RESEARCH ARTICLE

Potential Activity of Crude Alkaloids against *Echinococcus Granulosus* in Adult Albino Male Rats

Abeer A. Ali[1], Ahmed H Saleh[2]

[1] *Technical college of Kirkuk/North Technical University/Iraq*
[2] *Medical Analysis department/ Al-Qalam College /Kirkuk/Iraq*

Received: 07th July, 19; Revised: 13th August, 19, Accepted: 08th September, 19; Available Online: 12th September, 2019

ABSTRACT

The study was designed to show the activity of crude alkaloids against the toxicity of *E. granulosus*. In this study, a total of 20 adult albino male rats were used and divided randomly to following groups (each group consist 5 rats); control group received ad libidium, positive group injected with 2,5 X 10^3 of *E. granulosus* protoscolices third group injected with protoscolices and treated with 0.25 mg/ml crude alkaloids, fourth group injected with protoscolices and treated with 0.25 mg/mL crude alkaloids. The results show high Scavenging activity of crude alkaloids extracts reaches 88.6%. Otherwise, The results show a highly significant increased ($p < 0.05$) in levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) in the group injected with protoscolices compared with the control group. Oxidative stress factors in the group injected with protoscolices show significant increased ($p < 0.05$) in levels of malonedialdehyied (MDA) and significantly decreased ($p < 0.05$) in levels of glutathione (GSH) and catalase compared with control group. While, after used crude alkaloids with *E. granulosus*, the results showed non-significant changes ($p < 0.05$) in liver functions and MDA, GSH and catalase also showed non-significant changes ($p < 0.05$) compared with the control group. It was concluded that crude alkaloids has been a potential role against the toxicity of *Echinococcus granulosus* in male rats.

Keywords: Catalase, Crude alkaloids, Echinococcus granulosus, Glutathione (GSH), Liver function, Malonedialdehyied (MDA).

International Journal of Drug Delivery Technology (2019); DOI: 10.25258/ijddt.v9i3.11

How to cite this article: Ali, A.A. and Saleh, A.H. (2019). Potential activity of crude alkaloids against *Echinococcus granulosus* in adult albino male rats. International Journal of Drug Delivery Technology, 39(3): 386-389.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Hydatid cyst is a potential zoonotic disease of man and animals, caused by the metacestode of the dog tapeworm Echinococcus granulosus.[1] The adult form of the parasite lives in the intestine of canids as definitive hosts. Humans and herbivore animals, as intermediate hosts, can be infected by ingesting the parasite's eggs. Hydatid cyst is an important debilitating disease in humans, which often affects vital organs such as liver and lungs.[2-3] E. granulosus (s.l.) is found throughout the world, and canids are the definitive host. Hydatid cysts may develop in the lungs, liver, brain, or other internal organs of intermediate hosts.[4] Rosmarinus officinalis L. is a medicinal plant that belongs to the Lamiaceae family and is commonly known as rosemary.[5] Many compounds have been isolated from Genista microcephala and Rosmarinus officinalis, including glycoside, flavones, alkaloids, turbines, saponins.[6] Alkaloids are naturally occurring compounds containing carbon, hydrogen, nitrogen, and usually oxygen and are primarily found in plants, especially in certain flowering plants.[7] The parts of a plant as stems, leaves, and roots are used in the treatment of several diseases; they act as antibacterial, antifungal, antiviral, anthelmintic, anti-inflammatory, antioxidant, antidiabetic, antidiarrheal agent.[8]

MATERIALS AND METHODS

Animal model

In this study, twenty adult male albino rats (wt 200-250 gm with age 4–6 months) obtained from Science college/ Tikrit University and kept on standard pellet diet for two weeks to ensure it's normal and there isn't any infection.

Alkaloid extract

Alkaloid extraction from *R. officinalis* leaves was done according to.[9] 10 gm of dried and milled leaves were extracted in Soxhlet extractor for 24 hours at 40°C with 200ml of ethanol as a solvent, then it was evaporated at 50°C by a rotary evaporator. The dried extract was dissolved in 5 mL of 80% ethanol, 30mL of sulfuric acid was added, then it was dried in an electric oven to removed ethanol. The acidic extract was adjusted to pH = 9 using a 10% ammonium hydroxide solution.

