ml of distilled water a minute ago before oral administration [11].

Experimental Animals

All experimental protocols were approved by the Ethics Committee of the College of Medicine /AL Nahrain University. Twenty four healthy, local, domestic rabbits aged 3-4 months and weighing (600-1300) gm. of both sexes were used in this study. Before starting the study, the animals were left for 72 hours to acclimatize to the animal room conditions and were maintained on an environment of controlled temperature with a 12 hours light / dark cycle. All rabbits have free access to food and tap water.

Rabbits were divided into 3 groups randomly, each group including 8 animals: Group 1 (control): Rabbits were left without treatment. Group 2 (cisplatin): Rabbits were given cisplatin injection as an intraperitonial dose of (6.5 mg/kg) on day 3 of the experiment continued for three successive days. Group 3 (Thyme + cisplatin): Rabbits were given aqueous extract of thyme in a dose of 500 mg/kg orally once daily for 7 days, cisplatin was given intraperitonially in a dose of (6.5 mg/kg) 72 hours before sacrificing the animals . At the end of experiment, the rabbits were subjected to blood collection under anesthesia by ether inhalation, the blood collected directly from the heart, centrifuged to get serum which stored at -20°C for biochemical analysis.

Histological Examination

After scarification the liver tissue were excised by thoracic section and fixed in 10% formalin for 24 hours and embedded in paraffin. Blocks were cut by microtome into 5 mm thick sections,and then following staining with hematoxylin-eosin stains, sections were examined by light microscope and scored to assess histopathological changes according to the following grading system: 0, normal; 1,mild hydropic degeneration, no Kupffer cell proliferation and no necrosis; 2, moderate hydropic degeneration, Kupffer cell proliferation and no or little necrosis; 3, severe hydropic degeneration, Kupffer cell proliferation and necrosis [12].

Statistical analysis

Statistical analysis was performed using IBM SPSS version 21 statistical software and Microsoft Excel 2010. Descriptive statistics for the numerical data were formulated as mean and standard error. Parametric independent samples t-tests were carried out for comparison between two groups whenever data were normally distributed, while non-parametric Mann-Whitney U tests were carried out whenever the data were not normally distributed. The significant difference level (p-value) is below 0.05.

RESULTS

Table 1 shows the effect of administration of thyme extract on liver function tests, biochemical, oxidative stress, inflammation markers and histopathological parameters compared to cispaltin and control groups.

Cisplatin administration (6.5 mg/ kg I.P.) resulted in significant increase in the activity of liver enzymes serum alkaline phosphatase, serum alanine aminotransferase ,and aspartate aminotransferase compared to the negative control. Treatment with thyme extract resulted in a significant reduction in serum level of these enzymes and restoring to the normal range (table 1).

The serum albumin levels significantly decreased in cisplatin treated group while total serum bilirubin level significantly increased compared to control group, thyme extract administration significantly increased serum albumin level, the result that is not seen with total bilirubin.

Treatment with thyme extract significantly ameliorated the depletion of the antioxidant defense mechanisms (GSH level) and suppressed lipid peroxidation (MDA level) in liver tissue resulted from cisplatin administration (table 1). Concerning the inflammatory marker, cisplatin cause very highly significantly elevation in tumor necrosis factor alpha tissue level compared to control group, administration of thymus extract restore the TNF-α to normal range indicating its strong anti-inflammatory effect.

Effects of thyme extract on liver histopathology

Hepatic sections of the positive control group showed liver damage mostly in form of moderate hydropic degeneration, Kupffer cell proliferation and little necrosis, administration of thyme extract resulted only in mild degeneration while there was no Kupffer cell proliferation and no necrosis (table 1, figure 1)

Table 1: Effects of thyme extract on liver function tests, biochemical, oxidative stress, inflammation and histopathological parameters compared to cispaltin and control

Variables	Gp1 (N=8)	Gp2 (N=8)	Gp3 (N=8)	*P* value
S. Albumin (g/dl)	2.92 ± 0.03	0.92 ± 0.02	1.73 ± 0.03	< 0.01
S. Total Bilirubin (mg/dl)	0.11±0.10	0.13±0.11	0.14±0.12	< 0.878
S.ALP (U/l)	59.25±6.02	128.13±22.52	94.77±21.54	< 0.01
S. ALT (U/l)	48.97 ± 13.54	78.44 ± 22.36	52.11± 34.24	< 0.01
S. AST (U/l)	116.43 ± 29.75	263.65±113.21	101.26 ± 31.72	< 0.01
T. GSH (nmol/l)	35.76±3.6	13.22±0.63	21.73±1.51	< 0.01
T. MDA (ng/l)	122.28±0.69	145.2±4.3	122.55±4.55	< 0.01
T. TNF-α (pg/l)	42.91 ± 1.84	322.55 ± 5.33	37.16 ± 2.00	< 0.0001
Necrosis score	0	2	1	< 0.01

Results represent Mean±SD , Gp1= Control (no treatment) , Gp2= cisplatin treated group, Gp3=cisplatin+thymus extract treated group; S.ALP=serum alkaline phosphatase; S. ALT =Serum alanine aminotransferase ;S. AST= Aspartate aminotransferase ; T. GSH=tisuue glutathione; T. MDA=tissue malondialdehyde; T. TNF-α= tissue tumor necrosis factor alpha.

