Pure Sciences International Journal of kerbala

Year:2024 Volume : 1 Issue : 2 ISSN: 6188-2789 Print 3005 -2394 Online

Zahraa Fadel Thabet , Heba A. Abd-Alsalam Alsalame, Evaluation the effectiveness of *Nephelium lappaceum (Nl)*peel aqueous extract against hepatocellular carcinoma induced by thioacetamide (TAA) in Male Albino Rats.
, Pure Sciences International Journal of Kerbala, Vol. 1, No. 2, (2024) 43-48

2024 ,Volume 1 , Issues 2 PSIJK 43-48

Pure Sciences International Journal of Kerbala

Journal Homepage:https://journals.uokerbala.edu.iq/index.php/psijk

Evaluation the Effectiveness of *Nephelium lappaceum (Nl)* Peel Aqueous Extract Against Hepatocellular Carcinoma Induced by Thioacetamide (TAA) in Male Albino Rats

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P A P E R I N F O

Paper history:
Received 23 April 2024
Accepted 26 May 2024
Published 30 June 2024

Keywords:
Hepatotoxicity , liver enzyme , Rambutan ,Thioacetamide.

A B S T R A C T

This study aims to evaluate the effectiveness of *Nephelium lappaceum (Nl)* peel aqueous extract against liver cancer induced by Thioacetamide (TAA). This study has been conducted in the College of Education for Pure Sciences / University of Karbala and the animal house of Pharmacy College / University of Karbala for the period from the beginning of November 2023 until February 2024 , and the Physiological tests have been done in Al-Husseini Teaching Hospital in the Holy Karbala Governorate / Department of Clinical Chemistry. 40 male rats (12 weeks old , weighing 250 grams) are divided into four groups. First group (G1) is administered distilled water, the second group (G2) is injected 200 mg/kg Thioacetamide (TAA) while the third group (G3) is administered 25mg/kg *Nephelium lappaceum (Nl)* peel aqueous extract. Fourth group (G4) is administered 25mg/kg *Nephelium lappaceum (Nl)* peel aqueous extract with 200mg/kg Thioacetamide (TAA). After 90 days, blood samples are collected to study the following parameters: alkaline phosphatase (ALP), aminotransaminase transfer (ALT), aspartate transaminase (AST) and total bilirubin (TB) in addition to investigate the histological changes. The results show that TAA causes a significant increase ($P < 0.01$) in ALT, AST, ALP and T–BIL in (G2) compared with the (G1). While there is no significant increase ($P > 0.01$) in ALP, ALT, AST and T -BIL in G3 and G4 compared with the control. The results of the histological examination in G2 group reveal that treatment with TAA for 90 days leads to many noxious effects on liver cells with the appearance of many tumor nodules, vacillation and necrosis with infiltration of inflammatory cells. The study concludes that peel aqueous extract of *Nephelium lappaceum (Nl)* has a protective role against liver cancer induced by TAA in male rats.

1. INTRODUCTION

Thioacetamide (TAA) is a white or yellowish crystalline organic compound that is soluble in water. It contains sulfur, with a chemical molecular formula (CH3CSNH2). It is one of the chemical substances that is widely used in many experimental studies due to its high toxicity which causes cellular damage, especially liver damage, liver tumors, and cirrhosis in laboratory rats [1]. The existence of pollutants and toxins in the environment, such as TAA, which is commonly found in the food and beverage industries, laboratory treatments, and in the paper and automobile fuel industries [1,2]. In addition to its use as a fungicide and also in the textile industry,TAA is metabolized in the liver via cytochrome p 450 to produce highly reactive TAA-S-oxide and TAA-SS-oxide resulting the inflammation and oxidative stress in addition to their role as a carcinogen for man body's organs that include the liver [3,4]. Hepatocellular carcinoma (HCC) is the most common primary liver tumor and the fourth highest cause of cancer- related death globally, as it is directly linked to the increased production of free radicals [5,6]. Currently, medicinal plants are getting more importance because they cover many effective compounds that are utilized as complementary and alternative therapies with other treatments [7]. These plants comprise numerous antioxidant chemical compounds produced in the form of secondary metabolites that are protected from free radicals and oxidative stress associated with the appearance of cancer [8,9]. Among the types of these plants is the Rambutan (*Nephelium lappaceum NI*).This plant is considered one of the most antioxidant medicinal plants, whose fruits are characterized by being rich in many

