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ASSESSMENT STUDY OF ALPHA-FETOPROTEIN LEVEL AFTER TREATMENT WITH *URTICA DIOICA* PHENOLIC EXTRACT IN MALE RAT INDUCED BY CARBON TETRA-CHLORIDE

Murtadha M. Jawad[1,*], Arshad Noori Aldujaili [2] and Merza H. Homady[3]

[1]Technical Institute of Diwaniyah, AL-Furat AL-Awsat Technical University, AL-Najaf, Iraq.
[2,3]Department of Biology, Faculty of Science, University of Kufa, AL-Najaf, Iraq.

Abstract

The present study was conducted on 70 males of Wistar rat weightings (190-300 g), aged (15-17) weeks, at the animal house Science College, Kufa University during the period from December 2015 to July 2016. This study included some physiological and histological criteria to evaluate the defensive role of the phenolic compound of Urtica dioica leaves (250 and 500mg/kg) against hepatotoxicity induced by carbon tetrachloride. The animals experimental are divided into 22 groups (n= 5 rats per each group (22×5=110) for duration of two and three months. The levels of biomarker protein (Alpha-Fetoprotein), were measured in control groups, groups induced at carbon tetrachloride and groups treated by the phenolic extract of *Urtica dioica*. The results showed a significant increase ($P \leq 0.05$) in serum levels of Alpha-Fetoprotein in carbon tetrachloride groups as compared with the control group. A significant decrease ($P \leq 0.05$) in the serum levels of Alpha-Fetoprotein in groups treated with phenolic extract of *Urtica dioica* as compared with carbon tetrachloride group.

Key words : *Urtica dioica*, liver, carbon tetrachloride, alpha-fetoprotein.

Introduction

Liver damage produced by c hemicals or irresistible specialists may prompt progressive liver fibrosis and at last cirrhosis and liver failure (Field *et al*., 2008). Nonetheless, no successful treatment that postpones illness movement and complexities has yet been found. Late investigations propose that customary herbs and micronutrients, for example, carotenoids and selenium might be valuable for this reason (Sakka, 2007).

Excessive and large exposure to the poisons of all kinds, which disrupt liver function and therefore in breach of its functions manufacturing or storage that refers to hepatic toxicity (Schrem *et al*., 2002). The toxins that are exposed to the liver can be classified as external sources, which are exposed to the body in general through food, drink and inhalation, while the internal ones are produced from the metabolism of substances found inside the body, such as medicines and food additives (Singh *et al*., 2011).

Carbon tetrachloride can induce induced hepatotoxicity in laboratory animals by metabolizing it to trichloromethyl free radical, which causes of fat peroxidation in the liver cells (Pushplata *et al*., 2014). As a result of the toxic effects of carbon tetrachloride, the synthesis of RNA is stopped and subsequent production of proteins that lead to hepatic necrosis.

Urtica dioica L. ordinarily known as Stinging Bramble is a herbaceous lasting plant that develops in tropical regions around the globe (Bliddal and Christensen, 2009; Gül *et al*., 2012). Stinging Plant has been among the key plants of the European Pharmacopoeia since antiquated circumstances. It has a place with Urticaceae family in the request of Rosales that contains around Sixty genera and more than Seven hundred species. *U. dioica* is a dioecious herbaceous, compasses to 1-2 m high (Hadizadeh *et al*., 2009; Hammod *et al*., 2016b; Hammod *et al*., 2016).

The alpha-fetoprotein is a glycoprotein produced at all stages of the fetus to the elderly, which regulates most important functions of the body as the differentiation of

Author for correspondence : E-mail: murtadham.alkhafaji@student.uokufa.edu.iq

cells and regulate the metabolism of fat and glucose is an important sign of chronic liver disease (Bertino *et al*., 2011). The aim of this research was to investigate the protective role of the phenolic extract of *U. dioica on* liver injury induced by CCL_4.

Materials and Methods

Animals

Seventy male rats *Rattus norvegicus* weighting (Average weight: 190-300 g) and aged between (15-17 week) were obtained from the Faculty of Science, Thi-Qar University. The study started from Dec. 2015 to July 2016. Animals were housed in the animal house of faculty of Science/University of Kufa under control condition; light 12 and 12 dark hours and a temperature range from (20-24°C).

Preparation of CCL_4

Carbon Tetrachloride (CCL_4) were obtained from [Merck Ltd., Coimbatore, Tamilnadu (India)]. CCL_4 [(1 ml/kg body weight) combined with olive oil (1:1)] (Al-Seeni *et al*., 2016). The procedure of CCL_4 doses daily administration to male animal rats was orally for two and three months.

Preparation of *Urtica dioica* extract

U. dioca leaves was obtained from botany gardens of Baghdad University. The phenolic extract was prepared by using Sexholate and evaporated by rotary evaporation. Then, the solution was transferred for purification of phenolic compounds, after that dry, weighted and stored in a refrigerator until using (Harborne, 1984). Take 2g of phenolic extract powder were diluted in 10 mL of distal water as a stock solution for preparation of 250mg/kg and 500mg/kg concentrations of phenolic extract of *Urtica dioica* (Phenolic extraction).