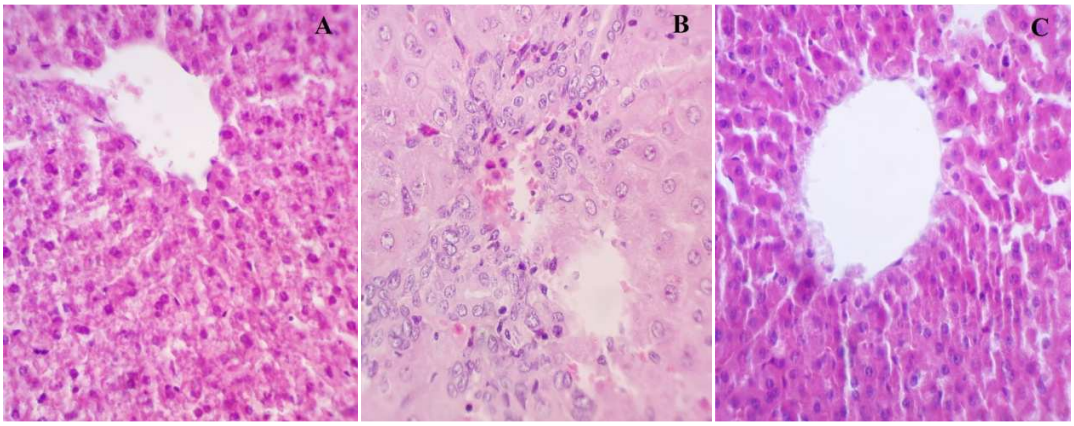

Figure 1: Section of liver of a White rabbits of study groups (A: Normal group, B: Cisplatin group, C: Thyme group) on day 8 of experiment 72 hours after administration of 6.5mg/ kg cisplatin I.P. 40X, H&E

DISCUSSION

Cisplatin is a potent anticancer agents that cause a wide range of adverse effects and organ toxicity including hepatotoxicity ,nephrotoxicity and vascular endothelial dysfunction that mediated by different mechanisms such as inflammation and oxidative stress [13]. Cisplatin-induced hepatotoxicity is a major problem in the cancer therapy [14] . In this study, using an experimental model of cisplatin induced hepatotoxicity in rabbits (single dose of 6.5 mg/ kg I.P.) characterized by alterations in liver function tests and TNF-α as well as increased MDA and decreased GSH levels compared to normal group. It has been reported that cisplatin induced hepatotoxicity is closely associated with an increase in lipid peroxidation manifested by increased MDA as well as a decrease in anti-oxidant activity with depletion of GSH [15]. The present study shown increased tissue content of inflammatory mediators together with inflammatory cell infiltration, suggesting that inflammation plays an important role in cisplatin induced liver injury [16]. Although the precise inflammatory mechanisms are unknown, marked attenuation of cisplatin induced liver damage by inhibition of tumor necrosis factor alpha (TNF- α) indicates that TNF-α has a central role of mediation cisplatin induced liver injury [17]. Histopathological results of this study showed that cisplatin had induced hepatic damage characterized by different degrees of degeneration and necrosis of hepatocytes and inflammatory cell infiltration that could be correlated with the harmful effects of cisplatin parallel to high MDA and low GSH levels.These results are in agreement with the results in other studies [18].

Results of the present study showed that thyme extract alleviates inflammation effectively. Thyme extract highly significantly reduced serum TNF-α level to the normal level as well as inflammatory cell filtration.The present study is in agreement with other previous studies1 [19,20]. Previous studies stated that thyme extracts significantly reduced production and gene expression of the pro-inflammatory mediators TNF-*α*, IL-1B, and IL-6 and highly increased in the anti-inflammatory IL-10 cytokine [21].

Thyme extract exerts inhibitory effects on leukocyte migration to the injury site. Thyme extract may inhibit prostanoid through suppression of COX-1 and COX-2 release and acts as activator of peroxisome proliferator-activated receptors. Thyme extract was able to inhibit *in vitro* chemotaxis induced by fMLP and LTB4. Leukotriene is a potent chemotactic agent derived from arachidonic acid. fMLP is a chemotactic agent involved in the release of cytokines. Upon binding to its G-proteincoupled receptor, it activates multiple signaling cascade pathways. These pathways include the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI-3K) cascades, which are important for the development of the functional responses of neutrophils in inflammation[22].

