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Hepatoprotactive activity of *Ruta chalepensis* ethanolic extract and histo-architecture of liver on CCL_4 damaged albino male mice

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Abstract

Medicines derivative from plants broadly famous owing to their safety, obtainability and little Leaves, roots, bark, kernels and florae of plant are mostly prepared that included herbal medicines. They are administered orally, inhaled or directly applied in the skin. *Ruta chalepensis* is take on in the outdated medicine of many countries as a natural herb of the Mediterranean region. Pharmacological properties responsible by using phytochemical screening has shown the attendance of bioactive molecules price. The current study meant to investigate the effect of *R. chalepensis* ethanolic extract on liver function enzyme (Aspartate Amino-Transferase (AST), Alanine Amino-Transferase (ALT) and Alkaline Phosphatase (ALP) and histological examination of liver section. Obtained showed the ability of plant extract to protect liver from any xenobiotics and provided protection against CCL4 damage on albino male mice.

Key words: Medicinal plant, Liver, CCL_4 , Xenobiotics .

Introduction

Medicinal plants have formed the basis of health care. Many herbal drugs are frequently used in their crude form by a large number of people (1). It may lead to a large-scale exposure of human to natural products. A great amount of aromatic, therapeutic plants and needs biological possessions very stimulating who find applications in many various arenas such as the pharmaceutical trades, agribusiness, manufacturing and the medication (2). These plants signify a newfangled basis of active composites due it has different secondarymetabolites which are the topic of frequent *invivo* and *invitro* researches, counting searchas for novel ordinary constituents such as phenolic compounds, saponins and essential oils (3,4). One of such medicinal plant is *Ruta chalepensis. Ruta chalepensis* is kinds of flowering plant in citrus domestic known by the public term fringed rue (5). It is native to Eurasia and North Africa. In outdated medicine, the plant is used as an herbal medicine for a number - ++ *of illnesses, such as fever and inflammation. 16 compounds were characterized in this plant representing 99.99 % of the essential oil with 2-acetoxytetradecane (58.44 %), 2-acetoxytridecane (19.07 %) and 2-tridecanone (6.39 %) as major components of this plant (6). This deviation from the common chemo-types may be attributed to the effect of the factors that specifically affect the composition and yield of the essential oil, which include seasonal, and maturity variation, geographical origin, genetic variation, growth stages, postharvest drying and storage. Although some times, *R. chalepensis* shows ebryotoxic effects on mouse exposed during postimplantational period (7). Liver has crucial part in life through its able metabolic and detoxifcation competence and due to the detail that it was exposure to several xenobotic and endogenous agents, a countless of intermediary and end productsare produced and can cause hepatocellular damageand death that establish the principal reasons of liver illness (8). So it would be extremely authoritative to prove the efficiency of the plant extracts in the attendance of different chemical-encouraged hepatotoxicity like (CCL_4 and paracetemol *etc*.) This work allowable determining the effect of the chemical arrangement presented in ethanolic extract of plant on liver function enzymes in addition to histological evaluation of plant on CCL_4 damaged albino male mice (9).

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Material and methods

Plant collection, identification and extraction

Plant leaves of *Ruta chalepensis* was collected from Baghdad local market during September 2019, which previously identified by national herbarium of Iraq. The plant leaves washed thoroughly in consecutively tap water. Air dry (in shade) Leaves were kept in an oven for about 48 hours at 37°C, make it powder by glass mortar with the aid of a pestle. The powdered leaves were formerly soxhlated by 80% ethanol (v/v) at 65°C using a soxhlat apparatus for about 3 hours for full extraction. Extract obtained was finally dried on water bath (at 60°C) to convert it in the form of a powder and then dissolving to prepare require doses (50 and 100 mg\kg) (10).The experimentations were done on 6 to 8 week old healthy research laboratory Swiss albino male mice of body weight 22-28gram which supplied by Biotechnology research center\al-Nahrain university\Iraq.

- **Treatment protocol (biochemical assessments of liver function enzyme (L.F.E.):**

To assess the L.F.E. parameters and liver histo-architecture, mice groups were divided to:

1. Control group: The mice of control groups were given 0.1ml distilled water per day by syringe with curved needle (negative group).
2. Treated group: Each time freshly prepared plant extract was administered for 7consecutive days according to mice groups:

Group A: mice treated with plant extract at a dose of 50mg\kg.

