Original Article

Investigating the protective effect of *Thymbra spicata* extract on hepatotoxicity induced by acetaminophen in male rats

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ABSTRACT

Introduction: Thymbra spicata has flavonoid, alkaloid compounds and antioxidant properties. In the current study, the protective effect of hydroalcoholic extract of Thymbra spicata (TS) was investigated on acetaminophen-induced liver toxicity. **Materials and Methods:** The hydroalcoholic extract of the plant was extracted by Soxhlet extractor. In this experimental study, 150 adult male rats were randomly divided into five groups (n=50). Animals were initially treated with different amounts of TS (100, 200 and 300 mg/kg, gavage) for seven consecutive days, then they were exposed to a toxic dose of acetaminophen (700 mg/kg, intraperitoneally). In the control group 1, distilled water was administered for seven days. In the group 2 (Ace), the rats received distilled water for seven days and the seventh day a toxic dose of acetaminophen was injected with. Twenty-four hours later blood and liver tissue samples were isolated for biochemical and antioxidant factors analysis. **Findings:** The results showed that AST-ALP-Bili-SOD levels in the treatment groups had a significant difference compared to the control group, but the ALT enzyme in TS with a dose of 300 mg/kg did not have significant difference with the control group but in the another treatment group it had a significant difference, which indicates that the positive effect of Thymbra spicata has a better effect with increasing plant dose. **Conclusion:** The results indicate that hydroalcoholic extract of Thymbra spicata has a protective role in the improvement of acute liver toxicity induced by acetaminophen.

Keywords: Thymbra spicata, Acetaminophen, Toxic liver

Introduction

Acetaminophen is one of the most popular painkillers in the world. Although, using it is safe in therapeutic doses, excessive consumption can cause severe liver damage [1]. Because of its low side effects and less interference with other drugs, acetaminophen gained a significant worldwide popularity among painkillers and became the most commonly prescribed drug in children [2]. Acetaminophen can damage human health by producing free radicals [3]. Acetaminophen toxicity begins with the production of a reactive metabolite that binds to the protein [1]. Formation of an excess protein, mitochondrial dysfunction, oxidative stress, and the fragmentation of the nucleus DNA are of vital cellular events in liver cells [4]. Biochemical studies showed that acetaminophen toxicity can alter the morphology and function of the liver mitochondria that leads to mitochondrial oxidative stress by binding to the mitochondrial protein. Currently, the only effective treatment for the excessive use of acetaminophen is N-acetyl cysteine that should be consumed by patient between 12 and 24 hours after ingestion of acetaminophen, but probability of a positive result is reduced in longer time intervals [1].

For determining the hepatotoxicity by acetaminophen, it is important to measure blood levels of intracellular liver enzymes among which, Serum Glutamic Pyruvate Transaminase (SGPT) or Alanine Transaminase (ALT) is important. Serum Glutamic Oxaloacetic Transaminase (SGOT) or Aspartate aminotransferase (AST) also change [5, 6]. Alkaline phosphatase is a trans-peptidase and increases in diseases associated with bone and liver [7]. The enzymatic activity of GGT (γ-Glutamyl Transferase) and concentration of plasma bilirubin are among

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other factors that increase during liver cell dysfunction [3]. During the liver damage, these enzymes and substances release and increase in the blood, and their measurement is an indicator for detecting liver damage [5, 6]. On the other hand, the antioxidant capacity is reduced by acetaminophen [8]. As a result, examination of the status of oxidation and peroxidation of the liver tissue and blood seems necessary which one of the methods is the thiobarbituric acid (TBA) test [9].

Today, using herbal medicines and application of these drugs in the treatment and prevention of diseases in the world and especially Iran have been increased significantly. Many plants contain different antioxidant compounds, including polyphenols. Polyphenols, especially flavonoids, have a protective effect against liver damage caused by toxins. The oxidation of flavonoids by free radicals leads to formation of more radicals with less activity and increased stability. Increasing the reaction of the hydroxyl group in flavonoids deactivates radicals [10]. Many herbal sources have antioxidant and phenolic properties, including Thymbra spicata.

