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Hepatoprotective effect of *hyoscyamus albus* leaves on carbon tetrachloride-induced an acute hepatotoxicity on rats

Massinissa Yahia, Mouloud Yahia, Afaf Benhouda, Nassiba Chafaa, Hachani Khadraoui, Djahida Benhouda, Boutaghrine Sihem

Biotechnology's Laboratory of the Bioactive Molecules and the Cellular Physiopathology, Department of Biology, University of Batna, Algeria

Abstract
Objective: The goal of this present work is to evaluate the anti-hepatotoxicity activity *in vivo* of the methanolic extract of *Hyoscyamus albus'* leaves (HAMeOH) on Wistar rats against CCl4 induced liver damage.
Methods: The animals were divided into seven groups with five rats in each group. CCl4 (2 mL Kg^{-1} b.w.) was injected subcutaneously in the second and the third day of the experiment, and the methanolic extracts of HAMeOH (100 and 200 mg/ kg b.w.) was given by gavage fifth day of the experiment. The extracts prevented liver damage caused by CCl4, as noted by the significant decrease in serum aminotransferases release and the alkaline phosphatase activities. This extracts decrease the necrosis effect of CCl4 in the histological examination.
Results: Serum ALAT and ASAT and ALP activities significantly decreased in a dose-dependent manner in treatment groups with extracts. Histological examination showed liver damage in HAMeOH-treated groups lower.

Keywords: *hyoscyamus albus*, methanolic extract, hepatotoxicity, carbon tetrachloride

1. Introduction
The liver is a vital part in the body present in vertebrate and other animals. It lays out a large range of functions, including the detoxication, the synthesis of proteins, and the production of biochemical products necessary for digestion [1, 2]. The model of toxicity by carbon tetrachloride (CCl4) is largely studied like a toxicity caused by a chemical product [3]. *H.albus* is a plant which belongs to Solanaceae family; it used in traditional medicine as a nervous sedative and para sympatholytic [4]. Mahmood *et al*., (2001) could isolate some tropane alkaloids such as scopolamine, hyoscyamine, and atropine and also with spectral technics they isolated 2, 3–dimethyl nonacosane.
This present work is for goal to evaluate the activity of anti-hepatotoxicity of the methanolic extracts of *H.albus*.

2. Materials and methods
2.1 Chemicals products
Carbon tetrachloride (Prochima Sigma), quercetin (prochima sigma), Methanol (Prochima sigma), Chloform (Prochima sigma).

2.2 Plant Material and preparation of extracts
The leaves of this plant were collected from Bouzina city, Batna, Algeria. It was identified by Dr. OUDJHIH, Laboratory of Botanic, Department of Agronomy, Batna Algeria. Plant leaves were dried for 40 days at an ambient temperature under shade, after; the leaves were crushed to obtain a fine and homogeneous powder and conserved in dry place. 1 Kg of powdered leaves was extracted with petroleum ether three times 5 L for each time. Then, the marc was dried and extracted with chloroform three times 5 L for each time and with methanol three times 5 L for each time and the supernatants were filtered sequentially using cotton wool, and Whatman filter paper. The solvents were then evaporated under reduced pressure (204 mbar) and controlled temperature (30°C) using a vacuum rotary evaporator (Buchi Rotavapor).

2.3 Phytochemical Screening
The phytochemical screening of HAMeOH was performed using standard method [6]. Phytochemical constituents such as phenolic compounds, terpenoids, saponins, alkaloids, steroids, flavonoids and tannins were qualitatively analyzed.

2.4 Animals
Wistar rats weighted (140-170g) provided by the Pasteur Institute – Algiers. These rats were allowed favorable conditions before and during the experiment: Temperature (23±2) °C, relative humidity 50 -55 %, with 12 hours day / 12 hours night cycle respectively. The food and water were given ad libitum.