Group B: mice were adminstrate with a single dose of 0.2% CCL_4 recieved distilled water (0.1 ml) as single daily dose in 7 days.

Group C: mice treated with CCL_4 in first day and up to 7 day with (50mg\kg) of plant extract.

Group D: mice treated with (100mg\kg) of plant dose and finally.

Group E: mice treated with CCL_4 at first day and up to 7 day with (100mg\kg) of plant extract.

Each tested group was administrated orally using curved needle with 0.1ml as single dose per day. On day 8 of the experiment, the animals were sacrificed to carry out laboratory biochemical assessments (11). Afterward puncturing of heart, blood keep into Eppendorf tube and ting it for 15 minutes at the room temperature formerly serum was separated by using centrifuge device at 3,000 rpm for 10 min. The biochemical assay determined following the enzymatic colorimetric method of Ref. (12), using commercial kits (Randox Company) to evaluate the liver function enzyme which included (Aspartate Amino-Transferase (AST or GOT), Alanine Amino-Transferase (ALT, GOT) and Alkaline Phosphatase (ALP). Finally, statistical analysis of data in this study were presented as mean ± S.D. using GraphPad Prism version 5.0 and differences were measured significant at ($p \leq 0.05$).

Liver Preparation for Histological section

By using 10% formalin samples remained stable for 24h and dehydration with a gradual series of alcohol (30-to100%) for (5) min. Formerly tasters unfurnished in two xylene changes previously entrenched in paraffin wax for sectioning.the Cross sections were prepared of (5)µm thickness and stained with hematoxylin(Harison) and eosin as stated by standard method. Under light microscope histopathlogical changes stand achieved as compared to control group.

Statistical analysis: one mode examination of variance ANOVA (Duncan) was made to test whther group alternation was important or not, statistical significance was defined as * $p \leq 0.05$ and ** $p \leq 0.01$. Date was carried out using Graph Pad Prism version 8.

Results

- **Hepatoprodutective activity Ruta chalpensis on liver function enzyme:**

As shown in table 1, ethanolic extract of plant possess the ability to protect liver from CCL_4 damage in a dose dependant manner. Treated mice with 50 and 100 mg\kg showed a decrease in (L.F.E.) as compared to control negative group (21.67±1.15, 10.68 ± 1.15 U \L.) (32.65±3.21, 20.01±2.23 U\L) (46.34 ± 2.13, 40.01 ± 4.58 U \L.) for GOT,GPT and ALP correspondingly, while the results of interactions group showed that CCL_4 and H_2O (75.03 0.81, 70.41±1.63, 117±9.21), CCL_4 and plant with a dose at 100

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mg\kg more protect liver than CCL_4 with 50 mg\kg of plant dose (55.01±2.64, 44.62±3.05 U \L.) (38.21 ± 3.03, 36.52±4.02 U \L.) (74.61±5.85, 76.68±4.22 U\L) for GOT, GPT and ALP respectively (13).

Table (1): Effect of *Ruta chalepensis* ethanolic extract on L.F.E in sera of albino male mice with or without carbon tetrachloride (CCl_4)

Mice Groups	GOT (mean±S.D.) U\L	GPT (mean±S.D.) U\L	ALP (mean±S.D.) U\L
Negative control	30.01±4.21^{D}	48.46±4.67^{B}	57.23±3.59^{C}
Group A	21.67±1.15^{E}	32.65±3.21^{D}	46.34±2.13^{D}
Group B	75.03 0.81^{A}	70.41±1.63^{A}	117±9.21^{A}
Group C	55.01±2.64^{B}	38.21±3.03^{C}	74.61±5.85^{B}
Group D	10.68 ± 1.15^{E}	20.01±2.23^{E}	40.01±4.58^{D}
Group E	44.62±3.05^{C}	36.52±4.02^{C}	76.68±4.22^{C}

Different letter mean significant different between means.

- **Effect of *Ruta chalepensis* on histo-architecture of liver:**

The histological examination of mice liver represented different results according to treatment type as shown below in figure 2,3,4 and 5 in compared to figure 1 of negative control group which showed normal liver structure.

