



A Comparative Histopathological and Biochemical Study Between Crude Methanolic and Watery Extracts of Eggplant for Treating Hepatotoxicity in Albino Rats

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Abstract | Eggplant (*Solanum melongena*) is one of the most essential vegetable crops that it's grown on more than 1.7 million hectares globally. It has been believed that may reduce toxicity and serum cholesterol. It is extensively consumed in Iraq. The present study was to evaluate the effects of two extract of eggplant (*S. melongena*) on potassium dichromate ($K_2Cr_2O_7$) inducing liver toxicity. In order to do that, animals were divided into four groups, with six rats in each group: group negative control ; rats received distilled water, group two positive control; rats received potassium Dichromate ($K_2Cr_2O_7$) (2.5 mg/kg-day) orally, group three received crude methanolic extract of eggplant at dose 400 mg / kg + $K_2Cr_2O_7$ (2.5 mg/kg-day) orally, group four : received crude watery extract of eggplant at dose (400 mg/kg) + $K_2Cr_2O_7$ (2.5 mg/kg-day) orally. Blood was drawn during the sacrifice and was placed into heparin and EDTA tubes for biochemical analyses. As liver tissue was collected, and then fixed in 10% neutral buffered formalin solution for histological studies. The results showed that crudeWEE better than crude MEE in reducing potassium dichromate ($K_2Cr_2O_7$) mediated liver toxicity. Also, that treatment with crude WEE reduced bad lipid serum levels significantly. The histopathological results showed the negative group showed atypical texture without any significant obvious lesions. While positive group in the potassium dichromate ($K_2Cr_2O_7$), the liver showed severe hydropic degeneration and necrosis of hepatic cells. In case of crude methanolic extract of Eggplant (MEE), the histopathological results showed fatty change that appears as vacuoles in liver parenchyma. Liver cells appear empty with destructed nucleus. While crude watery extract of Eggplant (WEE) group showed normal architecture of hepatic tissue. This study confirmed general adverse crude watery extract of Eggplant (WEE) better than crude methanolic extract of Eggplant (MEE) in reducing potassium dichromate ($K_2Cr_2O_7$)-mediated liver toxicity.

Keywords | Rats, Herbal medicine, Potassium dichromate, Biochemical analysis, Histopathology

Received | October 18, 2024; **Accepted** | December 22, 2024; **Published** | February 18, 2025

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Citation | Al-Zubaidi HJ, Al-Safi SMM, Abdulzahra AA, Mehdyal-Zeiny SS, Al-Dawah NKJ, Al-Asadi ZA (2025). A comparative histopathological and biochemical study between crude methanolic and watery extracts of eggplant for treating hepatotoxicity in albino rats. Adv. Anim. Vet. Sci. 13(3): 668-675.

DOI | <https://dx.doi.org/10.17582/journal.aavs/2025/13.3.668.675>

ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331



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The liver plays a crucial role in controlling the filtration and clearing process of blood that is received from the digestive tract before being distributed to other body tissues and organs (De Abajo *et al.*, 2004). The liver has several other functions, such as detoxifying, transforming accumulating metabolites, mediating drug transformations, and metabolism. Therefore, the liver is highly susceptible organ to damage due to different toxins, infectious agents, and free radical activity (Lu *et al.*, 2018). Subsequently, liver damage often leads to many disorders, including metabolic disturbance and synthetic dysfunction, for example: transient elevation in hepatic enzyme levels, hepatic fibrosis, liver cirrhosis and hepatocellular carcinoma (Srivastava and Shivanandappa, 2010).