Plant Doses Selection

A previous study documented that 2 g/kg of the phenolic extract was not toxic (Adedapo *et al*., 2009), therefore the present study was selected a 250 mg/kg and 500 mg/kg of phenolic extract, respectively. All doses are orally administered for two; three months for 2 hours before CCL_4 administration.

Phytochemical screening

The preliminary phytochemical screening of *U. dioica* leaves was done according to Harborne method (Harborne, 1998).

Methods

Animals sacrificing

The animals were sacrificed at end of the experiments by using an anaesthetizing xylene and ketamine (3/1 mL). The abdominal lumen was opened and liver was removed and transferred to formalin fixative (10%) for histological preparation.

Blood samples

The blood was drawn through heart puncture by using disposable syringe (5 mL), then left at room temperature for clotting, and then centrifuged at 3000 rpm for 15 minutes, serum was isolated and stored at deep freeze (-20^0 C or -80^0C) in Al-Sadar medical city in Al-Najaf Al-Ashraf province until using for measurements biomarkers and liver enzymes.

Biomarker liver protein

The assessment of alpha-fetoprotein rats Elisa kits provided by (Elabscience, China) Sandwich immunoassay technique (enzyme-linked immunosorbent assay – automated microtiter plate), Elisa reader (Biokit ELX 800 reader, ELX50 washer/USA).

Statistical analysis

Results was expressed as a mean ± standard error (SE) and performed using multivariate ANOVA by Graph Pad Prism® software (Graph Pad Software, Inc., La Jolla, CA, USA) and comparison between groups using t-test. Statistical significance was $p \leq 0.05$ (Al-Rawi, 2000).

Results

Biomarker protein

The results of the table 1 showed a significant increase ($P \leq 0.05$) in the level of biomarkers (AFP) in the group treated by CCL_4 (18.06±0.56), as compared with the control group (8.11±0.28). Also, this table revealed the protective effects of both concentrations of the phenolic extract before and after treated by CCL_4. The Results also showed a significant decrease ($P \leq 0.05$) in the level of AFP, as compared with CCL_4 treated group.

Effect of duration of a phenolic extract of *U.dioica* leaves (two and three months) for hepatotoxicity male rats treated by CCL_4

Biomarker protein

Fig. 1 showed a significant increase ($P \leq 0.05$) in levels AFP in CCL_4 treated groups for both periods (two and three months) (16.26±0.71; 18.91±0.36), respectively, as compared with control group (8.31±0.31; 8±0.57). Moreover, these figures also showed a significant decrease ($P \leq 0.05$) in the levels of AFP before and after induction for both periods as compared with CCL_4 treated group for both periods.

Additionally, the results of these figures did not show any significant difference (P≤0.05) between the periods used.

Effect of interaction between doses and periods of phenolic extract of *U. dioica* leaves in hepatotoxicity male rats treated by CCL_4

Biomarker protein

Fig. 2 showed a significant increase (P≤0.05) in levels of Biomarker (AFP) in treated groups for both Periods (two and three months) (16.26±0.71; 18.914±0.36), respectively, as compared with control group (8.31±0.31; 8±0.57).

Also, the results of figs. 1 and 2 described the interaction between doses and periods, which showed a significant decrease (P≤0.05) in levels of AFP in treated groups for both periods as compared with CCL_4 group. Treatment with a dose (500mg/kg) also resulted in a significant decrease (P≤0.05), where no significant difference was seen between the periods.

Discussion

Biomarker protein

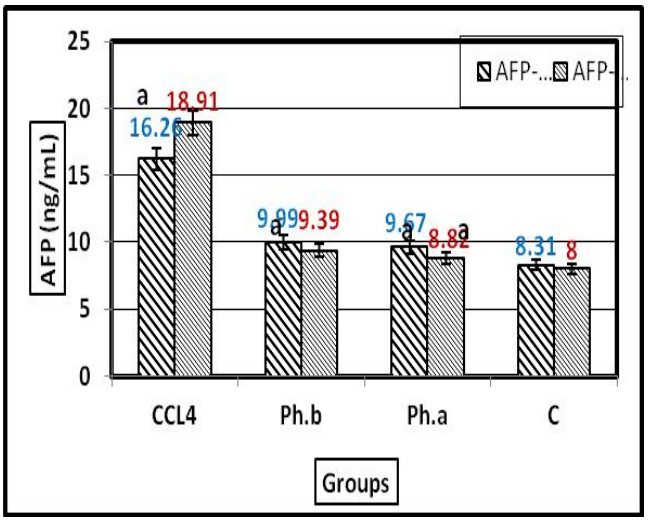
Alpha-fetoprotein protein

The results of table 1 and figs. 1 and 2 revealed a significant increase in alpha-fetoprotein level after induction by CCL_4. The present results are in agreement with the findings of Zhang (2004), who stated that increase in Alpha-fetoprotein level after induced by CCL_4, which is hepatotoxic causing liver necrosis, fibrosis, and cirrhosis. A recent study suggested that carcinogenesis by CCL_4 is secondary to its hepatotoxic effect (Provincial, 2010).