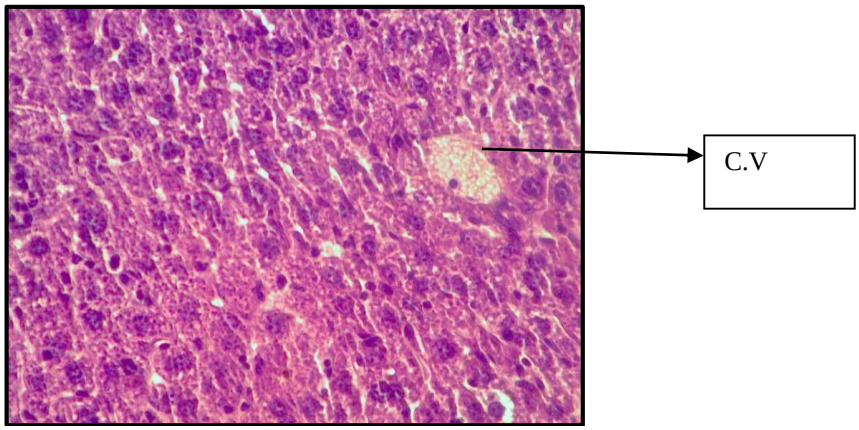

Figure 1: section of regular liver structure, which involves of central vein, surrounded by hepatocyte cells (H& E) X400.

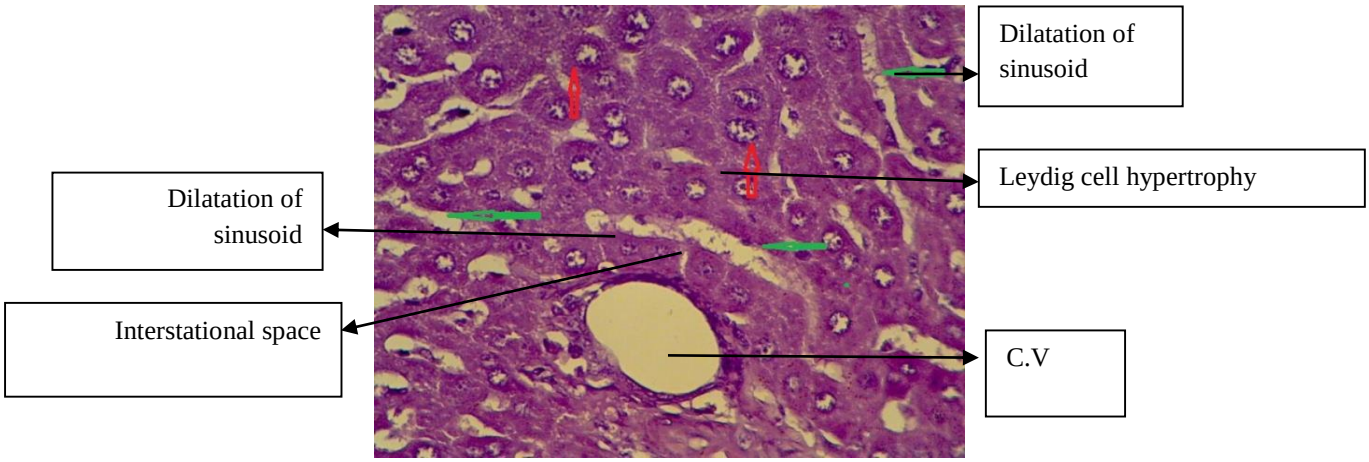

Figure 2: Section showing dilatation of sinusoid, hypertrophy of hepatocyte cells in mice treated with (50 mg\kg) plant extract. (X400)(H & E)