Thymbra spicata belongs to the mint family and is a persistent plant that preferably grows in arid and sunny areas of hills and dried meadows. The height of this plant is varied between 15 and 40 cm and has beautiful purple flowers [11]. The flowering and fertility season of Thymbra is May to June. Thymbra spicata plant is not cultivated anywhere in the world and it is collected by local people from the nature [12]. Due to the presence of thymol and carvacrol, which are important for biological and medicinal activities, the plant has a high reputation. This herb is used in the traditional medicine of Turkey, Greece, Egypt and Rome to treat asthma and bronchitis. It is also used for improving the taste of the food [13]. This plant is native to the province of Ilam in Iran, which has a lot of traditional uses and is of special importance among the people of the region [14]. The essential oil of this plant has antimicrobial, antioxidant, cholesterol decreasing and hepatoprotective activities [15]. The study of chemical compounds of essential oil showed 39 chemical compounds, the most important of which are carvacrol extract (60.26%), gamatripinene (15.09%), beta-myrcene (2.15%) and thymol (1.04%). The radical-detoxification activity of the essential oil of this plant is 77.81 ± 3.1 and its essential oil yield is 2.6% (v / w) [10].

Essence existed in different parts of the plant is a good source of antibacterial and antioxidant properties. So far, several studies have been done on antibacterial properties of the Thymbra essential oil, but there is lack of information on hepatoprotective effects of Thymbra cultivars in Ilam.

Regarding the aforementioned points, it is possible that the antioxidant compounds present in this plant, which is abundant in Ilam province, could have a protecting activity against acetaminophen poisoning, which today is one of the most prevalent poisonings. According to this, the hepatoprotective properties of these medicinal plants in Ilam province were examined in acetaminophen-induced hepatotoxicity in mice, which, in the case of confirmation, could be a good alternative to synthetic drugs having many side effects.

Methods

In this study, 150 white male rats (Albino) were used which their weight was in a range of 320 to 280 grams. These animals were kept in standard light conditions (12 hours of light and 12 hours of darkness) and had free access to water and food. The temperature was maintained at 24 ± 4 °C and they were kept in standard cages.

Animals were divided into 5 groups of 30 as follows:

- Control group 1: Control group which received normal saline solution for 7 days.
- Control group 2: The acetaminophen group which received normal saline 7 days before prescription of acetaminophen and 6 hours after receiving the last one, were intraperitoneally injected acetaminophen on the 7th day.
- Group 2 Ace + E1: The group receiving 100 mg / kg body weight hydroalcoholic extract of Thymbra plant for seven days and 6 hours after the last administration of the prescription, received intraperitoneal injection of 700 mg / kg body weight of acetaminophen.
- Group 4 Ace + E2: The group receiving 200 mg / kg bodyweight of hydroalcoholic extract of Thymbra plant for 7 days and 6 hours after the last administration of the prescription, received intraperitoneal injection of 700 mg / kg body weight of acetaminophen.
- Group5 Ace + E3: The group receiving 300 mg / kg body weight of hydroalcoholic extract of Thymbra plant for 7 days and 6 hours after the last administration of the prescription, received intraperitoneal injection of 700 mg / kg body weight of acetaminophen.

Based on the weight of the animal, the hydroalcoholic extract of the plant was given in a 1 ml volume of 0.9% physiological serum as an oral solvent via gastric tubes (gavage) for one week once daily as pretreatment. But the control groups of 1 and 2 received its solvent (normal saline) instead of the extract.

After 24 hours, animals were anesthetized (ketamine 100mg / kg, xylalin 16mg / kg intraperitoneal) and their blood and liver samples were collected. The blood was collected in glass tubes and centrifugation for 5 minutes at 4000 rpm for serum separation after 30 min in the laboratory environment and the separated serum samples were transferred into polyethylene tubes for storage of serum to measure ALT, AST, ALP liver enzymes and bilirubin and stored at -70 ° C until test. Liver tissue was used to evaluate the SOD activity.

Frozen tissue after weighing, were homogenized with a weight / volume ratio of 1 to 10 in phosphate buffer (pH = 7.4) and centrifuged in 15000 ×g for 15 min. Superficial solution was used for measurement of the studied indexes.

Data were analyzed by SPSS software version 22 and the level of significance was considered as $p < 0.05$. To analyze the overall comparison, one-way ANOVA was used and Duncan's complementary test was used to compare the groups. In all

experiments the significant difference was considered as $p < 0.05$.

Data analysis

ANOVA and Duncan's tests were used to analyze data. According to Duncan's test, groups with different colors ($P < 0.05$) are significantly different.

According to the Duncan's test, there was a significant difference between the treatment groups and the control group. Also, treatment groups were significantly different with each other and with the acetaminophen group.

