The effect of some secondary metabolites of alcoholic extract for eggplant fruits on liver enzymes in mice.

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Abstract:

The high-fat diet induces liver damage. atorvastatin has been traditionally used as a Lipid-lowering drug. this work studied the effect of *Solanum melongena* ethanolic extract on induced liver damage in mice that were treated with a high-fat diet for 14 days by collecting serum biochemical profiles (including GOT, ALP, and GPT), the treated animals showed a decrease in body fat along with necrotic cells in the liver region. *S. melongena* in high concentrations can lower serum biochemical profiles and hepatic fat formation. Added to this, *solanum melongena* had no acute oral toxicity in mice. These findings point to the potential of this extract as a hepatoprotective agent against liver damage brought on by a high-fat diet without causing any immediate oral toxicity. Its high total phenolic and flavonoid levels may have contributed, or at least in part, to these actions.

Keywords: eggplant, liver enzymes, atorvastatin, *Solanum melongena*.

Introduction:

Due to their rich nutritional content, the Consumption of eggplant fruits (*Solanum melongena* L.) is widespread worldwide. eggplant fruits are widely consumed around the world. The genus Solanum, which includes eggplant, is a member of the Solanaceae family (1). It is also referred to as aubergine (eggplant) and regarded as a basis of minerals (including calcium, iron, magnesium, potassium salt, and zinc), and vitamins C, B6, B12, A, E, D, niacin, thiamin, and K (2,3). In terms of its health benefits and economic value, it has been shown that eggplant has a high nutritional value and low-calorie count. Moreover, it has a very high-water content and is a rich source of fiber and vitamins (3). Also, the effectiveness of eggplant extract in lowering cholesterol levels has been demonstrated. Asthma, bronchitis, diabetes, arthritis, and hypercholesterolemia have all been treated with eggplant due to the presence of phenolic and alkaloid compounds. Eggplant is used in medicine (2). The amazing pharmacological and biological properties of herbal plants were the reason for the disease treatments' advancement and success. On the other hand, it is thought that natural and herbal medicines have a variety of therapeutic effects and fewer side effects (3). Alanine aminotransferase (GPT), alkaline phosphatase (ALP), and aspartate aminotransferase (GOT), among other enzymes, are some of the ones that carry out liver function. Because it can catalyze the hydrolysis of molecules in phosphate esters, ALP is in charge of manufacturing organic radicals and inorganic phosphate, whereas the activity of GPT

and GOT was indicated by reducing the activation energy of a chemical reaction to speed up the rate of the reaction (4-6). In this study, laboratory mice with particular liver damage from a high-fat diet for 30 days were used to examine the anti-hepatic effects of eggplant ethanolic extract.

Materials and Methods:

2.1 Plant Materials

In the summer of 2020, fresh *Solanum melongena* fruits were purchased from Baghdad which was previously identified by the National Herbarium of Iraq market. The best fruit specimens were chosen. The secondary metabolites lab at Alnahrain University's College of Biotechnology was used to achieve the work.

2.2 Experimental Animals albino male mice

The biotechnology research center of Al-Nahrain University provided albino male mice. Non-nulliparous mice that were healthy and had a white, smooth coat were chosen. The mice ranged in weight from (23-25) gm.

2.3 High-Fat Diet Preparation:

There was a prepared high-fat, high-cholesterol diet. The diet's main ingredients were lard (38%), dextrose (13%), milk powder (15%), common rodent pellets (29%), egg yolk (4%), and multivitamin (1%). The components were combined in a mixing bowl after the rodent pellets were ground into a powder. The ingredients were mixed, uniform paste that was incredibly firm and created by using distilled water that was progressively added. To create typical pellet shapes, utilizing plastic tubing with a tiny diameter, the evenly combined paste was molded. Next, the pellets were added. on absorbent paper and dried overnight in a 60˚C oven.

2.4 Experimental Set Up:

20 mice were divided into 5 groups at random and given a high-fat diet to cause hyperlipidemia. For the entire experiment, this diet was fed to the animals. The mice in the typical control group, on the other hand, were only given regular rodent pellets to eat. The mice in group 1 were subsequently given the extracts at the doses fourteen (14) days after starting on this diet, while those in group 2 (normal and positive controls) received distilled water or atorvastatin. Using gavage tubes, all therapies were administered orally, every day for 14 days.

