Study of the effect of the phenolic extract of the Urtica dioica the level of Des-gamma-prothrombin after treatment with carbon tetrachloride

Murtadha M Jawad, Arshad N Aldujaili

1Department of Nursing, Al-Dewaniyah Technical Institute, Al-Furat Al-Awsat Technical University, 51009 Dewaniyah, Iraq.
2Department of Biology, Faculty of Science, University of Kufa, 31001 AL-Najaf, Iraq
E-mail: murtadham.alkhafaji@student.uokufa.edu.iq

Abstract. The present study was conducted on 90 males of Wistar rat weightings (190-300 g), aged (15-17) weeks, at the animal house faculty of science / university of Kufa during the period from December 2016 to July 2017. This study included some physiological to evaluate the protective role of phenolic extract of Urtica dioica leaves (250 and 500 mg/kg ) against hepatotoxicity induced by carbon tetrachloride. The animals experimetal are divided into 22 groups (n= 5 rats per each group) for duration of two and three months. The results showed a significant increase (P ≤0.05) in serum levels of biomarker proteins (Des gamma carboxy prothrombin) in carbon tetrachloride groups as compared with the control group. A significant decrease (P ≤0.05) in the serum levels of biomarker proteins (Des gamma carboxy prothrombin) in groups treated with phenolic extract of Urtica dioica as compared with carbon tetrachloride group. The present study concluded that phenolic extract of Urtica dioica leaves had a protective effect on hepatotoxicity in carbon tetrachloride induced group.

Keywords: Carbon tetrachloride, Hepatotoxicity, Urtica dioica, Des-γ-Carboxy Prothrombin.

1. Introduction:

Des-γ-Carboxy-Prothrombin Protein Markers (DCP) is the prothrombin precursor maintaining some glutamic acid (Glu) residues in the N-terminal domain (Inagaki et al., 2011). In hepatocyte, prothrombin is synthesized depending on the presence of vitamin K-dependent γ-glutamyl carboxylase (Morimoto et al., 2014). In hepatocyte, these Glu residues must be converted into Gla residues before prothrombin obtains the coagulation activity, this conversion is the process of post-translational modification of prothrombin in the lumen of endoplasmic reticulum (Meguro et al., 2015). However, in HCC cells, there may occur vitamin K deficiency or vitamin K antagonists, leading to lower activity of γ-glutamyl carboxylase (Kamiyama et al., 2015). HCC cells are therefore unable to completely carboxylate these Glu residues into Gla, resulting some Glu residues leaving in N-terminal domain and these proteins are called prothrombin precursors or des-γ-carboxy prothrombin (DCP). As one of three most common markers, DCP has been used for diagnosis of HCC, other two plasma markers are alpha-fetoprotein (AFP) and Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) (Cheng et al., 2014).
Urtica dioica is a common species in two types of wet woodland, Mire vegetation, Mesotrophic and calcicolous grasslands, Maritime vegetation and Vegetation of open habitats (Rodwell, 2000). The leaves and stems are very hairy with non-stinging hairs and also bear many stinging hairs or trichomes (Golalipour et al., 2010), whose tips come off when touched, transforming the hair into a needle that will inject several chemicals including acetylcholine, histamine, 5-HT (serotonin), moroidin, leukotrienes and possibly formic acid (Casarett et al., 2008; Greenberg., 2003). From current pharmaceutical studies, additional pharmaceutical applications of U. dioica have revealed antioxidant (Jafari and Dehghan, 2012), Urtica dioica sting seems a safe treatment for musculoskeletal pain, it contains serotonin and histamine that are involved in the cascade of stimulation affecting levels of nerve growth factor which in turn increases activation of nociceptive pain neurones (Ozyigit and Akinci, 2009).

Carbon tetrachloride is said to induce hepatotoxicity in rats, rabbits and humans after being metabolised to trichloromethyl free radical which causes peroxidative degradation in the adipose tissue resulting in fatty infiltration of the hepatocytes (Pushplata et al., 2014).

2. Experimental:
   - General procedure:
     Preparation of CCL4:
     Carbon Tetrachloride (CCL4) were obtained from [Merck Ltd., Coimbatore, Tamilnadu (India)]. CCL4 [(1 ml/kg body weight) combined with olive oil (1:1)] (Kadhim et al. 2017). The procedure of CCL4 doses daily administration to male animal rats was orally for two and three months.

   Preparation of Urtica dioica extract:
   U.dioica 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 (Jawad et al. 2018).

3. Methods:
   - Phytochemical Screening:
   The preliminary phytochemical screening of U.Dioca leaves was done according to Harborne method (Jawad et al. 2017).