Results of measurement of AST enzyme in different groups

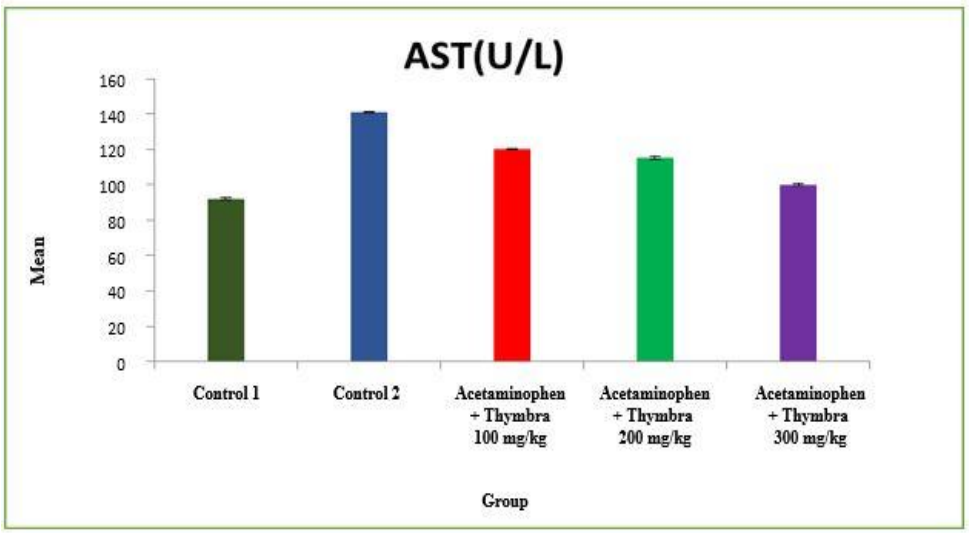

Figure 1: The effect of treatment groups on the level of AST enzyme and their comparison with the control group and the acetaminophen group

ANOVA and Duncan's tests were used to analyze data. According to Duncan's test, groups with different colors ($P < 0.05$) are significantly different.

According to the Duncan's test, there was a significant difference between the treatment groups and the control group. Also, treatment groups were significantly different with each other and with the acetaminophen group.

Results of measurement of ALT enzyme in different groups:

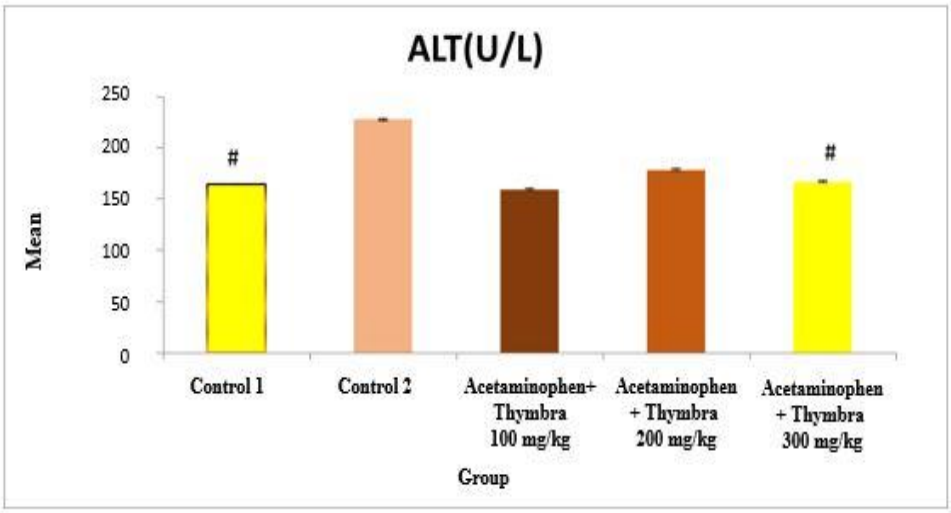

Figure 2: Effect of therapeutic groups on the amount of ALT enzyme and their comparison with the control group and the acetaminophen group

ANOVA and Duncan's tests were used to analyze data. Groups with similar letters and colors according to the Duncan's test ($P < 0.05$) showed similar effects and were not significantly different.

As shown in the figure, the control group and the third treatment group were not significantly different, while the other treatment groups were significantly different with each other and with the control group.