2.5 Preparation of ethanolic Extract:-

All *S. melongena* fresh fruits were cut into small pieces, removed from the pulps, and oven-dried at 40 ˚C for 36 hours with turnover instances to a constant weight. With a mortar and pestle, the dry sample was manually ground into a coarse powder. 50g of powder was steeped in 250ml of 70% ethanol in Erlenmeyer flasks for 24 hours while being sometimes shaken to create an ethanol extract. Whatman No. 1 filter paper was used to decant and filter the mixture. Using a rotary evaporator, the filtrate was concentrated to a certain volume, which was then dried at 45 °C using an oven to produce a fine powder or thick paste, which was then kept chilled at 4 °C. The hepatoprotective effects of three dosages (125, 250, and 500 mg/kg) were evaluated in 5 groups (4 mice in each group).

- Group I: For 14 days, mice in this group received a single dose of 0.1 ml of S. *melongena* extract containing 125 mg/Kg.
- Group II: For 14 days, mice in this group received a single dose of 0.1 ml (250 mg/Kg) of *S. melongena* extract.

- Group III: For 14 days, mice in this group received a single dose of 0.1 ml (500 mg/Kg) of *S. melongena* extract.
- Group IV For 14 days, mice received a single dose of 0.1 ml of D.W. every day. (As a negative control).
- Group V: For 14 days, one dose of distilled water (0.1 ml) per day was administered to the mice. after receiving a single dose of 0.2% atorvastatin in olive oil (0.1 ml) on day 1 (as positive control).

On day 15, mice were killed and dissected after receiving IP injections of the test substances. blood was drawn from a heart puncture and centrifuged for 10 minutes at 3000 rpm to separate the serum after it had been permitted to coagulate at room temperature for 15 minutes in an Eppendorf tube (7). Afterward, the liver was collected and preserved in formalin (10%) for histological exams, along with alkaline phosphatase (ALP), aspartate aminotransferase (GPT), and alanine aminotransferase (GOT). These enzymes were used to assess the liver's function (Fue et al., 2010) In mouse serum, Aspartate Aminotransferase (GPT) enzymes were computed using Reitman and Frankel's evaluation method (1957). AIP was measured in mouse serum using a particular kit.

2.6 Serum Lipid Profile Determination

Before beginning the high-fat diet, serum lipid profiles were taken from four mice per group at random on days 0 and 14 to confirm the induction of hyperlipidemia (after daily administration of the extracts or atorvastatin for 2 weeks). Blood samples were used to make the serum.

2.7 Statistical Analysis:

The impact of numerous elements on the research parameters was ascertained using the Statistical Analysis System- SAS (2018) application. In this study, the means were significantly compared using the least significant difference (LSD) test (ANOVA).

Results and discussion:

Histopathological activity of the liver:

The histopathological studies on the control negative group using DW, plant extract only at two concentrations of 125, 250, and 0.2% atorvastatin as shown in fig. 1, 2, 3, 4.

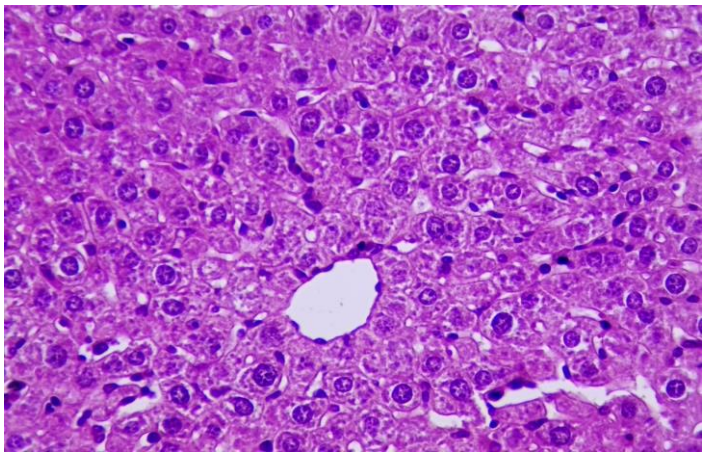

Figure 1: results of control negative of mice liver Section treated with single dose 0.1 ml of D.W for 14 days Section showing normal histological structure appearance of parenchymal hepatic tissue cells.