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active compounds such as phenols, flavonoids, ascorbic acid, geranin, and others which enable it to scavenge reactive oxygen species (ROS). These species have an essential role in the treatment of many disease especially cancer, diabetes, and cardiovascular diseases, as well as having anti-inflammatory, antimicrobial, and cholesterol-lowering activity [10-12]. Further more, the anti-tumor activity of the Rambutan fruit is known for its capacity to inhibit the growth of cancer cells by activating the programmed apoptosis pathway and destroying the DNA of cancer cells.Accordingly, numerous previous studies have shown the role of rambutan fruits in combating aging and regulating blood sugar levels [13,14].

2. MATERIALS AND METHODS

2.1 Animals

Forty male albino white rats (age: 10-12 weeks, average weight: 200-250 grams) were obtained from animal houses in Karbala, Iraq. They were placed in special plastic cages, and their floors were spread with fine sawdust. The rats were kept under standard laboratory conditions at a temperature25°C with controlled humidity and 12/12 light-dark cycle, with the lights turned on at 8:00 A:M. Rats were monitored daily, with a standard diet including concentrate pullet and tap water.

2.2 Liver cancer induction

Thioacetamide (TAA) was purchased from Mumbai, India, and injected subperitoneal at a concentration of 200 mg/kg into male albino rats for 90 days according to [4].

2.3 Experience design

Forty adult males were divided into 4 groups (10 animals per group). First negative Group(G1) was given distilled water, Second positive group (G2) was injected Sub peritoneal injections with TAA at a concentration of 200 mg/kg twice a week, while the third group (G3) was administered 25 mg/kg of *NI* peels aqueous extract.The fourth group (G4) was administered 25 mg/kg of *Nl* peels aqueous extract with Sub peritoneal injections 200 mg/kg of TAA. After 90 days, all the animals were anesthetized after being given a piece of cotton and placed in a closed transparent box. The animals were dissected, and the liver organ was removed and cut into small pieces longitudinally and transversely. They were preserved in formalin at a concentration of 10% at 48 h. Later, the specimens were processed during the stander prosedure by using histological techniques [15].

2.4 Aqueous extract Preparation

20 g of dry powder of rambutan peels were added with 300 ml of DW. The mixture was left for 24 hours at room temperature. The mixture was filtered by using several layers of medical gauze.Then, it was centrifuged for 10 minutes at 3000 rpm.After that, the extract was cleared by using Whatman No. 0.1 to obtain a clear solution and dried at room temperature for12 hours.Finally, it was kept in glass bottles in the refrigerator until its use [16].

2.5 Statistical analysis

SPSS program was employed to analyze the results and the researchers tested the correlation coefficient by means of the analysis of variance by complete randomized design (CRD). They used the least significant difference (L.S.D) to show the importance of the results [17].

3. RESULTS

The results revealed a significant increase ($P<0.01$) in the liver enzyme level (ALP, AST, ALT and T-BIL) in (G2), which was treated with TAA 200 mg/kg for 90 days compared with (G1). The outcomes of the third and fourth groups showed that there were no noteworthy differences ($P>0.01$) in enzymes liver and T-BIL, as shown in the table (1).

TABLE 1. The effect of thioacetamide and Rambutan peel aqueous extract on ALT,AST,ALP and T-BIL in the serum of male rat.