Results of measurement of ALP enzyme in different groups

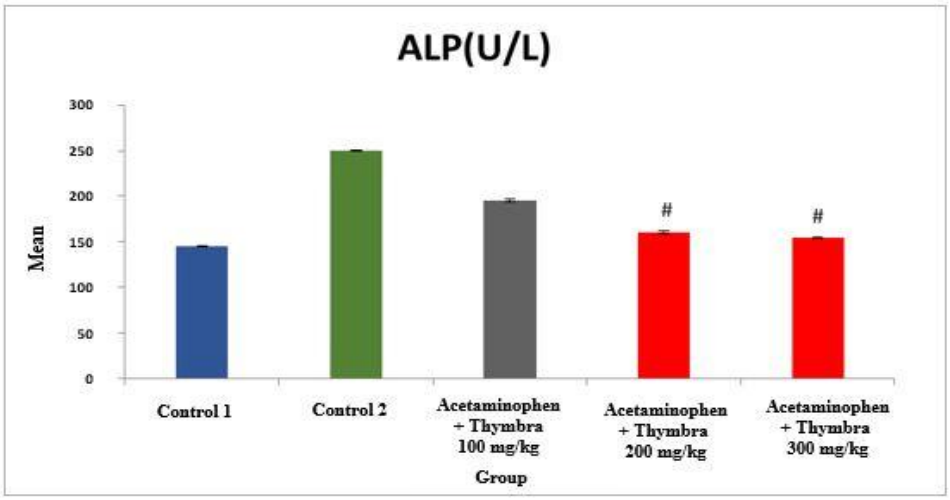

Figure 3: Effect of treatment groups on the amount of ALP enzyme and their comparison with the control group and the acetaminophen group

ANOVA and Duncan's tests were used to analyze data. Groups with similar letters and colors according to the Duncan's test ($P < 0.05$) showed similar effects and were not significantly different.

In order to study the protective effect of Thymbra plant on liver toxicity induced by acetaminophen, by comparison of the means, it was shown that the control group1 was significantly different with the treatment groups and the control group 2 was significantly different with the treatment groups, which indicates the effect of the treatment groups on the reduction of liver enzymes compared to the control group 2. There was no significant difference in treatment groups of acetaminophen + 200 mg / kg and acetaminophen + 300 mg / kg, indicating that the effect of both doses was almost the same.

The results of bilirubin measurements in different groups

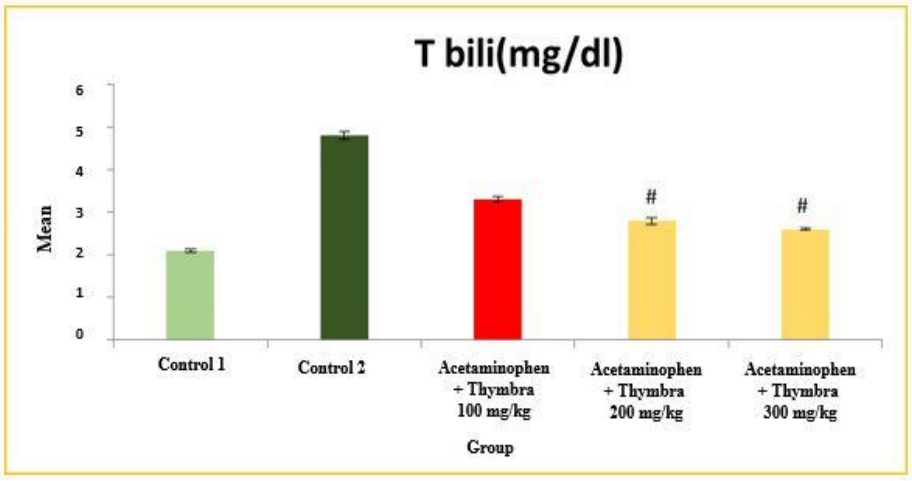

Figure 4: The effect of treatment groups on the amount of bilirubin and their comparison with the control group and the acetaminophen group

ANOVA and Duncan's tests were used to analyze data. Groups with similar letters and colors according to the Duncan's test ($P < 0.05$) showed similar effects and were not significantly different.

As shown, the control group 1 was significantly different with the treatment groups and the control group 2. There was also a significant difference between the control group 2 and the treatment groups, while the treatment groups of acetaminophen + 200 mg / kg and acetaminophen + 300mg / kg were not significantly different.