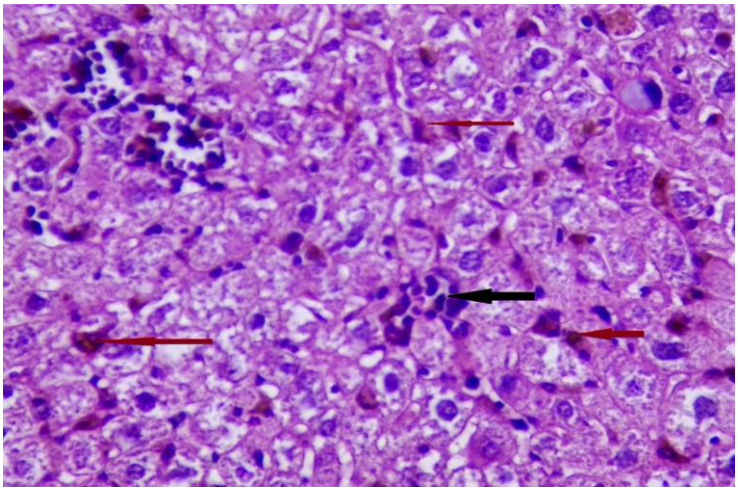

Figure 2: The results of a 14-day treatment of mice liver sections with a single dose of 0.1 ml of an *S. melongena* extract containing 125 mg/Kg showed minor glycoprotein depletion, bile pigment stasis, focal necrosis of hepatocyte cells, and infiltration of inflammatory cells.

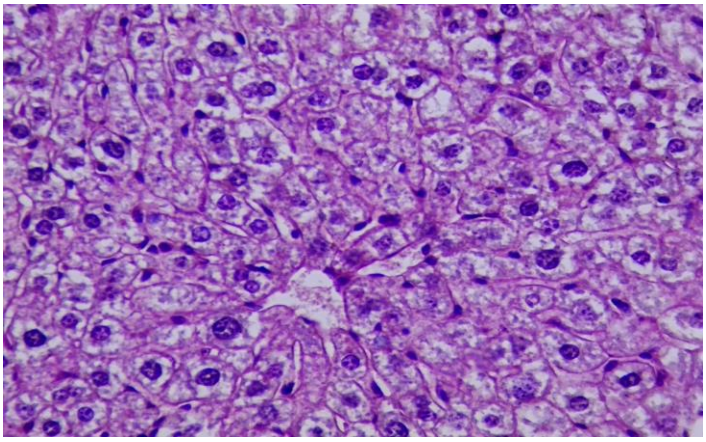

Figure 3: The results of the mice liver Section were administrated with 0.1 cc of 250 mg/Kg S. melongena extract given once a day for 14 days Showing depletion of glycoprotein with apoptotic cells. (X40)(H & E).

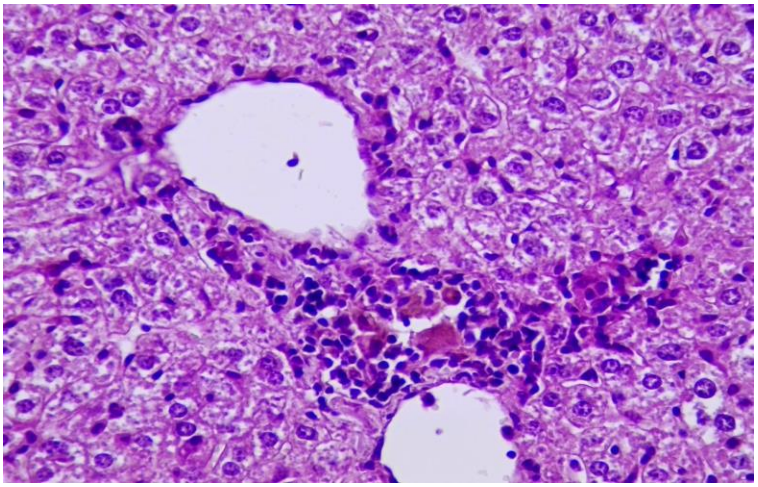

Figure 4: The results of mice liver Section Showing depletion of glycoprotein with a focal area of necrosis of hepatocyte cells and inflammatory cells infiltration after administration of a single dose of 0.2% atorvastatin in olive oil (0.1ml) on day 1 and subsequently received distilled water (0.1 ml) as the Control +ve (X40) for 14 days (H & E).