Metals, as environmental and occupational toxicants, are a major health concern. Consequently, long-term exposure is associated with the appearance of adverse health conditions. One of these metals is chromium, which is a widely distributed metal (Stohs and Bagchi, 2008). It is potentially giving rise to the risks of exposure in different environments, especially the work environment, that may affect human health (Permenter *et al.*, 2011). The toxicity of chromium (Cr) has made it a threat to human society (Salama *et al.*, 2021). The chemical formula, dose, and duration of exposure are factors that determine the toxicity of Cr. Toxicity with potassium dichromate ($K_2Cr_2O_7$) is causing severe damage in different tissues and vital organs (Shrivastava *et al.*, 2002). In one of these organs, the liver, exposure to potassium dichromate ($K_2Cr_2O_7$) induces hepatotoxicity due to high ROS levels (Patlolla *et al.*, 2009), lipid peroxidation (Liu and Shi, 2001), structural tissue injury (Acharya *et al.*, 2001) and inhibition of antioxidant enzymes (Soudani *et al.*, 2011). It has been revealed that natural and synthetic antioxidant have the ability to improve or prevent $K_2Cr_2O_7$ -mediated hepatotoxicity (Soudani *et al.*, 2011).

Solanum species (eggplants) belong to the family *Solanaceae* and the plant genus *Solanum*. The tropical region widely cultivates *Solanum melongena*, an economically important vegetable crop that is a good source of vitamins and phytochemicals (Agoreyo *et al.*, 2012). *Solanum melongena*'s leaves and fruits are used in traditional medicine and as vegetables (Bonsu *et al.*, 2008). *Solanum melongena* extracts were effective against a variety of diseases, which include hepatitis, high blood pressure, and microbes (Madhuri *et al.*, 2015). In addition, it acts as an antioxidant (Kandoliya *et al.*, 2015), antidiabetic (Kwon *et al.*, 2008), hypolipidaemic agent (Ossamulu *et al.*, 2014) and blood purifier (Grace-lin *et al.*, 2011) because of its phytochemical content. This plant possesses phytochemicals like alkaloids, saponins, cyanogenic glycosides and tannins. Therefore, it has been shown to possess antioxidant potential and ascorbic acid,

which makes it possible for them to act as reducing agents, metal chelators, and radical scavengers (Kasote *et al.*, 2015; Mbah and Egbunu, 2017). Because of their flavonoid components and fruit phenols, fruits are among the top ten vegetables in terms of antioxidant potential (Singh *et al.*, 2009), which have been linked to various health benefits (Hung *et al.*, 2004). Thus, the aim of this study was histopathology and biochemical comparison between two crude methanolic extracts of eggplant and a crude watery extract of eggplant in treating potassium dichromate ($K_2Cr_2O_7$) to induce toxicity in albino rats.

MATERIAL AND METHODS

ANIMALS

The animals were Albino rats about 6–8 weeks of age at (200±250g) of weight and kept in plastic cages. The animals were housed in a regulated 12-hour light-dark cycle at (25 ±2°C) with unlimited access to water and food. The animals were left in optimum conditions for one week for adaptation at the Faculty of Veterinary Medicine Animal House, according to Animal welfare guidelines.

ETHICS STATEMENTS

The study was approved with the Central Committee Bioethics for the Treatment and the Use of Animals. University of Kufa, Iraq, Faculty of Veterinary Ethics Committee. As documented through formal application. No. 12766 on May 19th 2024.

CHEMICAL

Potassium dichromate ($K_2Cr_2O_7$) was purchased from Sigma Aldrich 2.5 mg/kg-day orally (Mackenzie *et al.*, 1958).

PLANT COLLECTION

Fresh fruits from *Solanum melongena* were obtained from the local market to provide the required plant material.

PREPARATION OF CRUDE METHANOLIC EXTRACTION OF EGGPLANT

The fruits were shade dried at room temperature for 4 weeks and the dried samples were grounded to powder using a grinder. the extract was prepared by adding 50 grams of eggplant powder to 500 ml of methanol 70% mixed under constant magnetic stirring for 24 hour at 45°C temperature. The extract was filtered using a Buchner funnel and Whatman filterpaper No.1. the filtrate was concentrated at 40°C, 90rpm using a rotary evaporator by converting it into thick liquid to remove solvent According to (Harborne, 1973). Until it was needed for the experiment, the extract was put in a sample container and kept in a refrigerator at about 4°C. Rats have received the *Solanum melongena* extract at the dose of 400 mg/kg of body weight daily orally administration as dose described by (Manasa and Akondi, 2014).