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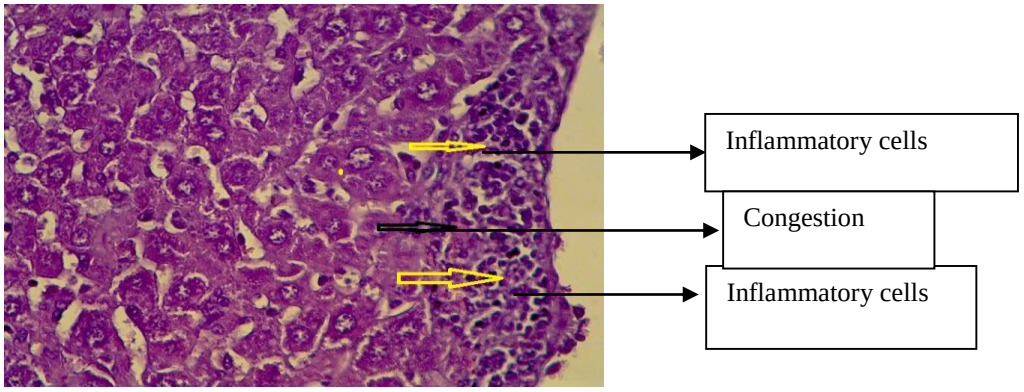

Figure 3: section showing necrosis of hepatocytes cells and numerous inflammatory cells infiltration with dilation of sinusoid in mice treated with CCL_4+50 mg\kg plant extract

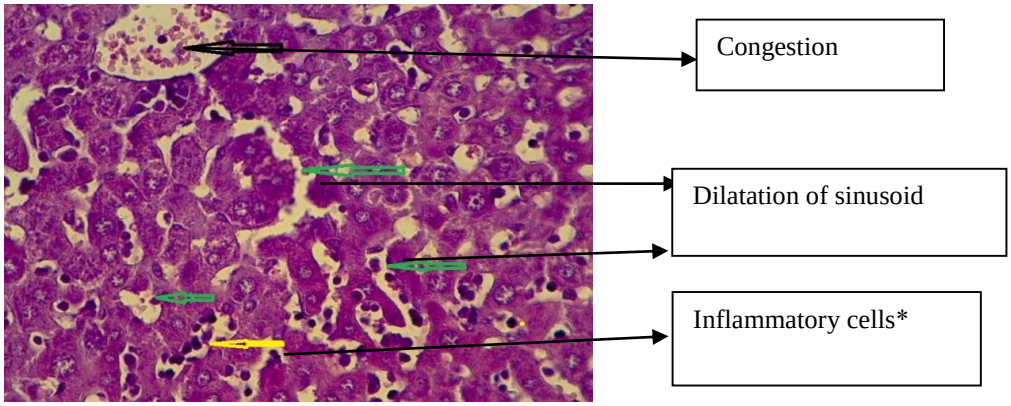

Figure 4: Section of mice treated with 100 mg\kg of plant extract showing mobbing, dilatation of sinusoid with inflammatory cells infiltration

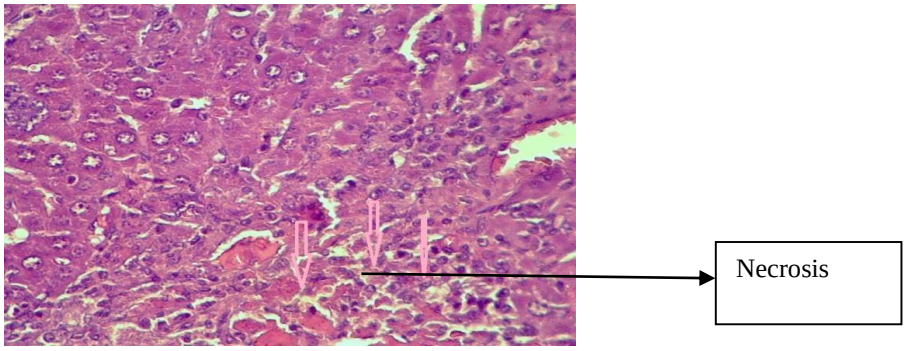

Figure 5: Section of mice preserved with CCL_4+100 mg\kg of plant extract showing wide area of necrosis and damage of parenchymal liver tissues

Discussion

Liver plays important role in all individual health. The need to test and utilize of botanical hepatoprotective agents is substantially increasing in last year's (14,15). CCl_4, a strong hepatotoxic agent, is the greatest widely used standard for evaluating the hepatoprotective activity of many plant extracts (16) Numerous studies designate that CCl_4 can produce centrizonal hemorrrhagic hepatic necrosis in human and experimental animals, the 80% ethanol extract displayed a decrease in the stages of "AST, "ALT, and "ALP in a dose-dependent manner (17).