Table 1. Comparison between different groups in Liver enzymes.

Group	GOT (Mean ± SE)	GPT (Mean ± SE)	AIK (Mean ± SE)
G1	154.1 ±7.32 a	69.25 ±2.93 a	71.35 ±3.05 b
G2	145.12 ±3.68 ab	67.09 ±1.74 a	70.85 ±8.01 b
G3	129.36 ±7.03 c	62.33 ±2.63 ab	68.25 ±2.52 b
G -	155.08 ±8.15 a	58.00 ±2.35 ab	99.18 ±3.02 a
G+	129.11 ±5.47 bc	50.07 ±2.93 b	42.26 ±2.17 c
LSD value	22.479 *	14.523 *	25.085 *

*** (P≤0.05).**

Got activity: According to Table 1's findings, untreated mice in the control group showed GOT activity of 155, 8.15 Unit/L. while using atorvastatin medicine decreased GOT value to 129.11±5.47 Unit/L. Interestingly, Significant reduction in GOT value which was 129.3±7.03 Unit/L after treatments with 500 mg/Kg of *S. melongina* plant extract given orally to the mice compared with the control negative group and no significant effect compared with control positive group, while concentrations at 125 and 250mg/Kg did not affect reducing GOT value. demonstrating that the plant extract helped the group that experienced the perturbation to positively regulate GOT activity.

GPT activity: As shown in Table (1), GPT activity is another parameter tested to determine the hepatoprotective activity of S. *melongena* plant extract. The GPT activity of untreated mice was 58.00 ±2.35 Unit/L, whereas, after treatment with S. *melongena* extract, there was a significant decrease in GPT value, which was 62.33 Unit/L at a dose of 500 mg/Kg.

AIP activity: Treated mice with atorvastatin in positive control reduced AIP levels compared to control negative 42.26±3.02 Unit/L (table 1). But, when the treated mice with S. *melongena* extract of 125, 250, and 500mg/Kg; the AIP levels reduced to 71.26±3.05, 70.85±8.01 and 68.25±2.52; which had a significant effect in reducing ALP values Due to its capacity to keep the value close to the normal range, the plant extract appears to have had a favorable impact once more in mending ALP activity when compared to the group that received atorvastatin treatment. as in figure 3.

SLE (extracts obtained with solid-liquid extraction) is one of the most popular and straightforward techniques, requiring only the direct extraction of plant material from a suitable solvent. Phenolics are mass transferred from the cell walls into the extraction solvent during this sort of extraction (8).

EtOH was chosen because of the phenolic compounds' high rate of recovery, its nontoxicity, and its appropriateness for polar molecules (9).

According to the results above and Because mice consume more non-esterified cholesterol on a high-fat diet and absorb it more easily via their intestines, this results in hyperlipidemia. Triglycerides and total cholesterol levels rise as a result, and the hepatic portal vein subsequently transports these substances to the liver. The excess of total cholesterol in the liver promotes the VLDL generation, followed by endothelial cell-associated lipoprotein lipase's conversion of VLDL to LDL, which in turn stimulates the creation of LDLs through the LDL receptors (7,8). As the major organ for detoxification, the liver is well-known to produce considerable hepatic shut-off after drug use due to the production of pro-oxidant reactive oxygen species (ROS), which in turn stimulates cellular abnormalities that impair specific biomolecules (9,10).

In contrast to atorvastatin, which lowers total cholesterol synthesis in peripheral body tissues and increases cholesterol and triglyceride excretion through bile salts in feces, the mechanism of action of *S. melongena* extracts is unknown (7) In this study, group 1, group 2, and group 3 (figs. 2 and 3) that received a *solanum melongena* fruit infusion have less hepatocyte necrosis than the positive and negative groups, which have more necrosis (fig. 1 and 4). This aligns with the findings of the study by (2). which demonstrated the fruit of *Solanum melongena* has hepatoprotective and antioxidant properties, which demonstrated the fruit of *Solanum melongena* has hepatoprotective and antioxidant properties. Another study by (10,11,12) also showed that flavonoids found in *Solanum melongena* fruits have hepatoprotective activities. Based on these findings, it is possible to conclude that giving *Solanum melongena* fruit infusion to all mouse groups can have hepatoprotective benefits against liver damage that is caused by a high-fat diet. This impact might have been brought on by the plant's phytochemicals.