PREPARATION OF CRUDE WATERY EXTRACTION OF EGGPLANT

The fruits were shade dried at room temperature for 4 weeks and the dried samples were grounded to powder using a grinder. the extract was prepared by adding 50 grams of *Solanum melongena* powder to 500 ml of distilled water and mixed by magnetic stirrer for 24 hours at 45°C temperature, then filtered using a Buchner funnel and Whatman filter paper No. 1. and the filtrate was concentrated at 40 °C, 90 rpm, using a rotary evaporator by converting it into thick liquid to remove solvent. A brownish residue is kept in airtight bottles in a refrigerator until use. According to Harborne (1973). Rats have received the *Solanum melongena* extract at the dose of 400 mg/kg of body weight daily orally administration as dose described by (Abdul *et al.*, 2012).

EXPERIMENTAL DESIGN

Twenty-four male Albino rats were randomly divided into four groups, six rats in each group namely;

- Group 1: control group rats received Feed +distilled water.
- Group 2: Positive control; rats received potassium Dichromate ($K_2Cr_2O_7$) (2.5 mg/kg-day).
- Group 3: crude methanolic extract of eggplant received (MEE) at dose 400 mg / kg + $K_2Cr_2O_7$ (2.5 mg/kg-day) orally.
- Group 4: crude watery extract of eggplant received (WEE) at dose (400 mg/kg) + $K_2Cr_2O_7$ (2.5 mg/kg-day) orally.

All group except the control group received $K_2Cr_2O_7$ after 2 hrs. From giving the extract according to the previously mentioned groups. Blood was collected during the sacrifice and placed into EDTA and heparin tubes for biochemical assays. While liver tissue was collected, cleaned, and then fixed in a 10% formalin solution for histological studies.

BIOCHEMICAL ANALYSIS

Blood samples in the EDTA and heparin tubes were centrifuged at 2,000 rpm for 5 min. Then plasma was collected into sterile tubes and stored at -20°C until it subjected to biochemical analysis which include, liver function biochemical test such as Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT), Alkaline Phosphatase (ALP). While Lipoprotein fractions Enzymatic determined of levels of total cholesterol, triglyceride's, Low Density lipoprotein (LDL) and High Density lipoprotein (HDL) was estimated using FujiFilm auto analyzer (Biochemistry analyzer, Japan).

HISTOPATHOLOGICAL EXAMINATIONS

Each organ has been cut into small pieces, 3-5 mm, then washed with PBS and fixed with 10% formalin and stored

in sterile containers. After 24 hrs. In order to maintain the concentration at 10%, the 10% formalin was subsequently replaced with a new one. Then the fixed tissues were dehydrated in varying ethanol concentrations. After that, the tissues were immersed in xylene three times for an hour each. Finally, the tissues were preserved at 60°C in liquid paraffin. Originally, liquid paraffin was used to embed the tissue samples in tissue cassettes. Subsequently, the blocks were adhered on glass slides after being sliced into 5-micron slices by a microtome. for staining, hematoxylin and eosin staining technique's according to (Gibson-Corley *et al.*, 2013, Luna, 1968).

STATISTICAL ANALYSIS

Ordinary one-way analysis of variance (ANOVA) or F test with multiple comparisons and the Fisher test (least significant difference) ($P \leq 0.01$) were carried out to compare positive control (C+) with other groups (crude methanolic extract of Eggplant and crude water extract of Eggplant) according to (Fisher, 1925). Statistics was conducted using prism Graphpad 6 software (La Jolla CA, USA).

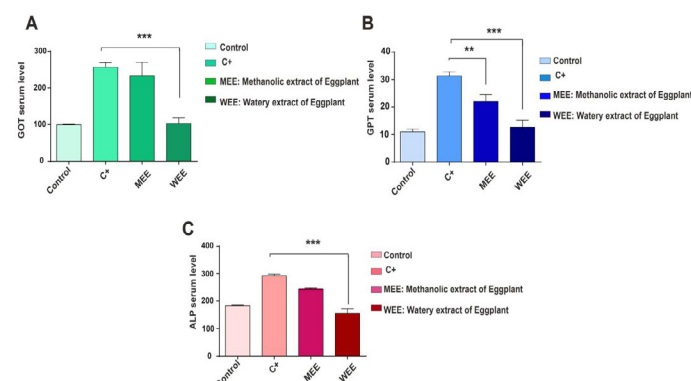


Figure 1: Watery extract of Eggplant enhanced marked decrease in liver Function Enzymes.