4. Results:
   Effects of two concentrations of U.Dioica leaves phenolic extract in hepatotoxicity male rats treated by CCL4.

   The results of the table (1) showed a significant increase (P ≤ 0.05) in the level of biomarker (DES) in the group treated by CCL4 (20.81±0.54), as compared with the control group (7.08±0.29). Also, this table revealed the protective effects of both concentrations of the phenolic extract before and after treated by CCL4. The Results also showed a significant decrease (P ≤ 0.05) in the level of biomarker (DES), as compared with CCL4 treated group.
Table (1): showing the effects of two concentrations of phenolic extract of U.dioica leaves on the level of biomarker (DES) in hepatotoxicity male rats treated with CCL4.

| Parameters | Mean ± SE |
|------------|-----------|
| Control   | 7.08±0.29 |
| CCL4      | 20.81±0.54a |
| 250ph.b   | 18.67±0.31ab |
| 250ph.a   | 17.89±0.22ab |
| 500 ph.b  | 14.17±0.65abcd |
| 500ph.a   | 11.04±0.65abcde |

L.S.D ≤0.05 1.21

(Ph: Phenolic extract, a: After administered CCL4, b: Before administered CCL4. Similar letters indicate non-significant, while the different letters indicate significant compared treated groups vs control group) (n=20 for CCL4 group and n=10 for other groups).

Effect of duration of a phenolic extract of U.dioica leaves( two and three months) for hepatotoxicity male rats treated by CCL4.

![Graph showing effects of two concentrations of phenolic extract](image1)

Figure (1): Effect of duration of phenolic extract of U.dioica leaves (two and three months) on DES level in hepatotoxicity male rats treated by CCL4.

(Ph: Phenolic extract, A: After administered CCL4, B: Before administered CCL4. Similar letters indicate non-significant, while the different letters indicate significant compared treated groups vs control group) (n=20 for CCL4 group and n=10 for other groups).

Effect of interaction between doses and periods of phenolic extract of U.dioica leaves in hepatotoxicity male rats treated by CCL4.

Figures (2) showed a significant increase (P ≤0.05) in levels of Biomarker (DES) in treated groups for both Periods (two and three months) (19.66±0.35 ; 21.94±0.94), respectively, as compared with control group (8.5±0.54 ; 9.08±0.27).
Also, the results of figures (2) described the interaction between doses and periods, which showed a significant decrease (P ≤0.05) in levels of Biomarker (DES) in treated groups for both periods as compared with CCL4 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.

![Figure (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 DES level in hepatotoxicity male rat treated by CCL4.](image)

(Ph: Phenolic extract, A: After administered CCL4, B: Before administered CCL4, C: control. Similar letters indicate non-significant, while the different letters indicate significant compared treated groups vs control group) (n=20 for CCL4 group and n=10 for other groups).

5. Discussion:

The results of a table (1) and figure (1); (2) showed a significant increase in des-gamma-carboxy prothrombin level after induction by CCL4.

The present findings of the high level of DCP in serum after induced by CCL4 may consider prognostic and diagnostic markers for liver disease. DCP stimulate angiogenesis in HCC occurs by activation of many other kinase cascades and through activation of MMP including MMP-2 and MMP-9 suggesting that activation vascular endothelial cell by DCP may stimulate (MMP-2) to break down extracellular matrix for proliferative and migration of HCC, therefore, activation or release of MMPS from HCC cell after DCP stimulate might a critical stage to trigger other kinase cascades to angiogenesis these findings are in agreement with the finding of (Gao et al., 2012; Matsubara et al., 2012).

The previous studies show that DCP affected by the etiology of liver disease for the diagnostic of an early stage of hepatocellular carcinoma (HCC) and elevated in plasma in HCC (Fujikawa et al.,2007).

Many studies demonstrated that the elevation of DCP correlates with the presence of intrahepatic metastasis and vascular invasion (Liebman et al.,1984; Hakamada et al.,2008).
However other studies have reported that DCP was an independent prognostic factor for recurrence and survival after hepatic resection in hepatic injury, liver transplantation and chemotherapy (Suehiro et al., 1995; Shimada et al., 2005; Soejima et al., 2007).

Des-gamma-carboxy prothrombin (DES) level depend on or combined with the score of stage and liver damage (Omagari et al., 2004).

Whereas Suzuki et al., (2005) reported that DCP exerts the mitogenic effect on hepatic cell via (Met-janns kinase) signaling pathway.

The results of the table (1) and figures (1); (2) revealed a significant decrease in des-gamma-carboxy prothrombin level after oral administration with U.dioica. Up to our knowledge, there are no research work concerns the effect of U.dioica on DPC level in laboratory animals.