The Solanaceae family of vegetables' most abundant source of phenolic acids is the pulp of the eggplant (13). The purple color of the eggplant peels is given by the glycosides of delphinidin (14). Though *S. melongena* extracts contain antihyperlipidemic and weight-reduction qualities, The study's results suggest High levels of total cholesterol, particularly LDL, in the blood, cause plaque buildup in the capillaries, which causes them to narrow (15,16). This increases the risk of developing hypertension and other cardiovascular illnesses (2). According to our study results *Solanum melongena* ethanolic extract has antioxidant and hepatoprotective activities (17,18).

Conclusion:

The management of obesity and hyperlipidemia using eggplant as a source of alternative treatments is highlighted in this study, particularly at the maximum dose of 500mg/kg of ethanolic extract. Therefore, the presence of *Solanum melongena* in the diet may have therapeutic benefits and preventative effects against these two non-communicable diseases in people (obesity and hyperlipidemia).

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We are extremely appreciative of the technical personnel at the College of Biotechnology and the Biotechnology Research Center Labs at Al-Nahrain University for their tireless work and assistance during this project.

Authors Declaration:

- - Interest Conflicts: None.
- -We thus attest that the manuscript's Figures and Tables are fully original to us.
- -The statement on animal welfare has our signature.
- -We have consented to ethical considerations.
- - Ethical Clearance: The project was given the go-ahead by the local ethical committee at the College of Biotechnology at the University of Al-Nahrain.

Authors Contribution:

The authors use their initials to explain their contribution (L. Zaineb Sabeeh Omran collected the Fruit samples and extracted them to prepare concentrations used as treatments for several mice, Professor assistant Dr. Enas Hamid coordinated with the Biotechnology Research Center to administer doses to mice and L. Hadeel Khalaf wrote the manuscript. From the inception of the research to the submission of the MS, each authorship is anticipated to significantly contribute to a portion of the MS. Each author has carefully read and approved the final draft of their MS.

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دراسة تأثير بعض مركبات الايض الثانوي للمستخلص الكحولي لثمار الباذنجان المحلي على إنزيمات الكبد للفئران

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الخلاصة

النظام الغذائي الغني بالدهون يؤدي إلى تلف الكبد. تم استخدام أتورفاستاتين تقليديًا كأدوية لخفض الدهون. في هذه الدراسة، قمنا بفحص تأثير المستخلص الكحولي لنبات الباذنجان *Solanum melongena* وتأثيره على معالجة تلف الكبد الناجم عن تغذية الفئران بنظام عالي الدهون بسبب زيادة الكوليسترول في الدم. لوحظ تغيير المؤشرات البيوكيميائية في مصل الدم بما في ذلك (GOT، ALP، GPT) المرتبطة بزيادة الدهون في كبد الفئران التي عولجت بنظام غذائي عالي الدهون. كان التركيز العالي من مستخلص الباذنجان الكحولي *Solanum melongena* قادراً على تقليل المؤشرات البيوكيميائية في الدم وتراكم الدهون في الكبد. يضاف إلى ذلك، أن المستخلص الكحولي لثمار الباذنجان *Solanum melongena* لم يكن ذو تأثيرات سمية حادة على الفئران التي تم تجريعها للمستخلص عن طريق الفم. تشير هذه النتائج إلى احتمالية التأثير الايجابي لهذا المستخلص كعامل وقائي للكبد التالف نتيجة اتباع نظام غذائي غني بالدهون دون أي تأثيرات سمية حادة كونه يمكن تجريعه عن طريق الفم. قد تساهم المحتويات العالية من الفينول والفلافونويد الموجودة في ثمار الباذنجان في تحسين مستويات المؤشرات البيوكيميائية للكبد التالف.

الكلمات المفتاحية: النشاط الوقائي للكبد، مركبات الفلافونويد ، الكبد ، ثمار الباذنجان.