RESULTS AND DISCUSSION

LIVER FUNCTION STUDY

In order to explore the effect of both extracts, namely the crude methanolic extract of eggplant and crude watery extract of eggplant, on liver function following, we investigated the effect of these extracts on liver function enzymes, namely GOT (Glutamic Oxaloacetic Transaminase), GPT (Glutamic Pyruvic Transaminase), and ALP (Alkaline Phosphatase), following potassium dichromate-mediated liver toxicity. The results indicated that GOT and ALP showed a significant decrease in serum levels in the watery extract of the eggplant treated group compared with the control positive group (C+) (Figure 1A and C). While GPT showed a significant decrease in serum levels in both groups of methanolic and watery crude extracts of eggplant treated groups compared with the (C+) (Figure 1B). Taken together, these results showed that crude WEE better than crude MEE in reducing potassium dichromate ($K_2Cr_2O_7$)-

mediated liver toxicity. Serum samples were collected from all experiment groups, namely Control, C+ (control positive exposed to Potassium Dichromate($K_2Cr_2O_7$), MEE (crude methanolic extract of eggplant+($K_2Cr_2O_7$)) and WEE (crude watery extract of eggplant+($K_2Cr_2O_7$)). Then, the liver function enzymes namely A: GOT, B: GPT and C: ALP were tested. *** $P \leq 0.001$, ** $P \leq 0.01$ Error bars = mean $\pm$ standard error of mean (n=6).

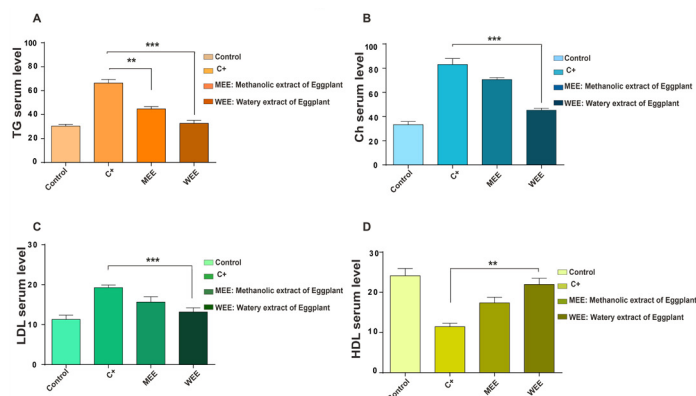


Figure 2: Significant decrease of bad lipid flowing treatment with Watery Extract of Eggplant.

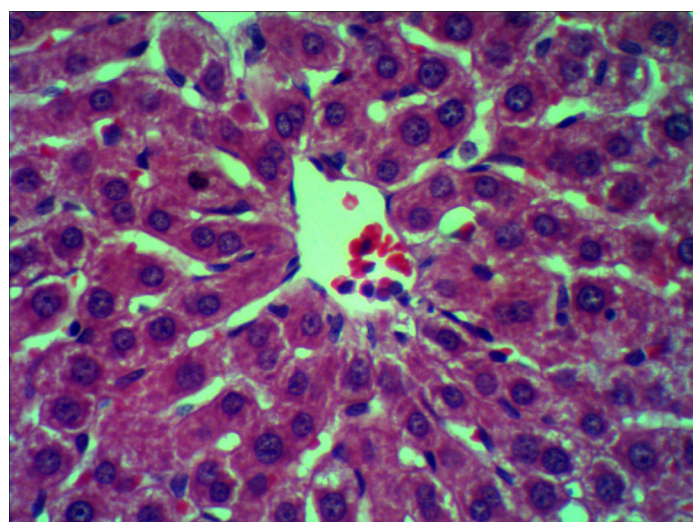


Figure 3: Microphotograph of liver negative control: No pathological changes. Observed in hepatic tissue (H and E,400X).