**Author for Correspondence: ahmedeagle72@gmail.com*

It was then extracted with chloroform four times (10 mL each time) in the separation funnel. The extracts were collected, and 10 gm of anhydrate sodium carbonate was added to removed humidity. Then it was filtered through filter paper (Whatman No.1). The extract was dried by rotary evaporator and the concentrated extract was stored in a refrigerator until the next stage of the experiment.

Echinococcus granulosus

Hydatid cysts were collected and obtained from infected sheep livers. They were put in plastic bags and transported to the Department of Microbiology, College Technical, North Technical University, where protoscolices were isolated from livers according to 10 method. Protoscolices indicate the fertility of the hydatid cyst, and it's were counted according to the method cited by[11]. The viable protoscolices for parasite were counted in 1ml from the supernatural based on the formula: Viability in 1 mL = number of protoscolices in (10 µL) × 100.

Experimental design

Twenty adult male albino rats were used and divided as follow (each group consist four rats):

- Control group: rats were received standard pellet diet only for seven days and then killed.
- Positive group: Rats injected with protoscolices and then killed.
- Third group: rats injected with protoscolices and treated with 0.05ug/mL alkaloid extract for a month, and then killed.
- Fourth group: rats injected with protoscolices and treated with 0.1ug/mL alkaloid extract for a month, and then killed.

Prepare of blood solution.

Blood was collecting from rats by cardiac puncher, under anesthesia, and put in test tubes. After clotting, the tubes were centrifugation for 10 minutes to obtain sera. The serum was taken and stored by deep freezing until used.

Homogenization

Liver samples were removed immediately, and the put-in glass dish contents 0.9% NaCl buffer for washing and removed the blood. To oxidative stress factors determination, 10% of organ weight was dissolved with buffer (PH 7.4), and the organ tissue was crashed by use of ceramic mortar. Then the mixture was centrifugation for 10 minutes. The supernatant was taken and stored by deep freezing until used.[12]

MEASUREMENTS

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of *R. officinalis* extracts (alkaloid, and ethanolic) was determined according to the method of 13 a solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (50 µg/mL) in distilled water were added to a H_2O_2 solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. The blank solution contained the phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging of *R. officinalis* extracts and standard compound (α- tocopherol) was calculated as:

$$H_2O_2 \text{ scavenging effect (\%)} = \frac{control - samp \times 100}{control}$$

Where: $control$ is the absorbance of the control. $sample$ is the absorbance of the sample of extracts and standard.

ALT, AST and ALP

Serum ALT, AST and ALP were measured by technique according to the instructions of manufacturer company kit (Randox).

Plasma Peroxidation levels (MDA)

Malonedialdehyied (MDA), was measured based on the colorimetric reaction with thiobarbituric acid (TBA) using spectrophotometer.[14]

Glutathione (GSH) and Catalase

GSH level estimated bymixed 2.3 mL buffer with 0.2mL of the sample and then added 0.5ml of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB). The mixture was analyzed by spectrophotometer.[15] Catalase was measured by using the procedure of Biovision-USA kits.

Statistical analysis

The Data were analyzed using a statistical Minitab program. A statistical difference between the means of the experimental groups was analyzed using one-way analysis of variance (ANOVA).

RESULTS & DISCUSSION

Hydrogen peroxide scavenging activity

In these results, alkaloid compounds at 50 µg/mL and ethanolic extract exhibited 77.53% and 52.1% scavenging activity of H_2O_2, respectively. that is in agreement with Maiza-Benabdesselam et al. (2007) who referred that the scavenging activity of alkaloid extract of *F.bastardii* reached to 86%.[16]

Liver function tests

AST (99.14 ± 6.17), ALT (93103.12 ± 8.15) and ALP (134.83 ± 11.34) in second group show high significant increased ($p < 0.05$) compare with control group; AST (8.18 ± 1.7). ALT (7.52 ± 1.37) and ALP (43.83 ± 4.05). In third group, AST (32.34 ± 7.03). ALT (29.25 ± 4.15) and ALP (61.65 ± 15.27) show significant changes ($p < 0.05$) compared with control group. AST (10.28 ± 0.93), ALT (11.06 ± 1.32) and ALP (42.21 ± 5.45) in fourth group show non-significant changes ($p < 0.05$) compare with control group as shown in Table 2. The infection

Table 1: Scavenging activity of *R. officinalis* extracts (alkaloid, and ethanolic) and standard antioxidant compound (α- tocopherol) at concentration 50 µg/mL.