2.5 Hepatotoxicity activity
The experiment carried out according to the method described previously by Jain *et al*., (2011) with modifications. The rats were divided randomly into five groups (n=5). Groupe I (normal control): the rats received distilled water (1 ml/kg /day), during 5 days of the experiment and olive oil (1 ml/Kg b.w. injection subcutaneously) in the second and the third day of the experiment. Group II (CCl4): the rats received distilled water (1ml/Kg/b.w. day) during the 5 days of the experiment. Group III: was treated with HAMeOH with an amount of (100 mg/Kg by oral way) during 5 days of the experiment. Group IV: was treated with HAMeOH (200 (mg/kg, b.w.), during the 5 days of the experiment. Group V: was treated with quercetin (50 mg/Kg b.w.) during the 5 days of the experiment. All rats

in the groups II to V received the mixture of CCl4 and the olive oil (v/v, 1:1, 2 ml/Kg b.w.) by injection subcutaneously in the second and the third day thirty minutes after different treatment.

2.6 Biochemical analysis

In the sixth day, all rats were scarified, the blood collected in the heparins tubes and centrifuged 3500 rpm during 5 min.After centrifugation the analyses of the biochemical parameters were made such as: Aspartate aminotransferase (ASAT), Alanine aminotransferase (ALAT), Alkaline phosphatase (ALP).

2.7 Histopathological study

The histopathological study was done according to the method of 11. Organs (liver and Kidney) were fixed in a formalin solution 10%, after these organs are embedded in the paraffin, these tissues were cut with microtome 5 µm of thickness and are mounted on slides and stained with the hematoxylin-eosin.

2.8 Statistical analysis

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as signifiant.

3. Results

3.1 Phytochemical Screening

The phytochemical screening of HAMeOH revealed the presence of alkaloids, terpenoids, saponins, condensed tannins, steroids, polyphenol compounds and also favonoids (Table 1).

Table 1: Phytochemical constituents of methanolic extract from *H.albus's* leaves.

Sample	Phytochemical constituents	Result
HAMeOH	Alkaloid	+++
	Saponin	+++
	Flavonoid	++
	Tannins and polyphenolic compounds	++
	Terpenoids	+++
	Steroid	++

+++: Very positive reaction, ++: positive reaction, -: negative reaction

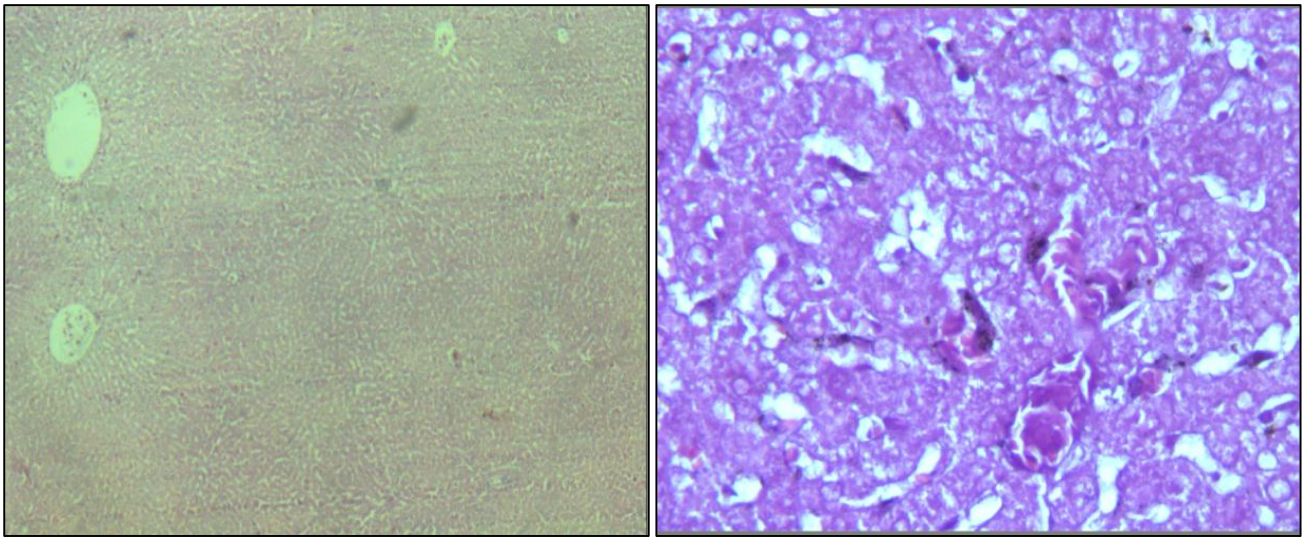
3.2 Effects of HAMeOH extracton serum ALAT, ASAT, ALP and BT activities in CCl4 intoxicated rats

Table 2: Effects of HAMeOH on CCl4 induced hepatotoxicity in rats.