LIPIDS PROFILE STUDY

To determine whether the effects of both extracts under study on the different lipids in serum levels, namely TG (Triglyceride's), Ch. (Cholesterol), LDL (Low Density Lipoprotein), and HDL (High Density Lipoprotein), following potassium dichromate-mediated liver toxicity. The results revealed that the crude WEE treated group showed a significant decrease of adverse lipid profiles (TG, Ch., and LDL) in serum levels compared with the C+ group. (Figure 2A, B and C). However, HDL results revealed a significant increase with the crude WEE treated group comparing (C+). However, the crude MEE treated group

showed no significant effect compared with (C+) (Figure 2D). Taken together, these results suggested that treatment with crude WEE reduced bad lipid serum levels significantly. Serum samples were collected from all experiment groups, namely control, C+ (control positive exposed to Potassium Dichromate ($K_2Cr_2O_7$), MEE (crude methanolic extract of eggplant+($K_2Cr_2O_7$)) and WEE (crude watery extract of eggplant+($K_2Cr_2O_7$)). Then, different lipid levels, namely A: TG, B: Ch., C: LDL and D: HDL were tested. Serum level measured based on mg/dl. *** $P \leq 0.001$, ** $P \leq 0.01$ Error bars = mean $\pm$ standard error of mean (n=6).

HISTOPATHOLOGY STUDY

In order to explore the changes in liver, histopathological study was took place using light microscope. The liver histopathological section showed different lesions in it's texture according to different manipulate. In the control negative group, the liver showed typical texture without any significant obvious lesions (Figure 3). While in the potassium dichromate ($K_2Cr_2O_7$) group (C+), the liver showed severe hydropic degeneration and necrosis of hepatic cells (Figure 4). In case of crude methanolic extract of eggplant (MEE), the histopathological results showed fatty change that appears as vacuoles in liver parenchyma. Liver cells appear empty with destructed nucleus (Figure 5). While crude watery extract of eggplant (WEE) group showed normal architecture of hepatic tissue (Figure 6).

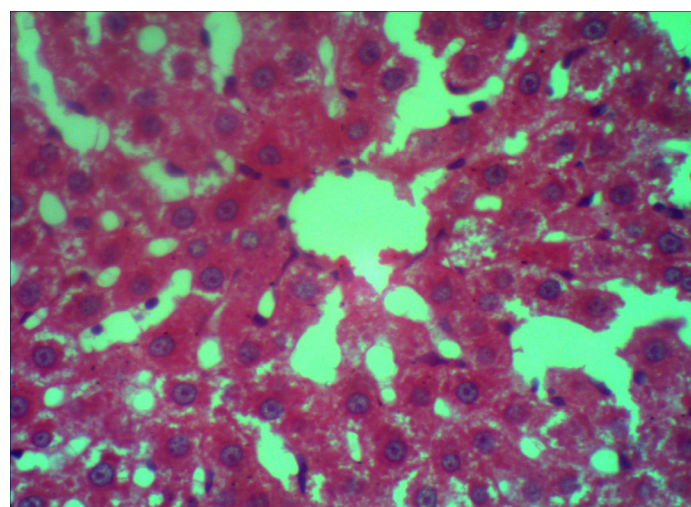


Figure 4: Microphotograph of liver positive control: severe hydropic degeneration and necrosis, Liver cells appear empty with destructed nucleus (Observed in hepatic tissue (H and E,400X).

Liver is playing a vital role in biotransformation of different xenobiotic compounds. Therefore, liver is the major site for toxicants where the transformation process take place to more or less toxic intermediate products (Elshazly *et al.*, 2016). It has been reported that the hexavalent form of Cr is $K_2Cr_2O_7$, which induces toxicity in different organs, notably the liver, through various doses and routes