The results of SOD measurements in different groups

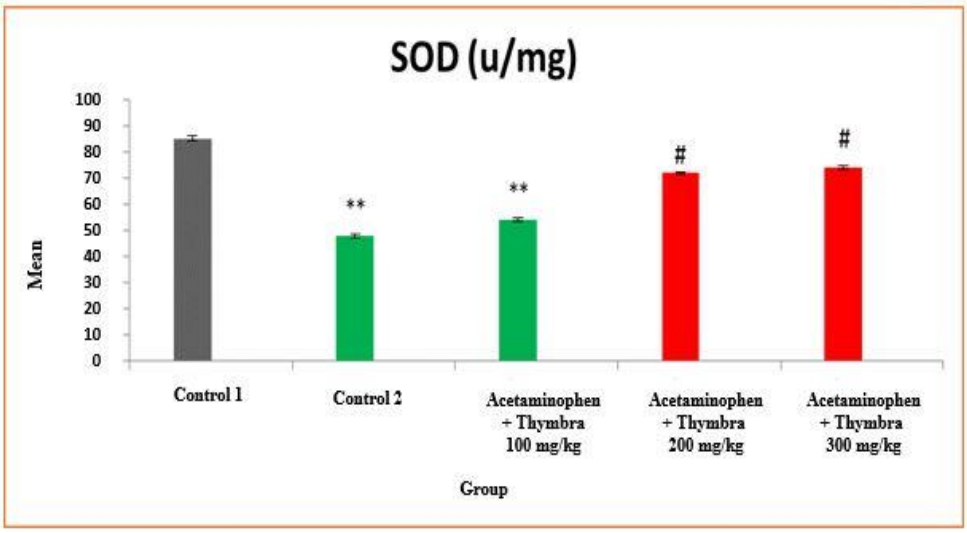

Figure 5: Effect of treatment groups on amount of SOD and their comparison with control group and acetaminophen group

ANOVA and Duncan's tests were used to analyze data. Groups with similar letters and colors according to the Duncan's test ($P < 0.05$) showed similar effects and were not significantly different.

As shown, the control group 1 was significantly different with the treatment groups and the control group 2. Also, the control group 2 was not significantly different with the treatment group of acetaminophen + 100 mg / kg, as well as the treatment groups of acetaminophen + 200 mg kg / kg and acetaminophen +300 mg / kg were not significantly different.

Discussion and Conclusion:

In present study, probably the strong inhibiting effects of hydroalcoholic extract of Thymbra against toxic effects of acetaminophen was related to the antioxidant active compounds of polyphenols, flavonoids, thymol and carvacrol in the hydroalcoholic extract of Thymbra, which could increase the antioxidant capacity in the cell. Additionally, the extracts of other medicinal plants in preventing liver damage have been previously studied and it has been proven that the seeds of the silymarin plant containing silybine, silicristin, silydianine and isosilybinin flavonoids, had an inhibitory effect on the detoxification system of cytochrome P450 and could prevent the metabolism of toxic compounds such as thioacetamide, acetaminophen and carbonic trichloride, which was consistent with the results of the present study. According to the results, administration of hydroalcoholic extract of Thymbra in all three doses significantly increased the serum SOD concentration in the treatment groups compared to the untreated control group. In addition, hepatoprotective effects of some medicinal plants have been investigated, among which *Cynara scolymus, Feniculum vulgare, Solanum nigrum, Terminalia chebula* and *Carum copticum* could be pointed out and the hepatoprotective effects of these plants was attributed to their glycosides, flavonoid, triterpene and phenolic compounds [16-18].

Although the hepatoprotective effects of the mentioned medicinal plants have been identified, and the results of this study also indicated the hepatoprotective effects of *Thymbra spicata*, it is suggested to investigate the combined effects of the effective substances of these medicinal herbs or their effects in combination with chemical drugs in further studies to design and find medicinal products of plant origins. Also, in many studies about the hepatoprotective effects of medicinal plants, carbon tetrachloride has been used to induce poisoning. Although the consumption and poisoning induced by carbon tetrachloride is normally very low, but the its poisoning effects in tissue is similar to the effects of poisoning induced by some drugs, but using drugs directly, in diagnosis of the mechanism and toxic metabolites is better [18, 19]. Studies have shown that acute doses of toxic substances and some drugs such as acetaminophen or the long-term use of some substances and drugs could produce high amounts of active radicals that these radicals could overcome the antioxidant defense system and cause liver damage that is in agreement with the results of this study [20]. In general, the results of this study indicated that the hydroalcoholic extract of *Thymbra spicata* had a significant antioxidant effect which played a protective role in recovery from the acute liver toxicity induced by acetaminophen and after further studies in the food and pharmaceutical industries, it could be used with more confidence.