Also,some results indicated that when mice treated with 80% ethanol the level of enzyme return to normal and the plant has the ability to steady liver cell membranes and mimic the leak of enzymes (18). Avoiding the creation of free radicals and neutralizing them in addition to the protection potential of this plant against hepatotoxins can be other likely details for the healing effect of *ruta chalepensis* leaf extract

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(19). The active principle(s) responsible for the hepatoprotective activity are flavonoids and essential oils plant contained which were found to have antioxidant activity (20).

Possibly, the ability of plant extract to flavonoids and essential oil present in the crude leaf extract to scavenge free radical led to hepatoprotective effect by prevent lipid peroxidation (21).The histopathological examinations of liver sections in CCl_4-treated mice revealed a regeneration of hepatic cells after treatments with the plant extract especially at a dose of 100 mg/kg; therefore it is possible to suggest that the plant extract being able to condition the hepatic cells to a state of accelerated regeneration (22). The observed protective effect of *ruta chalepensis* against the hepatotoxin CCl_4 may be attributed to the presence of flavonoids and terpenoids, which are among the important plant constituents (23). many compound related to ruta skimmianine, psoralen and isopimpinellin exhibit cytotoxic activity other than that leaves extracts of ruta were found to be commonly used in treatment many diseases, isolated compounds from the ethanolic extract of roots, aerial part of *ruta* species stimulated the inhibition of platlet agriggation property and exibited cytotoxicity (24).

References

1. Kipkore W, Wanjohi B, Rono H, Kigen G. A study of the medicinal plants used by the Marakwet Community in Kenya. J Ethnobiol Ethnomed. (2014); 10(1): 22.

2. Enyew A , Asfaw Z , Kelbessa E , Nagappan R. Ethnobotanical study of traditional medicinal plants in and around Fiche District, Central Ethiopia. Curr Res J Biol Sci. (2014); 6(4) :154–167.

3. Matu EN. Clutia abyssinica Jaub. & Spach. In: Schmelzer GH, Gurib-Fakim A, editors. PROTA (Plant Resources of Tropical Africa / Ressources Végétales De l'Afrique Tropicale). Wageningen, Netherlands; 2008. Available from: https://uses.plantnet-project.org/en/Clutia_abyssinica_(PROTA). Accessed January 2020.

4. Kigen G, Some F, Kibosia J. Ethnomedicinal plants traditionally used by the keiyo community in Elgeyo Marakwet County, Kenya. J Biodivers Biopros Dev. (2014); 1: 3.

5. Pascaline J, Charles M, Lukhoba C, George O. Phytochemical constituents of some medicinal plants used by the Nandis of South Nandi district, Kenya. J Anim Plant Sci. (2011); 9(3): 1201–1210.

6. Koech S, Maoga J, Sindani A, Ireri M, Mwonjoria J. Anti-inflammatory activity of dichloromethanolic root extract of Clutia abyssinica in Swiss Albino Mice. J Pharmacogn Nat Prod. (2017); 3(132): 615–634.

7. Andemariam SW. Legislative Regulation of Traditional Medicinal Knowledge in Eritrea via-a-vis Eritrea's Commitments under the Convention on Biological Diversity: issues and Alternatives. Law Env't Dev J. (2010); 6: 130.

8. Lampertico P, Agarwal K, Berg T EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol. (2017); 67(2): 370–398.

9. Connor HD, Thurman R, Galizi M, Mason R. The formation of a novel free radical metabolite from CCl4 in the perfused rat liver and in vivo. J Biol Chem. (1986); 261(10): 4542–4548.

10. Al-Naimy EH, Al-Amery MM, Al-ezzzy RM. antibacterial and cytogenetic effect of Cassia italica LEAF extract on albino male mice. Journal of Al-Nahrain University. (2010); 13(10): 132-139.

11. Alchalab RN, Ibraheem RM , Adnan R , Al-bayati SH. the antibacterial effect of ethanolic extract of*allium sativum* on biofilm forming *staphylococcus aureus* - which cause folliculitis. int. j. adv. res.
(2017); 5(5): 1175-1182.