(Avila *et al.*, 2016). The activity of serum enzymes is an important indicator for diagnosing damage and recognising the severity of injuries that involve organs and tissues. GOT (known as AST) and GPT (known as ALT) are the common serum plasma sensitive biomarkers that are used to examine hepatic integrity and function. (Anandasagopan *et al.*, 2017). For instance, Cr (VI) uptake is leading to an increase in the plasma activities of GOT and GPT. Thus, these enzymes are indicating liver damage as a result of oxidative stress (Bayraktar *et al.*, 2015). Previously, this observation was reported in various lab animals, such as rabbits, rats and mice (Mary Momo *et al.*, 2019). Similarly, our results indicate that the potassium dichromate-mediated liver toxicity group (C+) showed an increase in serum levels for the liver function enzymes GOT, GPT, and ALP (Figure 1 A, B and C). In correlation with these results, histopathological results showed liver damage caused by severe necrosis of hepatic cells with a record of hydropic change following potassium dichromate-induced liver toxicity (Figure 4).

tioned sever hydropic degeneration and necrosis enhanced in a few hepatocytes as a result of potassium dichromate toxicity (Wang *et al.*, 2010).

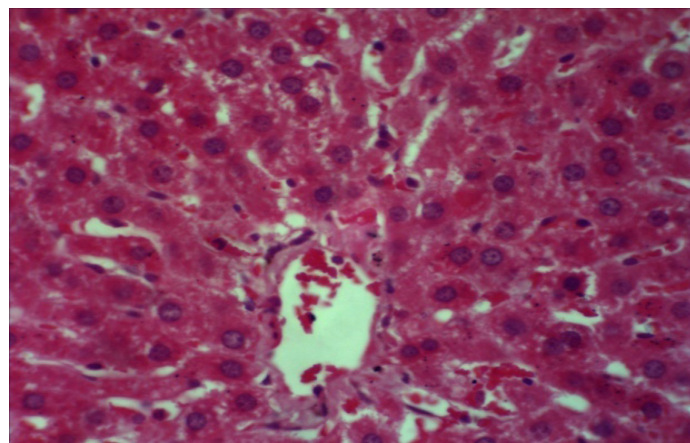


Figure 6: Microphotograph of Liver treated with crude Watery Extract of Eggplant (WEE) group rat showed normal architecture of hepatic tissue. (H and E, 400x).

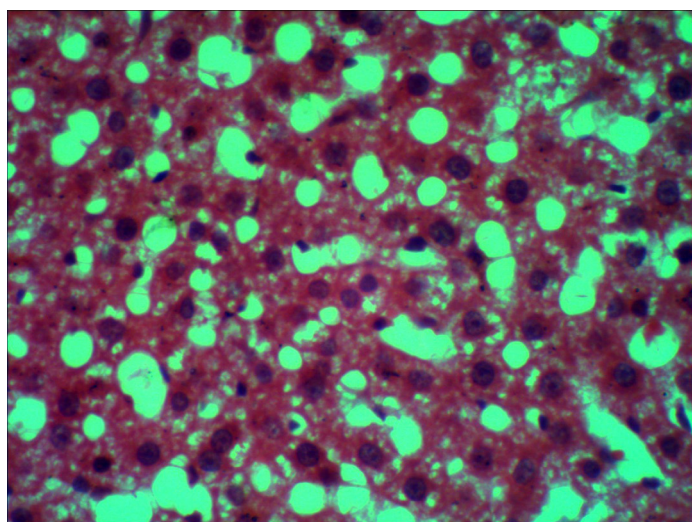


Figure 5: Microphotograph of liver treated with crude Methanolic Extract of Eggplant (MEE) group rat: fatty change that appear as vacuoles in liver parenchyma. (H and E, 400x).

Alternative medicine or herbal medicine, is used worldwide as a treatment strategy for a variety of diseases and to improve health (Vivekanand *et al.*, 2020). Therefore, in this study, two crude extracts were investigated for their effects on potassium dichromate-induced liver toxicity. Crude WEE showed a significant decrease with all three enzymes in serum levels compared with (C+) (Figure 1A, B and C), as well as histopathological results. While MEE showed a significant decrease in the case of GPT (Figure 1B). It has been recorded that high exposure to dichromate's enhanced liver cell damage, necrosis, histocytic, and lymphocytic infiltration. In addition, Kupffer cells increased according to the severity of damage, which may lead to hepatic failure (Neki *et al.*, 2016). Furthermore, some observations men-