Parameter	G1control group	G2 treated with 200mg/kg TAA	G3 treated with 25mg/kg NI peel aqueous extract	G4 treated with 200mg/kg TAA +25mg/kg NI peel aqueous extract
ALP(U/L)	231.50 ± 7.46 a	501.80 ± 33.89 b	232.40 ± 4.77 a	247.40 ± 10.47 c
AST(U/L)	128.10 ± 5.57 a	311.70 ± 39.72 b	124.30 ± 5.70 a	132.80 ± 4.37 a
ALT(U/L)	44.80 ± 5.31 a	114.40 ± 9.62 b	44.50 ± 4.01 a	48.20 ± 4.10 a
T_BIL	0.17 ± 0.03 a	0.64 ± 0.12 b	0.14 ± 0.04 a	0.18 ± 0.08 a

Mean± standard error

The histological structure of the control group shows several lobules containing a central vein and regularity of hepatocytes with normal bands and sinusoids as well as clear nuclei along with the central vein and sinusoids as in Figure (1).On the other hand , the results of histological of

the G2 group injected with a TAA showed the appearance of clear tumor nodules and the occurrence of degenerative changes as Figure (2). The consequences of histological treated with TAA and *NI* peels aqueous extract exposed enhancements in the changes induced by TAA, the normal structure of the liver and no apoptotic cells as Figure (3). Histological result of G4 group exhibited the normal structure of the liver cell as Figure (4).

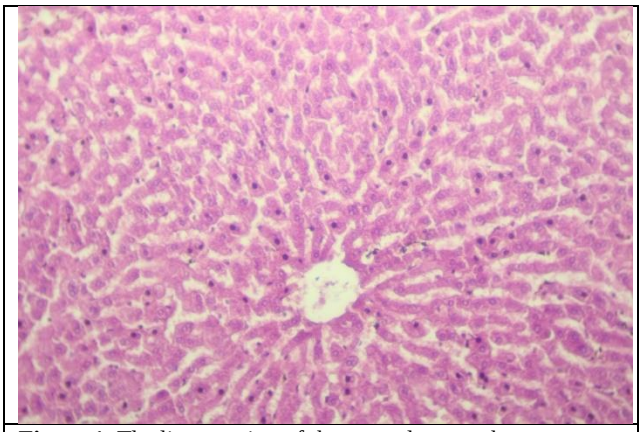

Figure 1. The liver section of the control group shows a central vein and regularity of hepatic lobules (H&E 100×).

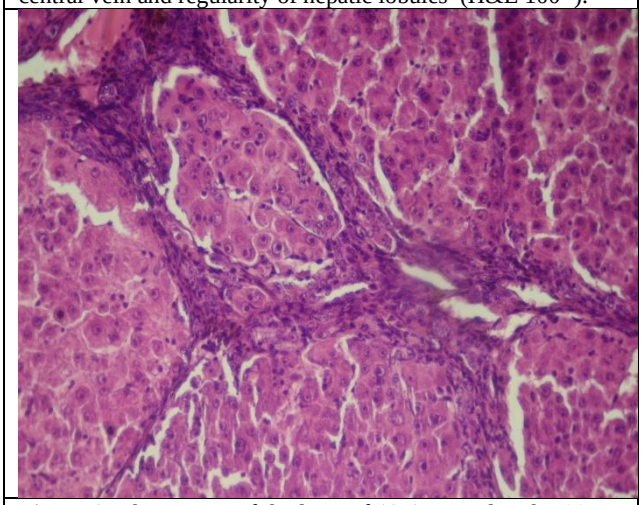

Figure 2. The section of the liver of (G2) treated with 200 mg /kg of TAA shows degenerative changes in the liver tissue and the appearance of large tumor nodules and infiltration of inflammatory cells with the occurrence of cell necrosis hepatic cells and congestion of central vein (H&E 200×).