Type of compound	*H_2O_2 Scavenging activity (%)*
α- tocopherol	44.7%
ethanolic extract	52.1%
Alkaloid compounds extract	77.53%

Table 2: The levels of AST, ALT, and ALP in the serum of the groups

Parameters / Groups	AST (mg/dL)	ALT (mg/dL)	ALP (mg/dL)
Control	8.18 ± 1.7 c	7.52 ± 1.37 c	43.83 ± 4.05 c
Second	99.14 ± 6.17 a	103.12 ± 8.15 a	134.83 ± 11.34 a
Third	32.34 ± 7.03 b	29.25 ± 4.15 b	61.65 ± 15.27 b
Fourth	10.28 ± 0.93 c	11.06 ± 1.32 c	42.21 ± 5.45 c

Note: Same letters mean non-significant changes, and different letters mean significant changes.

Table 3: The levels of MDA, GSH, and catalase in the liver extract of the groups

Parameters / Groups	MDA (mmol/L)	GSH (mol/L)	Catalase (mol/L)
Control	1.53 ± 0.08 c	0.443 ± 0.024 a	1.62 ± 0.11 a
Second	2.39 ± 0.24 a	0.339 ± 0.028 c	0.73 ± 0.06 c
Third	1.81 ± 0.14 b	0.408 ± 0.033 b	1.28 ± 0.08 b
Fourth	1.51 ± 0.03 c	0.451 ± 0.021 a	1.58 ± 0.13 a

by *E. granulosus* leads to an increase in the levels of the liver that back to the ability of *E. granulosus* to induce hepatocytes degeneration with infiltration of mono-nucleated cells and hemorrhage with different changes in nuclei of hepatocytes.[17] In this study, alkaloids show potential activity in the treatment of enzyme disorders, and this results in agreement with Domitrovic et al.[18] that referred that alkaloid compounds have potential effects on liver enzymes. Where after rats administrated with CCl_4, lead to increase the levels of liver enzymes but after treatment with alkaloid compounds, liver enzymes back to normal ranges. They suggest that alkaloid has antioxidant activity.

MDA, GSH, and catalase in liver extract

MDA (2.39 ± 0.24), GSH (0.339 ± 0.028) catalase (0.73 ± 0.06) in rats exposure to X rays show high significant increased (MDA) and decreased (GSH) ($p < 0.05$) compared with control rats (1.53 ± 0.08; 0.443 ± 0.024 and 1.62 ± 0.11 respectively). MDA (1.81 ± 0.14), GSH (0.408 ± 0.033) and catalase (1.28 ± 0.08) in third group show significant changes ($p < 0.05$) compared with control rats. MDA (1.51 ± 0.03), GSH (0.451 ± 0.021) and catalase (1.58 ± 0.13) in fourth group show non-significant changes ($p < 0.05$) compared with control rats as shown in table 3. In this study, the infection by *E. granulosus* lead to an increase in in the levels of MDA and a decrease in GSH and catalase levels but after treatment with alkaloid compounds back to normal ranges. The results of the present study are in agreement with Rashed (2011), who referred that alkaloid compounds lead to decrease MDA levels and increase GSH levels in mice liver, this ability of alkaloid back to its activity as antioxidants[19]. Also, Al-Saadon (2007) referred that alkaloid has the ability as an antioxidant. Where, the administrated mice with alkaloid lead to decrease MDA levels and increased GSH levels, this ability back to remove free radical by alkaloid compounds[20].