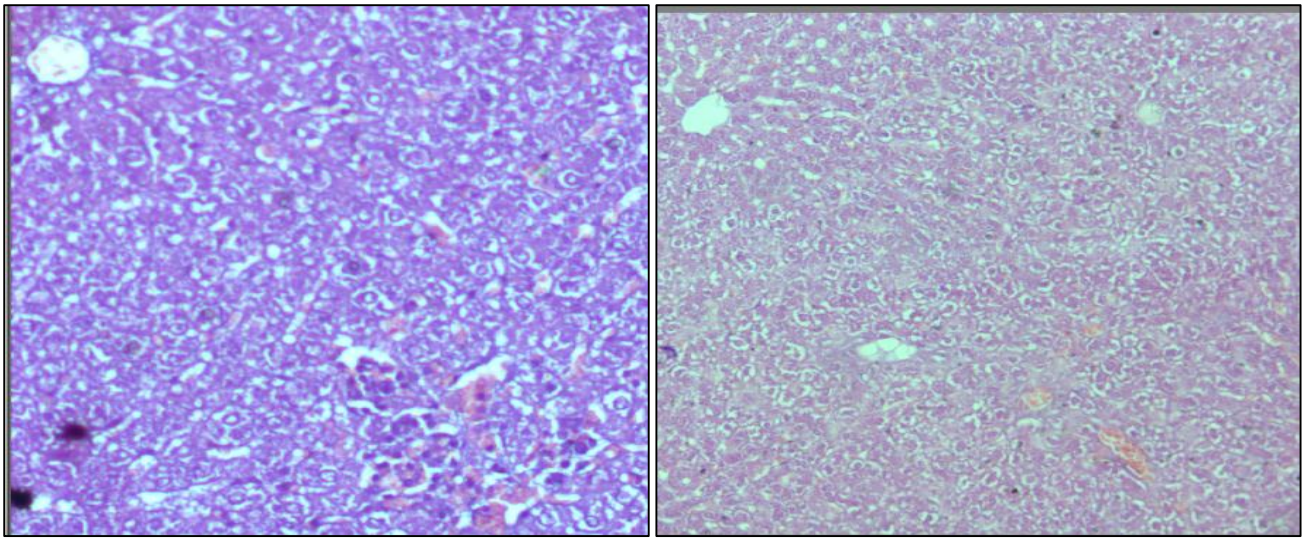
	Liver Weight (g)	ASAT (U/L)	ALAT(U/L)	ALP(U/L)	BT (mg/L)
Temoin	6,160±0,028 ***	4,750±3,606***	6,900±4,243***	41,44± 3,472**	0,9400.±0,07***
CCl4 control	9,285±0,233	174 ,0±15,56	154,1 ± 15,41	239,5± 67,18	16,75± 3,88
HAMeOH (100mg/k)	7,330±0,141 ***	36,10±5,515***	34,10±1,556***	55,93± 1,336**	1,665± 0,47***
HAMeOH (200mg/kg)	6,700±0,070 ***	14,90± 0,42***	14,25± 3,74***	47,97± 0,183**	1,005± 0,021***
Quercetin (50mg/kg)	6.560±0,12***	12,90±8,48***	19,00± 6,788***	45,88± 0,14**	1,605± 0,38***

Each value represents the mean± SD, **P < 0.001 when compared with CCl4 group in ALP analyses. ***P < 0.0001 when compared with CCl4 group.

3.3 Result as of liver histological sections



[a] [b]



[c] [d]

Fig 1: Photomicrography of liver sections of rats. a. liver sections of normal rats treated with olive oil vehicle only; b. liver section of the control rat treated with CCl4 with the olive oil only; c. liver section of the CCl4-treated rat treated by HAMeOH at 100 mg/kg; d. liver section of the rat treated by HAMeOH at 200 mg/kg; e. (H&E stain, original magnification ×100).