Kim et al., (2010c) suggested that the decreasd level of DCP after treatment with U.dioica and as a result of anti-oxidant and chemo-protect effect of both U.dioica and by scavenger free radical and led to prevent DNA damage, mutagenesis, and carcinogenesis in rat.

On the other gand Fabiani et al., (2002) reported that any blocking in the cell progression at G1 phase inducing apoptosis and protect cells from hydrogen peroxidase (H2O2) by catalase activation after treatment with olive leaf.

The increase activation of antioxidant enzyme such as glutathione-S-transferase (GST) and glutathione- peroxidase (GPX) after treatment with (5%) aqueous extract of neem Azadirachta indica lead to decrease level of alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) due to the repealing the damage in the liver tissue and carcinogenesis (Manal et al., 2007).

In the present study, The decreased level of DCP after treatment with U. dioica may consider as anti-cancers by reducing hepatocytes aggregation; decreases liver damage and expression of CCL4 due to the inhibitory effect on cells proliferation.

6. References

[1 ] Casarett LJ, Klaassen CD, Doull J (2008). Casarett and Doull's toxicology: the basic science of poisons. McGraw-Hill Professional: p.1104.
[2 ] Cheng J, Wang W, Zhang Y, Liu X, Li M, Wu Z, Liu Z, Lv Y, Wang B (2014). Prognostic role of pretreatment serum AFP-L3% in hepatocellular carcinoma: systematic review and meta-analysis. PLoS One;9:e87011.
[3 ] Fabiani, R. A. De Bartolomeo, P. Rosignoli, M. Servili, G. F. Montedoro, and G. Morozzi (2002). Cancer chemoprevention by hydroxytyrosol isolated from virgin olive oil through G1 cell cycle arrest and apoptosis. Eur J Cancer Prev 11,351-8.
[4 ] Fujikawa T, Shiraha H, Ueda N, Takaoka N, Nakanishi Y, Matsuino T, Tanaka A, Sakaguchi K, Shiraton Y(2007). Des-γ-carboxy prothrombin-promoted vascular endothelial cell proliferation and migration. J Biol Chem;282:8741-8748.
[5 ] Gao J, Feng X, Inagaki Y, Song P, Kokudo N, Hasegawa K, Sugawara Y, Tang W(2012). Des-γ-carboxy prothrombin and c-Met were concurrently and extensively expressed in hepatocellular carcinoma and associated with tumor recurrence. Biosci Trends;6:153-159.
[6 ] Golalipour MJ, Ghafari S, Afshar M (2010). Protective role of Urtica dioica L. (Urticaceae) extracts on hepatocytes morphometric changes in STZ diabetic Wistar rats. Turk. J. Gastroenterol. 21(3):262-269.
[7] Greenberg MI (2003). Occupational, industrial, and environmental toxicology. Elsevier Health Sci. p.180.

[8] Hakamada K, Kimura N, Miura T, Morohashi H, Ishido K, Nara M, Toyoki Y, Narumi S, Sasaki M (2008). Des-gamma-carboxy prothrombin as an important prognostic indicator in patients with small hepatocellular carcinoma. World J Gastroenterol;14(9): 1370-1377.

[9] Inagaki Y, Tang W, Makuuchi M, Hasegawa K, Sugawara Y, Kokudo N (2011). Clinical and molecular insights into the hepatocellular carcinoma tumour marker des-γ-carboxyprothrombin. Liver Int;31:22-35.

[10] Jafari, Z. and Dehghan M. (2012). Anatomical Structure Study of Aerial Organs in Four Populations of Urtica dioica L. Journal of Medicinal Plants and By-products. 2: 133-137.

[11] Kamiyama T, Yokoo H, Kakisaka T, Orimo T, Wakayama K, Kamachi H, et al (2015). Multiplication of alpha-fetoprotein and protein induced by vitamin K absence-II is a powerful predictor of prognosis and recurrence in hepatocellular carcinoma patients after a hepatectomy. Hepatol Res;45: E21–31.

[12] Kim, YS, Song, MY, Park, JD, Song, KS, Ryu, HR, Chung, YH, Chang, HK, Lee, JH, Oh, KH, Kelman, BJ, Hwang, IK, Yu, IJ (2010c) Subchronic oral toxicity of silver nanoparticles. Part. Fibre Toxicol. 7, 20.

[13] Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, Coleman MS, Furie B (1984). Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. N Engl J Med; 310: 1427-1431.