References

1. McGill MR, Sharpe MR, Williams CD, Taha M, Curry SC, Jaeschke H. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. The Journal of clinical investigation. 2012 Apr 2;122(4):1574-83.
2. Bentley E, Mackie IC. Trends in prescriptions of paracetamol for children. Bmj. 1995 Aug 5;311(7001):362.
3. Sakeran MI, Zidan N, Rehman H, Aziz AT, Saggu S. Abrogation by Trifolium alexandrinum root extract on

hepatotoxicity induced by acetaminophen in rats. Redox Report. 2014 Jan 1;19(1):26-33.

4. Jaeschke H, Williams CD, Ramachandran A, Bajt ML. Acetaminophen hepatotoxicity and repair: the role of sterile inflammation and innate immunity. Liver International. 2012 Jan;32(1):8-20.
5. Vouffo EY, Donfack FM, Temdie RJ, Ngueguim FT, Donfack JH, Dzeufiet DD, Dongmo AB, Dimo T. Hepatho-nephroprotective and antioxidant effect of stem bark of Allanblackia gabonensis aqueous extract against acetaminophen-induced liver and kidney disorders in rats. Journal of Experimental and Integrative Medicine. 2012 Oct 1;2(4):337-44.
6. Rahmani, A. H., The protective effect of red lentil extract on hepatotoxicity induced by carbon tetrachloride in small white mice, Journal of Babol University of Medical Sciences, 2013; 16(2): 55-49.
7. Fakurazi, S., Sharifudin, S.A. and Arulselvan, P., 2012. Moringa oleifera hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. Molecules, 17(7), pp.8334-8350.
8. Kaushik V, Chaudhary G, Ahmad S, Saini V, Pandurangan A. Diverse Sources of Hepatotoxicity in Rats–A Review. International Journal of Pharmaceutical Sciences Review and Research. 2013;21(1):254-9.
9. Khorsandi, L. S., Taheri Mobarakeh, M., Kalantari, H., Protective effect of Curcuma Longa extract on acute liver toxicity induced by acetaminophen in rat, Journal of Rafsanjan University of Medical Sciences, 2007; 4: 226-219
10. Mohammad Zarin Abadi, M., Seyyedi, M., Movahedi, Kh. Chemical composition and anti-bacterial and antioxidant properties of essential oils of Thymbra spicata. Journal of the 7th Iranian Horticultural Science Congress, Isfahan University of Technology, September 2011:2550-2548.
11. Dorman HJ, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of applied microbiology. 2000 Feb;88(2):308-16.
12. Mosleh Arani, A., Naderi, M., Gholam Nia, A., Piri, A.-S., Kahzadian, M., Study of the effects of salinity and drought stress on germination and early growth of the medicinal species of Thymbra spicata L, Bi Quarterly Journal Khoshk Boom, Spring and Summer 2014, 4 (1).
13. Ali-Shtayeh MS, Yaniv Z, Mahajna J. Ethnobotanical survey in the Palestinian area: a classification of the healing potential of medicinal plants. Journal of Ethnopharmacology. 2000 Nov 1;73(1-2):221-32.
14. Tümen G, Ermin N, Özek T, Kürkçüoglu M, Baser KH. Composition of essential oils from two varieties of Thymbra spicata L. Journal of Essential Oil Research. 1994 Sep 1;6(5):463-8.
15. Akkol EK, Avcı G, Küçükkurt I, Keleş H, Tamer U, Ince S, Yesilada E. Cholesterol-reducer, antioxidant and liver protective effects of Thymbra spicata L. var. spicata. Journal of ethnopharmacology. 2009 Nov 12;126(2):314-9.
16. Adewusi EA, Afolayan AJ. A review of natural products with hepatoprotective activity. Journal of Medicinal Plants Research. 2010 Jul 4;4(13):1318-34.
17. Heidarian E, Jafari-Dehkordi E, Seidkhani-Nahal A. Effect of garlic on liver phosphatidate phosphohydrolase and plasma lipid levels in hyperlipidemic rats. Food and chemical toxicology. 2011 May 1;49(5):1110-4.
18. Kim SH, Cheon HJ, Yun N, Oh ST, Shin E, Shim KS, Lee SM. Protective effect of a mixture of Aloe vera and Silybum marianum against carbon tetrachloride–induced acute hepatotoxicity and liver fibrosis. Journal of pharmacological sciences. 2009;109(1):119-27.
19. Sidana J, Deswal G, Nain P, Arora K. Liver toxicity and hepatoprotective herbs. International Journal of Pharmaceutical Sciences Review and Research. 2011;9(1):116-21.
20. Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. The Journal of laboratory and clinical medicine. 1975 Feb 1;85(2):337-41.