Results from the histological studies were in agreement with the measured activities of serum enzymes. There were no abnormalities or histological changes in the livers of normal rats (Figure 1a). Severe hepatocyte necrosis, inflammatory cells infiltration and fatty degeneration were found in rats treated by CCl4 with olive oil (Figure 1b). Fatty change, lymphocyte infiltration and hemorrhage were improved in the histological sections of rat treated by HAMeOH (Figure 1c). The second dose of HAMeOH (200mg/kg, b.w.) also attenuated the fatty change and the lymphocyte infiltration in the liver section induced by CCl4 (Figure 5d). These results indicated the effects of HAMeOH against CCl4-induced acute liver damage in a dose-dependent manner.

4. Discussion

The liver is an important site for the synthesis of many serums. The oxidative lesions of certain amino-acids are regarded as the major cause of metabolic disorders of hepatic lesions [9]. The model of toxicity by carbon tetrachloride (CCl4) is very known by its hepatoxicity [10].

In the present investigation, the increased levels of ASAT, ALAT have been observed in CCl4 control group. Many research confirmed this elevation after CCl4 hepatotoxicity in rats [11-14]. This elevation indicating the high permeability of liver cells [15, 16]. The ASAT and the ALAT levels are the better indexes of liver damage diagnosis. These markers is releasing in liver injury and liver necrosis cases in the blood [17], because these enzymes are located in the cytoplasm [18]. The results of the treatment groups indicate the decrease levels of these markers according to dose manner of our extract (HAMeOH). Thabrew and Joice, (1987) [19] showed that the conversion of enzymes levels towards the normal level indicate the hepatocytes regeneration.

The ALP is the best parameter for the detection of the hepatic lesions. The elevation of the ALP serum level is an index of the loss of functional integrity of the celluar membranes of the liver [20].

The CCl4 increase the ALP level when previous experimental studies showed that CCl4 increases significantly the the level of ALP serum [21]. In the treatment groups, the ALP level decrease according to dose administrated, this decreasing indicates the stability of the biliary dysfunction in the liver during chronic hepatic injury [22].

In this study, the weight results indicated the hepatotoxic role of CCl4, where it increases this parameter. Orisakwe *et al*., 2003 [23], reported that the increase or the reduction of organ weight after chemical products administration is a toxic effect of this product.

Ahmed *et al*., (2012) [24] reported that the CCl4 induced histopathological changes.

The bilirubin is an endogenous anion derived from the regular degradation of hemglbin of the red and excreted globules of the liver in the bile [25].

The hyperbilirubinemy is a very sensitive test to justify the functional integrity of the liver and the severity of necrosis which increases the excretory abilities of the hepatocytes and which is proportional with the erythrocytes degeneration in the spleen [26]. In our study, the extract HAMeOH increase the BT level in a dose dependent manner, this reduction can be according to the hepatic cells regeneration.

The histological results indicate the presence of fatty changes, necrosis and sinusoidal spaces are flooded with inflammatory cells, all this observations are reported in many researchs [21]. The HAMeOH doses eliminate the necrotic effects of CCl4, and reduce the fatty changes in the liver section with reduction of inflammatory cells in sinusoidal spaces.

The results of biochemical markers lead to the histological results, where the necrosis releases the ALAT and ASAT [20, 27] and ALP [28] in the blood circulation.

The hepatoprotective activity of *Hyoscyamus albus* extract reveal to the presence of many compounds as polyphenols [29, 30], saponines [31, 32, 33], tannins [34], the flavonoïdes [35-39] and terpenoids [40-41].

Conclusion

In conclusion, the results of this study demonstrate that methanolic extract of *Hyoscyamys albus* has a potent hepatoprotective action against CCl4 induced hepatic damage

in rats. Further studies are important to isolate the principal compounds actives the hepatoprotective activity.