Further analysis took place for lipid profile detection; crude WEE enhanced a significant decrease in lipid serum levels, namely TG, Ch. and LDL (Figure 2A, B and C). While HDL showed a significant increase (Figure 2D). These results have been approved by Nwozo *et al.* (2018), who found that eggplant controlled the levels of lipid profiles and enhanced liver function. LDL and HDL are considered plasma lipoproteins that are involved in lipid metabolism and the exchange of triglycerides, cholesterol, and cholesterol ester between tissues (McNamara, 1992). Most body tissues, rather than hepatic tissue, have a requirement for cholesterol due to their low cholesterol biosynthesis pathway activity. Therefore, these requirements are provided by LDL which is internalized by receptor mediated endocytosis. The major function of HDL is to scavenge cholesterol from other body tissue and then transported to the liver (Das, 2003). In a rat model of histopathology, infusion of eggplant fruit exhibits hepatoprotective benefits against some liver conditions (Nabhan *et al.*, 2015).

Eggplant's potential mechanism of action on cholesterol metabolism is still unclear (Guimarães *et al.*, 2000). Since the eggplant has induced 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity, eggplant may have shown protective effects on hyperlipidemia via the enhancement rate of degradative processes of cholesterol or induction of lipoprotein lipase activity, and the effective reduction in lipids absorption from the intestine (Fateme *et al.*, 2021).

HMG-CoA reductase is an enzyme in the liver that catalyzes the formation of cholesterol from HMG-CoA (Res-saissi *et al.*, 2017). Therefore, it might be suggested that eggplant reduced cholesterol levels via some other mech-

animals, including the enhancement rate of degradative processes of elimination of cholesterol and the effective reduction in its absorption from the intestine (Sudheesh *et al.*, 1997).

However, the therapeutic effect of Eggplant may be related to antioxidant compounds such as flavonoids, phenols, alkaloids, tannins, saponins, and terpenoids. The fruits of eggplant are powerful antioxidants due to their phenolic compounds and ascorbic acid activity (Vinson *et al.*, 1998). Soluble fibers of Eggplants decrease cholesterol levels in both humans and experimental animals by 5 to 15% (Guimarães *et al.*, 2000; Kishimoto *et al.*, 1995).

CONCLUSIONS AND RECOMMENDATIONS

Eggplant fruit are a vital source of several beneficial components. According to this study, these results suggested that crude WEE better than MEE in reducing potassium dichromate (K₂Cr₂O₇)-mediated liver toxicity in all parameters in the current study. Also, treatment with WEE reduced bad lipid serum levels significantly. According to our scientific view we recommended time dependent increase concentrations of both potassium dichromate (as toxic factor) and the extracts of eggplant (as treatment).

ACKNOWLEDGMENTS

The authors are thankful Asst. prof Dr. Aoula Al-zebeeb and Miss Azhar Ahamed Al-Kaby both in Department of Pathology and Poultry diseases/Faculty of Veterinary Medicine/University of Kufa for their help in tissue processing and proof reading.

LIST OF ABBREVIATION'S

Abbreviation	Meaning
S. melongena	<i>Solanum. melongena</i>
MEE	Crude methanolic extract of Eggplant
WEE	Crude watery extract of Eggplant
Cr	chromium
GOT	Glutamic Oxaloacetic Transaminase
GPT	Glutamic Pyruvic Transaminase
ALP	Alkaline Phosphatase
LDL	Low Density lipoprotein
HDL	High Density lipoprotein
TG	Triglyceride
Ch.	Cholesterol
C+	positive control

NOVELTY STATEMENT

The novelty of the study is focusing on the evaluation of

the effect of extracts of eggplant on the potassium dichromate mediated –liver toxicity the main results showed watery extract of eggplant better than methanolic extract in both parameters under study, namely histopathological study and biochemical study in reducing the toxic effect of potassium dichromate and reduced bad lipid serum.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally.

CONFLICT OF INTEREST

No conflicts of interest have been declared by the authors.

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