Table 1 : The effects of two concentrations of phenolic extract of *U. dioica* leaves on the level of AFP in hepatotoxicity male rats treated with CCL_4.

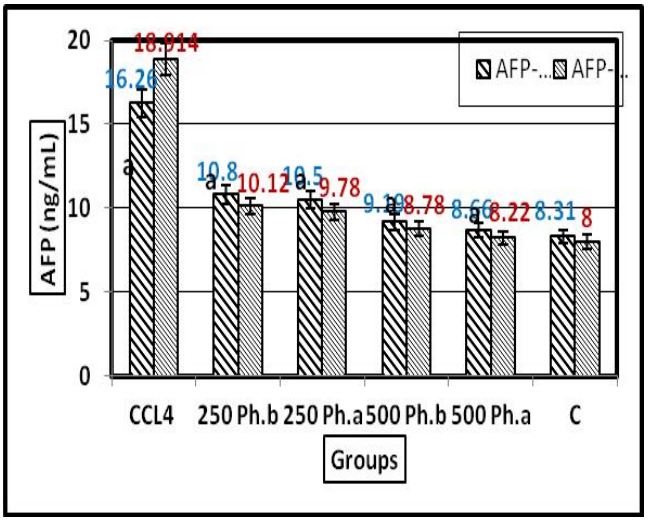
Parameters / Treated	Mean ± SE AFP (ng/mL)
Control	8.11±0.28
CCL_4	18.06±0.56**a**
250ph.b	10.47±0.31**ab**
250ph.a	10.21±0.31**ab**
500 ph.b	9.19±0.33**abcd**
500ph.a	9.14±0.27**abcd**
L.S.D ≤ 0.05	**0.9**

(Ph: Phenolic extract, a: After administered CCL_4, b: Before administered CCL_4. Similar letters indicate non-significant, while the different letters indicate significant compared treated groups vs control group) (n=20 for CCL_4 group and n=10 for other groups).



(Ph: Phenolic extract, A: After administered CCL_4, B: Before administered CCL_4. Similar letters indicate non-significant, while the different letters indicate significant compared treated groups vs control group) (n=20 for CCL_4 group and n=10 for other groups).

Fig. 1 : Effect of duration of phenolic extract of *U. dioica* leaves (two and three months) on AFP level in hepatotoxicity male rats treated by CCL_4.



(Ph: Phenolic extract, A: After administered CCL_4, B: Before administered CCL_4. Similar letters indicate non-significant, while the different letters indicate significant compared treated groups vs control group) (n=20 for CCL_4 group and n=10 for other groups).

Fig. 2 : Effect of interaction between two doses (250 and 500mg/kg) and duration (two and three month) of phenolic extract of *U.dioica* leaves on AFP level in hepatotoxicity male rat treated by CCL_4.

Moreover studied by Manibusan *et al*. (2010) also supported the present findings, the inhalation of CCL_4 leads to increase in Alpha-fetoprotein level in rat and mice.

Previous studies have confirmed that the increase in AFP level is associated with acute and chronic liver injury

(Takikawa and Suzuki, 2002; Schiodt *et al*., 2006). ATP can be produced from liver progenitor cells as well as the ability to produce them from the major cells contributing to the regeneration process (Kuhlmann and Wurster, 1980; Fausto *et al.*, 2006). The AFP level is also associated with progression and severity of the disease (Eleazar *et al.*, 2004; Fujiwara *et al.*, 2011).

The results of a table 1 and figs. 1 and 2 showed a significant decrease in alpha-fetoprotein level of treated with *U. Dioica*. According to our knowledge, there was no any study deals with the effect of *U. dioica* on alpha-fetoprotein level.

Many of the medicines used in folk medicine, which is extracted from medicinal plants as well as *Cassia fistula* and *Ficus carica*, have effects on disease, anti-cancer and atherosclerosis (Bisht *et al*., 2011; Chawla *et al*., 2012).

Leung *et al*. (2002) stated that olive leaf contains the polyphenolic compound with high activity reduce liver enzyme after treatment, as well as decrease alpha-fetoprotein.

The AFP levels may be depleted after treatment with carbon tetrachloride due to interference phenolic compounds that possess antioxidant effects, Anti-cancer and Anti-necrosis, Studies have shown that phenolic compounds, in general, are important plant components for their ability to remove free radicals because of the hydroxyl group, which is antioxidant in many phenolic plant extracts (Kataki *et al*., 2012b; Oktay *et al*., 2003). A study that linked antioxidant activity to polyphenols due to its ability to stabilize lipid peroxidation and carcinogenesis, leading to its relationship to decreasing AFP level (Duh *et al*., 1999). Because of the richness of the phenolic compounds of the U.dioica, it is considered the important source and the main factor for the supply of antioxidants agents (Kataki and Murugamani, 2012).

Conclusion

The present study concluded that phenolic extract of *Urtica dioica* leaves had a protective effect on hepatotoxicity in carbon tetrachloride induced group.

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