References

1. Karan M, Vasisht K, Handa SS. Antihepatotoxic activity of Swertia chirata on carbon tetrachloride-induced hepatotoxicity in rats. Phytother. Res. 1999; 13:24-30.
2. Chaterrjee TK. Medicinal Plants with Hepatoprotective Properties. In Herbal Options; Books and Applied Allied (P) Ltd: Calcutta, India, 2000; p. 143.
3. Xiao-feng J, Jie Q, Yan-hua L. The role of hepatoprotective effect of a flavonoid-rich extract of *Salvia plebeian* R.Br. on carbon tetrachloride induced acute hepatic injury in mice. J. of Medicinal Plants Res. 2011; 5:1558-1563.
4. Jouzier E. Solanacees medicinales et philatelie Bull. Soc. Pharm. Bordeaux. 2005; 144:311-332.
5. Mahmood U, Yogendra S, Raghunath S, Thakur R. 2, 3-di methyl nonacosane and tropane alkaloids from *Hyoscyamuss albus*. Phytochemistry. 2001; 24(7):1618-1619.
6. Harborne JB. Phytochemical methods, a guide to modern techniques of plant analysis. Thirdth edition. New York: Chapman and Hall Int. Ed, 2001, 1998.
7. Jain NK, Lodhi S, Jain A, Nahata A, Singhai AK. Effects of *Phyllanthus acidus* (L.) Skeels fruit on carbon tetrachlorideinduced acute oxidative damage in liver of Wistar rats and mice. J Chin Integra Med. 2011; 9:48-55.
8. Lamb GM. Manual of veterinary techniques in Kenya. Kenya: Ciba-Geigy, 1981; pp.100-104.
9. Bandyopadhyay U, Dipak D, Banerji RK. Reactive oxygen species. Oxidative damage and pathogenesis. Curr. Sci. 1999; 5:658.
10. Lee KS, Lee SJ, Park HJ, Chung JP, Han KH, Chon CY. Oxidative stress effect on the activation of hepatic stellate cells. Yonsci Med J. 2001; 42(1):1-8.
11. Tribble DL, Aw TY, Jone DP. The pathophysiological significance of lipid peroxidation in oxidative cell injury. J Hepatol. 1987; 7:377-386.
12. Wang PY, Kaneko T, Tsukada H, *et al*. Time courses of hepatic injuries induced by chloroform and by carbon tetrachloride: comparison of biochemical and histopathological changes. *Arch Toxicol*. 1997; 71(10):638-645.
13. Mehmetcik G, Ozdemirler G, Koc N, *et al*. Role of carnosine in preventing thioacetamide-induced liver injury in the rat. Peptides. 2008; 29:425-429.
14. Arici OF, Cetin N. Protective role of ghrelin against carbon tetrachloride (CCl4 induced coagulation disturbances in rats. Regul Pept. 2011; 166:139-142.
15. Naik SR, Panda VS. Hepatoprotective effect of Ginkgoselect Phytosome in rifampicin induced liver injury in rats: evidence of antioxidant activity. Fitoterapia. 2008; 79:439-45.
16. Jain NK, Singhai AK. Ameliorative effects of Spinacia oleracea L. seeds on carbon tetrachloride (CCl4) - induced hepatotoxicity: In vitro and in vivo studies. Asian Pacific Journal of Tropical Biomedicine. 2012; S232-S237.
17. Shanmugasundaram P, Venkataraman S. Hepatoprotective and antioxidant effects of *Hygrophila auriculata* (K. Schum) Heine Acanthaceae root extract. J Ethnopharmacol. 2006; 104:124-128.
18. Sallie R, Tredger JM, William R. Drugs and the liver. Part I. Testing liver function. Biopharm Drug Disp. 1991; 12:251-259.
19. Thabrew M, Joice P. A comparative study of the efficacy of Pavetta indica and Osbeckia octanda in the treatment of liver dysfunction. Planta. Med. 1987; 53(3):239-241.
20. Drotman R, Lawhan G. Serum enzymes are indications of chemical induced liver damage. Drug Chem Toxicol. 1978; 1:163-171.
21. Khan AA, Alzohairy M. Hepatoprotective effects of camel milk against CCl4-induced hepatotoxicity in Rats. Asian J Biochem. 2011; 6(2):171-180.
22. Navarro VJ, Senior JR. Drug-related hepatotoxicity. N Engl J Med. 2006; 354:731-9.
23. Orisakwe OR, Afonne OJ, Chude MA, Obi E, Dioka CE. Sub chronic toxicity studies of the aqueous extract of Boerhavia diffusa leaves. J. Health. Sc. 2003; 49:444-447.
24. Ahmed MF, Rao AS, Ahemad SR, Ibrahim M. Phytochemical Studies and Hepatoprotective activity of *Melia azedarach* Linn, against CCl4 induced Hepatotoxicity in rats. Journal of Pharmacy Research. 2012; 5(5):2664-2667.
25. Saukkonen JJ, Cohn DL, Jasmer RM, Schenker S, Jereb JA, *et al*. An Official ATS Statement: Hepatotoxicity of antituberculosis therapy. Am J Respir Crit Care Med. 2006; 174:935-952.
26. Singh B, Saxena AK, Chandan BK, Anand KK, Suri OP, Suri KA, *et al*. Hepatoprotective activity of verbenalin on experimental liver damage in rodents, Fitoterapia. 1998; 69:135-140.
27. Chandrashekhar VM, Haseeb TA, Habbu PV, Nagappa AM. Hepatoprotective activity of *Wightia tinctoria* (Roxb) in rats. Indian Drugs. 2004; 41(6):366.
28. Zeashan H, Amresh G, Singh S, Rao CV. Hepatoprotective activity of Amaranthus spinosus in experimental animals. Food Chem. Toxicol. 2008; 46:3417-3421.
29. Lee HS, Keum KY, Ku SK. Effects of picrorrhiza rhizome water extracts on the subacute liver damages induced by carbon tetrachloride. J. Med. Food. 2007; 10(1):110-117.
30. Dhawal K, Deshpande YS, Purohit AP, Kadam SS. Evaluation of antioxidant activity of *Sida cordifolia*. Pharm. Biol. 2005; 43(9):754-761.
31. Sangita B, Krishnakumari S. Tephrosia purpurea (linn.) Pers: a Folk Medicinal Plant Ameliorates Carbon Tetrachloride Induced Hepatic Damage in Rats. Int. J. Pharma and Bio Sc. 2010; 1(2).
32. Roy CK, Kamath JV, Asad M. Hepatoprtective activity of Psidium guajava Linn. Leaf Extract. Ind. J. Exp. Biol. 2006; 44:305-311.
33. Xiong X, Chen W, Cui J, Yi S, Zhang Z, Li K. Effects of ursolic acid on liver-protection and bile secretion. Journal of Chinese Medicinal Materials. 2003; 26(8):578-581.
34. Duraiswamy B, Satishkumar MN, Gupta S, Rawat M, Porwal O, Murugan R. Hepatoprotective activity of Betula Utilis bark on D-galactosamine induced hepatic insult World journal of pharmacy and pharmaceutical sciences. 2012; 1(1):456-471.