12. Reitman S, Frankel S. "A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases." Am J Clin Pathol. (1957); 28: 56-63.

13. Singab ANB, Youssef DT, Noaman E, Kotb S. Hepatoprotective effect of flavonol glycosides rich fraction from egyptianVicia calcarata desf. against CCI 4-induced liver damage in rats. Arch Pharm Res. (2005); 28(7): 791–798.

14. Tauchen J, Doskocil I, Caffi C, *et al.* In vitro antioxidant and anti-proliferative activity of Ethiopian medicinal plant extracts. Ind Crops Prod. (2015); 74: 671–679.

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15. Teklay A, Abera B, Giday M. An ethnobotanical study of medicinal plants used in Kilte Awulaelo District, Tigray Region of Ethiopia. J Ethnobiol Ethnomed. (2013); 9(1): 65.
16. Mukazayire M-J, Minani V, Ruffo CK, Bizuru E, Stévigny C, Duez P. Traditional phytotherapy remedies used in Southern Rwanda for the treatment of liver diseases. J Ethnopharmacol. (2011); 138(2): 415–431.
17. Malhi H, Gores GJ. Cellular and molecular mechanisms of liver injury. Gastroenterology. (2008); 134(6): 1641–1654.
18. Jeong HG. Inhibition of cytochrome P450 2E1 expression by oleanolic acid: hepatoprotective effects against carbon tetrachloride-induced hepatic injury. Toxicol Lett. (1999); 105(3): 215–222.
19. Lee WM. Drug-induced hepatotoxicity. N Engl J Med. (2003); 349(5): 474–485.
20. Pritchard MT, Apte U. Models to Study Liver Regeneration. Liver Regeneration. Elsevier.(2015):15-40.
21. Lee WM. Drug-induced hepatotoxicity. N Engl J Med. (2003); 349(5): 474–485.
22. Koech S, Sindani A, Maoga J, Ouko R, Njagi N. Anti-pyretic potential of dichloromethanolic root extract of Clutia abyssinica in Wistar Albino Rats. Med Aromat Plants. (2017); 6(281): 2167–0412.1000281.
23. Rohini MV, Padmini E. Preliminary phytochemical screening of selected medicinal plants of polyherbal formulation. J Pharmacogn Phytochem. (2016); 5(5): 277.
24. Jamel J, Bouajila J, Aydi A, Barth D, Abderrabbaa M, Mejri M . Supercritical CO_2 extract and essential oil of Ruta chalepensis L Growing in Tunisia: A natural source of Undecan-2-one Analytical Chemistry Letters (2012); 2(5): 290.

الفعالية الحامية للكبد للمستخلص الايثانولي لنبات السذاب على ذكور الفئران البيضاء المستحثة برباعي كلوريد الكاربون

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الخلاصة

تنتشر الأدوية المشتقة من النباتات على نطاق واسع بسبب سلامتها وسهولة توافرها وتكلفتها المنخفضة. قد تشمل الأدوية العشبية اما أجزاء كاملة من النبات أو اجزاء من النبات محضرة في الغالب من أوراق وجذور ولحاء وبذور وأزهار النباتات. كما يتم تناول هذه الادوية عن طريق الفم أو الاستنشاق أو وضعها مباشرة في الجلد. نبات السذاب هو عشب محلي في ينتشر في منطقة البحر الأبيض المتوسط ويستخدم في الطب التقليدي في العديد من البلدان. أظهر الفحص الكيميائي النباتي وجود جزيئات نشطة بيولوجيًا مسؤولة عن خصائصه الدوائية .هدفت الدراسة الحالية إلى التحقق من تأثير المستخلص الإيثانولي لنبات السذاب R. chalepensis على إنزيم وظائف الكبد (Aspartate Amino-Transferase (AST or GOT)، Alanine Amino-Transferase (ALT or GPT) وAlkaline Phosphatase (ALP) والفحص النسيجي للكبد. أظهرت جميع النتائج التي تم الحصول عليها قدرة المستخلص النباتي على حماية الكبد من أي مادة كيميائية غريبة وتوفير الحماية ضد أضرار CCL4 على ذكور الفئران البيضاء.

الكلمات المفتاحية: النباتات الطبية ، الكبد ، CCL4 ، زينوبايوتك.