[14] Manal, M.E.T. P. Hanachi, I. Patimah, I.A. Siddig and O. Fauziah, (2007). The Effect of Neem (Azadirachta indica) Leaves Extract on Alpha-fetoprotein Serum Concentration, Glutathione S-transferase and Glutathione Peroxidase Activity in Hepatocarcinogenesis Induced Rats. International Journal of Cancer Research, 3: 111-118.

[15] Matsubara M, Shiraha H, Kataoka J, Iwamuro M, Horiguchi S, Nishina S, Takaoka N, Uemura M, Takaki A, Nakamura S, Kobayashi Y, Nouso K, Yamamoto K (2012). Des-γ-carboxyl prothrombin is associated with tumor angiogenesis in hepatocellular carcinoma. J Gastroenterol Hepatol;27:1602–1608.

[16] Meguro M, Mizuguchi T, Nishidate T, Okita K, Ishii M, Ota S, et al (2015). Prognostic roles of preoperative α-fetoprotein and des-γ-carboxy prothrombin in hepatocellular carcinoma patients. World J Gastroenterol;21:4933–4945.

[17] Morimoto Y, Nouso K, Wada N, Takeuchi Y, Kinugasa H, Miyahara K, Yasunaka T, Kuwaki K, Onishi H, Ikeda F, Miyake Y, Nakamura S, Shiraha H, Takaki A, Yamamoto K (2014). Involvement of platelets in extra-hepatic metastasis of hepatocellular carcinoma. Hepatol Res; doi: 10.1111/hepr.12315.

[18] Omegari K, Honda S, Kadokawa Y, Isomoto H, TakeshimaF, Hayashida K, Mizuta Y, Murata I, Kohno S (2004). Preliminary analysis of a newly proposed prognostic scoring system (SliDe score) for hepatocellular carcinoma. J Gastroenterol Hepatol; 19: 805-811.

[19] Ozuyigit, I. I. and S. Akinci. (2009). Effects of some Stress Factors (Aluminum, Cadmium and Drought) on Stomata of Roman Nettle (Urtica pilulifera L.). Not. Bot. Hort. Agrobot. Cluj. 37 (1): 108-115.

[20] Pushplata, C., Yadunath, J. and Ashish, J. (2014) ‘Protective effect of ethanol extract of centaurea behen linn in carbon tetra chloride-induced hepatitis in rats’, International Journal of Pharmacy and Pharmaceutical Sciences, 6(8), pp. 197–200.

[21] Rodwell, J.S. (2000). British Plant Communities. V. Maritime Communities and Vegetation of Open Habitats. Cambridge University Press, Cambridge, UK.

[22] Shimada M, Yonemura Y, Ijichi H, Harada N, Shiotani S, Ninomiya M, Terashi T, Yoshizumi T, Soejima Y, MacharaY (2005). Living donor liver transplantation for hepatocellular carcinoma: a special reference to a preoperative des-gamma-carboxy prothrombin value. Transplant Proc; 37: 1177-1179.
[23] Soejima Y, Taketomi A, Yoshizumi T, Uchiyama H, Aishima S, Terashi T, Shimada M, Machara Y (2007). Extended indication for living donor liver transplantation in patients with hepatocellular carcinoma. Transplantation; 83: 893-899.

[24] Suehiro T, Matsumata T, Itasaka H, Taketomi A, Yamamoto K, Sugimachi K (1995). Des-gamma-carboxy prothrombin and proliferative activity of hepatocellular carcinoma. Surgery;117: 682-691.

[25] Suzuki M, Shiraha H, Fujikawa T, Takaoka N, Ueda N, Nakanishi Y, Koike K, Takaki A, Shiratori Y (2005). Des-gammacarboxy prothrombin is a potential autologous growth factor for hepatocellular carcinoma. J Biol Chem; 280: 6409-6415.

[26] Jawad, M.M., Aldujaili, A.N. & Homady, M.H., 2018. ASSESSMENT STUDY OF ALPHA-FETOPROTEIN LEVEL AFTER TREATMENT WITH URTICA DIOICA PHENOLIC EXTRACT IN MALE RAT INDUCED BY CARBON TETRA-CHLORIDE., 18(1), pp.410–414.

[27] Jawad, M.M., Homady, M.H. & Aldujaili, A.N., 2017. Protective effect of phenolic extract of Urtica dioica leaves against carbon tetra-chloride induced hepatotoxicity in male rats. Research Journal of Pharmacy and Technology, 10(8), pp.2619–2627.

[28] Kadhim, M.M., Aldujaili, A.N. & Homady, M.H., 2017. Assessment of hepatoprotective role of phenolic extract of urtica dioica and silver nanoparticles in male rat induced by carbon tetra-chloride. Rasayan Journal of Chemistry, 10(2), pp.305–312.