35. Seevola D, Baebacini GM, Bona S. Flavonoids and hepatic cyclic monophosphates in liver injury. Boll. Ins. Sieroter. Milan. 1984; 63:777-782.
36. Back NL, Kim YS, Kying JS, Park KH. Isolation of antihepatotoxic agent from the roots of *Astragalus membra naceous*, Korean J. Pharmacog. 1996; 27:111.
37. Hesham RE, Shaeru N. Chemestry of bioflavonoids. Indian J Pharm edu. 2002; 36:191-4.
38. Orhan DD, Aslan M, Aktay G, Ergun E, Yesilada E, Ergun F. Evaluation of hepatoprotective effect of Gentiana olivieri herbs on sub-acute administration and isolation active principles. Life Sciences. 2003; 72:2273-83.
39. Sureshkumar SV, Mishra SH. Hepatoprotective activity of extracts from Pergularia daemia Forsk. Against carbon tetrachloride induced toxicity in rats. Pharmacognosy Magazine. 2007; 3(Suppl 11):187-91.
40. Patel PB, Patel TK, Shah P, Baxi SN, Sharma HO, Tripathi CB. Protective effects of ethanol extract of *Gymnosporia montana* (Roth) Bemth. in paracetamolinduced hepatotoxicity in rats. Indian J. Pharm. Sci. 2010; 72(3):392-396.
41. Hagerman AE, Reidel KM, Jone GA, Sovik KN, Ritchard NT, Hartzfield PW. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agri. Food Chem. 1998; 46:1887-1892.