Results of the present study showed that thyme extract had effectively maintained normal levels of oxidative stress parameters. Thyme extract reduced tissue MDA and increased tissue GSH levels in statistically highly significant difference almost to the normal levels, beside that histopathological examination of liver tissues in the present study showed that thyme extract had beneficial effect.

CONCLUSION

Administration of aqueous thyme extract (500 mg/ kg/ day, orally) had protective effects against cisplatin induced hepatotoxicity in rabbits. The treatment cause significant positive change in oxidative stress and inflammatory markers, restoring normal liver functions and preventing histopathological changes.

REFERENCES

[1] S.E. Friedman, J.H. Grendell, K.R. McQuaid , Current diagnosis & treatment in gastroenterology, Lang Medical Books/McGraw-Hill, New York, **2003**, 664-679.
[2] S. Y. Saad, T. A. Najjar, M. Alashari, , *Clin. Exp. Pharmacol Physiol*. 31, **2004**, 862-867.
[3] G. Wang, E. Reed, Q.Q. Li, , *Oncol. Rep.*, **2004**, 955-965.
[4] A. Zicca, S. Cafaggi , M. A. Mariggio, M.O. Vannozzi, M. Ottone, V. Bocchini, G. Caviglioli, M. Viale,. *Eur. J. Pharmacol*. 442, **2004**, 265-272.
[5] M. Ozguven , S. Tansi , *Tr. J. of Agriculture and Forestry*, 22(6), **1998**, 537-542.
[6] M. Domaracky , P. Rehak , S. Juhas , *J. Koppel, Physiol. Res*., 56(1), **2007**, 97-104.
[7] I. Rasooli, M.B. Rezaei , A. Allameh , , *International Journal Infectious Diseases*, 10(4), **2006**, 236-241.
[8] M. Höferl , S. Krist , G. Buchbauer , *Planta Med*., 72(13), **2006**, 1188-1192.
[9] H.G.D. Dorman , S.G. Deans , *Appl. Microbiol*., 88(2), **2000**, 308-316.
[10] R. Amarowicz , Z. Zegarska , R. Rafałowski , R.B. Pegg , M. Karamac , A. Kosin , *Eur. J. Lipid Sci. Technol*., 110(1), **2008**, 1-7.
[11] O. Kandil , N.M. Radwan , A.B. Hassan , A.M. Amer , H.A. El-Banna , W.M. *Amer, J. Ethnopharmacol*., 44(1), **1994**, 19-24.
[12] A. Kart, Y. Cigremis , M. Karaman , H. Ozen . , *Exp Toxicol Pathol*, 62, **2010**, 45-52.
[13] Z.H. Siddik, *Oncogene*. 22(47), **2003**, 7265-7279.
[14] D. Wang, J.L. Stephen , *Nature reviews Drug discovery*. 4(4), **2005**, 307-320.
[15] A.H. Eid , N.F. Abdelkader , O.M. Abd El-Raouf , H.M. Fawzy , E.S. El-Denshary , *Arch Pharm Res*. **2016** [Epub ahead of print].
[16] K.V. Athira , R.M. Madhana , E.R. Kasala , P.K. Samudrala , M. Lahkar , R. Gogoi , *J Biochem Mol Toxicol*. **2016** [Epub ahead of print].
[17] W. Li , M.H. Yan , Y. Liu , Z. Liu , Z. Wang , C. Chen , J. Zhang , Y.S. Sun , *Nutrients*. **2016**, 8(9). pii: E566.
[18] L. Yan , R. Hu , S. Tu , W.J. Cheng , Q. Zheng , J.W. Wang , W.S. Kan , Y.J. Ren , *Oncol Lett*. **2016,** 12(3):1981-1985.
[19] S.H. Abdel-Aziem, A. M. Hassan, E. S. El- Denshary, M. A. Hamzawy, F. A. Mannaa, M. A. Abdel-Wahhab , *Cytotechnology*. **2014**, 66(3), 457-470.
[20] A.K. Gulec, D. Danabas, M. Ural, E. Seker, A.Arslan, O. Serdar, ActaVeterinaria Brno, **2013,** 82(3), 297- 302.
[21] A. Ocaña, G. Reglero, *Journal of obesity.* **2012**,2012.
[22] Q. Fachini, C. Fernanda, C. Fernanda, K Raquel, F. Camila , S. Estevao, D. Maria , , Evidence-Based *Complementary and Alternative Medicine*. **2012**, 2012.