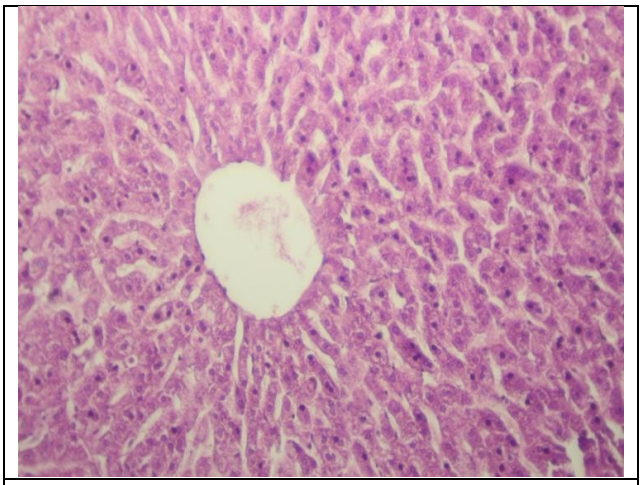

Figure 3. Show the effect of oral dosage with 25 mg/kg of *NI* peel aqueous extract which shows the normal structure of liver tissue and the normal appearance of hepatocytes with dilatation of the central vein (H&E 200×).

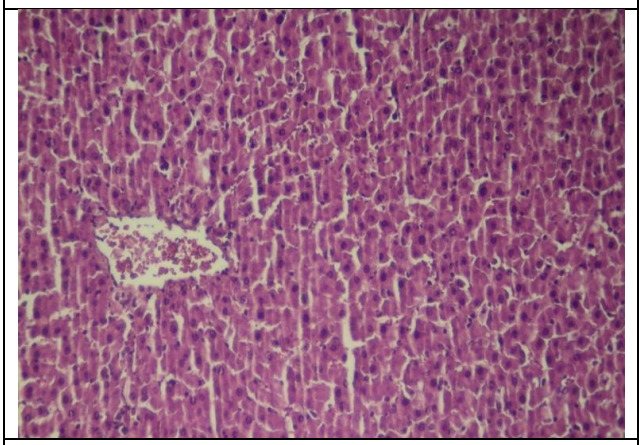

Figure 4. Section of the liver in rats injected with 200 mg/kg of TAA + 25 mg/kg of *NI* peel aqueous extract, showed the normal structure of the liver tissue was noticed with the reorganization of the hepatic chords with congestion of central vein (H&E 200×).

4. DISSCAION

The elevated level of liver enzymes due to acute toxic effect exerted by TAA, that causes severe oxidative stress to the liver cells and an increase in the level of free radicals (ROS) which occurs because of the depletion of the endogenous antioxidant GSH and SOD [18-20]. This leads to cellular damage, and necrosis in the liver cells, cirrhosis, as well as breakdown of the cell membrane, resulting in leakage of liver enzymes out of the liver cells, and an increase in the level of these enzymes in the blood [21-23]. Cytochrome 450 system participates in the bioactivation of the TAA.This leads to generating the toxic reactive metabolite (TAASO2). The toxic reactive metabolite forms acetylimidolysine derivatives, that cause cellular toxicity by binding to macromolecules in the liver