REFERENCE

1. Hama, A. A.; Wijdan M. S. M. and Jaladet M. S. (2018). Genotyping of Echinococcus Granulosus (Hydatid Cyst) Isolated from Domestic Animals in Kurdistan – Iraq. International Conference on Pure and Applied Sciences. Pp: 28-31.
2. Sarkari, B., Fatemie, S.A., Moshfe, A., Abdolahi, K.S., Savardashtaki, A., Hosseini, F. and Shahbazi, A. (2016) Clinical and molecular evaluation of a case of giant primary splenic hydatid cyst: A case report. Iran J. Parasitol., 11: 585-590.
3. Barazesh A.; Sarkari B.; Ebrahimi S. and Hami M. (2018). DNA extraction from hydatid cyst protoscolices: Comparison of five different methods. J. Veter. World. 11(2): 231-234.
4. Shima, A. L.; Constantin C. C.; Linda K. J. and Lee F. S. (2018). Echinococcus Granulosus Infection in Two Free-Ranging Lumholtz's Tree-Kangaroo (Dendrolagus lumholtzi) from the Atherton Tablelands, Queensland. J. Trop. Med. Infect. Dis. 3: 47-54.
5. Andrade, J. M.; Celia F.; , Catarina G.; Diogo L.; Catarina P R. and Patr´ıcia R. (2018). Rosmarinus officinalis L.: an update review of its phytochemistry and biological activity. J. Future Sci. 4(4): 1-15.
6. Mohamed, E. S.; Alafid F.; Imramovský A.; Altwair K.; Alkhumsi S. and Hrdina R. (2017). Phytochemical screening and antibacterial activity of genista microcephala and rosmarinus officinalis extracts from libyan's regions. Int. J. Res. Ayurveda Pharm. 8(4): 52-56.
7. Hussain, G.; Azhar R.; Haseeb A.; Nimra A.; Aroona R.; Wei W.; Muhammad A.; Jiang L. and Xiaomeng L. (2018). Role of Plant Derived Alkaloids and Their Mechanism in Neurodegenerative Disorders . Int. J. Biol. Sci. 14: 341-357.
8. Atala, M. L., and Muhammed A. A. (2017). Antibacterial activity of Rosmarinus officinalis and Dodonaea viscosa leaves extracts against Escherichia coli and Staphylococcus aureus. Iraqi J. Sci. 58(3B): 1393-1397.
9. Harborn , J.B. (1984). Phytochemical methods: A guide to modern techniques of plant analysis . Academic press , Chapman and Hall , 2nd . ed. New York . 288 p.
10. Smyth, J. D. (1964). The biology of hydatid organisms. Adv. Parasitol. , 2: 169-219.
11. Al – Humairy , A. KH. (2010). Evaluation of the Activity Datura stramonium Seeds Extracts on Growth and Development of Hydatid Cysts for Echinococcus granulosus in White Mice Balb/c (Therapeutic , Histologic and Immunologic Study).Ph. D. Thesis . College of Science , Kufa University.
12. Albir, A. A. (2013). Histological changes in the liver of mice treated with cadmium. J. Sci. 54(1): 50-54.
13. Ruch, R.J, J. Klauning and J.J.Cheng. 1989. Prevention of cytotoxity and inhibition of intracellular by antioxidant Catechins isolated from Chinese green tea. Carcinogenesis. 10:1003- 1008.
14. Abdul kareem, E. A., Abdul Monaim H. M., Rafah R. H. (2016). Biochemical and histological study of the effect of sterol extract from pistachio on the level of antioxidants in the liver tissue green. J. Pur. Sci. 5(21): 82-87.

15. Mahmood, N. A. (2010). Glutathion-S- transferase Enzyme and Malondialdehyde (MDA) in Colorectal Cancer and in Healthy Control. J. Can. Med. Gen. 3(1): 21-26.
16. Maiza-Benabdesselam F.; Sabiha K.; Khalida B.; Mohamed C.; Sandrine A.; Yves C.; Henry M. and Dominique L. (2007). Antioxidant activities of alkaloid extracts of two Algerian species of Fumaria : Fumaria capreolata and Fumaria bastardii. J. Rec. Nat. Prod. 1:(2-3) 28-35.
17. Al-Ammash, M. S. J.; Marwa S. M. and Ahmed H. A. (2017). The effect of Cinnamomum zeylanicum plant extracts and Albendazole drug in mice Balb/c infected experimentally with Hydatid cysts and follow some Histological and Physiological changes. Tikrit J. Pur. Sci. 10(22): 50-62
18. Domitrovic, R.; Hrvoje J.; Vanja V. M. and Biljana B. (2013). Resolution of Liver Fibrosis by Isoquinoline Alkaloid Berberine in CCl4-Intoxicated Mice Is Mediated by Suppression of Oxidative Stress and Upregulation of MMP-2 Expression. J. Med. Food. 16 (6): 518–528.
19. Rashed, S.H. (2011). Isolation and Studying some Extracts, Protenious and non-protenious Compounds from Sweet Majorum (Origanum majorana) in Mice Exposed to Oxidative Stress. J. Al-Rafeedin Sci. 22(2): 129-144.
20. Al-Saadon, M. B. (2007). Studying Some of the Extracts and Isolated Proteinous Compounds From Apple (Pyrus malus) in Mice Exposed to Oxidative Stress. J. Al-Rafeedin Sci. 19(1): 116-133.