especially lipid. This leads to an increase in lipid peroxidation, Plasma membrane breakdown and an increase of enzymes released from liver cell [24]. In addition, change in the permeability and fluidity of the cell membrane takes place for its association $TAASO_2$ with calcium ions (Ca^{+2}), whose concentration increases inside the cells causing a defect in the transport and exchange of ions.This causes swelling and cellular dissolution , leads to damage enzymes in the cytosol and then release these enzymes into the bloodstream [24,18]. Bilirubin is the breakdown product of heme and the liver organ responsible for filtering bilirubin from the blood. It binds to albumin and transports to the liver where it is conjugated with glucuronic acid and secreted in the bile through the intestine [25]. The current study showed increased bilirubin levels that may due to the toxic effect of TAA by preventing the conversion of bilirubin (insoluble) by glucuronic acid into the water-soluble conjugated forms, which are monoglucuronide bilirubin and diglucuronide bilirubin.This issue leads to their non-excretion in the bile duct and accumulation in the serum and tissues [26]. The use of Rambutan peel extract led to the preservation of the normal structure of the liver , repairing of damaged cell membranes and regulation of the level of liver enzymes because of the antioxidants that rambutan peels contain comprising phenolic compounds.These compounds have the ability to reduce the free radical effect. The compound acts as an electron donor for H+ from its hydroxyl group, which leads to reducing the production of free radicals and making them more stable [27,28]. The peels also contain many active compounds, such as ellagic, corellagen, gallic, and flavonoids, which can neutralize reactive oxygen species (ROS), increase activities of internal antioxidants enzymes such as GSH and SOD, and enhance their synthesis, in additionto scavenging free radicals [29]. More over, geranin, which constitutes the largest portion of the compounds found in the peels of rambutan, has high activity in scavenging free radicals and reducing oxidative stress caused by TAA, which indicates the stability of cell membranes and the preservation of the secretory functions hepatic cells with the integrity of the bile duct [30]. In conclusion, *NI* peel aqueous extract that improves the defense status against oxidative stress against hepatotoxicity, has high antioxidant activity, and reduces hepatic tissue damage. It has great preventive importance in preserving hepatic tissue from damage caused by TAA.

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Arabic Abstract

هدفت الدراسة إلى تقييم فعالية المستخلص المائي لقشور *Nephelium lappaceum (Nl)* ضد سرطان الكبد المستحث بواسطة الثيواسيتاميد (TAA). أجريت هذه الدراسة في كلية التربية للعلوم الصرفة / جامعة كربلاء وبيت الحيوان التابع لكلية الصيدلة / جامعة كربلاء للمدة مابين بداية تشرين الثاني 2023 ولغاية شباط 2024، وأجريت الاختبارات الفسيولوجية في مستشفى الحسيني التعليمي في محافظة كربلاء المقدسة / قسم الكيمياء السريرية. اربعين من ذكور الجرذان البيض (عمرها 12 أسبوع، تزن 250 جرام) قسمت إلى أربع مجموعات، المجموعة الأولى (G1) تم تجريعها بالماء المقطر، المجموعة الثانية (G2) تم حقنها بـ 200 ملجم/كجم من الثيواسيتاميد (TAA)، اما المجموعة الثالثة فقد جرعت بالمستخلص المائي لقشور(*Nephelium lappaceum (Nl)*، المجموعة الرابعة (G4) جرعت 25 ملغم/كغم بالمستخلص المائي لقشور *Nephelium lappaceum (Nl)* مع 200 ملغم/كغم من الثيوأسيتاميد (TAA). بعد 90 يوما، تم جمع عينات الدم لدراسة المستويات التالية: انزيم الفوسفاتيز القاعدي (ALP)، الانزيم الناقل للأمين (ALT)، الانزيم الناقل للأسبارتات (AST)، البيليروبين الكلي (TB) بالإضافة إلى دراسة التغيرات النسيجية. أظهرت النتائج أن TAA قد سبب ارتفاع معنوي ($P < 0.01$) في ALT وAST وALP وT-BIL في (G2) مقارنة مع مجموعة السيطرة (G1). بينما لم يكن هناك فرق معنوي ($P > 0.01$) في مستوى ALP وALT وAST وT-BIL في G3 وG4 مقارنة مع مجموعة السيطرة. أظهرت نتائج الفحص النسيجي لمجموعة G2 أن المعالجة بـ TAA لمدة 90 يوماً سبب العديد من التأثيرات الضارة على خلايا الكبد مع ظهور العديد من العقيدات الورمية وتفجي والنخر الخلوي مع ارتشاح الخلايا الالتهابية. نستنتج من هذه الدراسة أن المستخلص المائي لقشور نبات *Nephelium lappaceum (Nl)* كان له دور وقائي ضد سرطان الكبد المستحث بمادة TAA في ذكور الجرذان البيض.