# Can Luteolin Ameliorates Thioacetamide Induced Acute Hepatotoxicity in Rat Model: Biochemical and Histopathological Studies

**Zaenah Zuhair Alamri**

*Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, 21589 Jeddah, Saudi Arabia.*

**ABSTRACT**

Acute liver injury is a challenging clinical problem. Luteolin (Lut) is a natural herbal flavonoid. The current study investigated Lut's protective effect against thioacetamide (TAA) induced acute hepatotoxicity. Thirty male adult albino rats were allocated into five groups (n=6). Group I: received saline, and served as control. Group II: received a single intraperitoneal (IP) dose of TAA (500mg/kg) and sacrificed 24 hours later. Group III was supplemented with oral Lut (50mg/kg) for 2 weeks. Group IV: given Lut (50mg/kg) for 2 weeks, then given the same doses of TAA. Group V received oral metformin (250mg/kg) for 2 weeks, then given same TAA dose. Body and liver weights were recorded. Sera liver enzymes were measured. Malondialdehyde (MDA) and glutathione (GSH) were assayed in liver homogenate. Liver tissues were examined histologically. Results showed that TAA caused significant decline in body weight gain, and increased in liver index. In TAA group, serumalanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, alkaline phosphatase,total bilirubin and MDA were increased while total protein, albumin and GSH were decreased. Histopathology showed centrilobular necrosis. Altered parameters were partially improved by Lut. In conclusion, Lut provided potential protection against TAA induced hepatotoxicity, and may need longer duration or high dose to provide effective prophylaxis.

**Key Words:** Hepatotoxicity, Luteolin, Oxidative stress, Thioacetamide.

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## INTRODUCTION

Liver is susceptible to different harmful endogenous and exogenous agents. Liver diseases may result by oxidative stress or inflammation induced by exposure to toxic chemicals [1, 2]. The main liver functions are detoxification of xenobiotics and toxin. Thiono-sulfur is the substance present in Thioacetamide (TAA). It is used as a solvent, motor oil stabilizer and alsoas fungicide [3]. Liver injury and fibrosis could be induced by TAA treatment in experimental animals [4-8]. TAA is metabolized inside the body to thioacetamide-S-oxideor acetamide [9]. TAA causes membrane damage, oxidative stress and lipid droplets accumulation in the hepatocyte cytoplasm [10]. The toxic influences of TAA depend on its concentration, administration period and methods, animal's sex, strain, age and weight [11].

Complementary and alternative medicines are among therapeutic strategies for liver diseases. Using reasonable therapeutic strategies could protect or treat acute liver injury or fibrosis [12]. Bentayeb et al. (2018) studied the protective role of the date palm pollen (Phoenix Dactilyfera) on liver and haematological changes caused by the Diethyl Phthalate [13]. El-Halwagy et al.(2018) examined hepatoprotective effect of Alpha lipoic Acid versus intoxication with imidacloprid which is widely used in KSA in Albino Rats, and cocluded that both low and high doses of administration of Imidaclopride increased the generation of ROS which has been important in the cascade of events leading to liver toxicity [14]. Ayaz (2018) explored the adverse toxic impacts of

**Corresponding author:** Zaenah Zuhair Alamri
**Address**: Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, 21589 Jeddah, Saudi Arabia .
**E-mail:** ✉ zalamri@kau.edu.sa
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AuNPs (about 20nm) on the livers of experimental animals and the potential protective and therapeutic roles of N3 polyunsaturated fatty acids (N-3 PUFA), and based on the results, the use of N-3 PUFA as a protective or a therapeutic agent against liver damage caused by AuNPs toxicity was suppoted [15].

Luteolin (Lut) is a natural flavonoid which is abundant in manyherbs, fruits and vegetables [16]. Lut has been tried as an antioxidant for protection against oxidative- stress induced diseases such as cardiovascular diseases [17], liver diseases [18], kidney diseases [19], neurological diseases [20], and cancer [21].

This study was planned to examine the effectiveness of oral administration of Lut to provide protection against TAA-induced acute hepatic injury in adult male albino rats, and also to study the possible underlying mechanisms. The protective effect was compared with Metformin; a well-known hepato-protective agent.

## MATERIALS AND METHODS

### Material

Thioacetamide was purchased from Sigma–Aldrich Corp., St. Louis, MO, USA. Before using TAA, it was mixed thoroughly in distilled water till complete dissolving according to the manufacture protocol. Lut was supplied by Smart Nutrition, Guillaume, Luxembourg. Lut powder was also dissolved in distilled water, and mixed thoroughly until all the powder was dissolved.

### Animals

Thirty male adult Wister Albino rats (Rattusnorvegicus), with average weight of 175 - 250 grams were provided by Animal house of King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Saudi Arabia. The animals were left one week in the lab for acclimatization [humidity (65%), temperature (20 ± 1 °C) and light: dark (12/12h)]. The rats were allowed free access to standard rodent chow and water *ad libitum*. Animal dealing was conducted according to the ethical guidelines of the Animal Care and Research Ethical Committee of King Abdul-Aziz University.

### Study protocol

The animals were sorted into 5 equal groups (n=6). GI: Control group which received daily oral saline (0.9% NaCl) via oral gavage for 2 weeks; GII:TAA group which received single dose of TAA (500 mg/kg) via intraperitoneal (IP) route to induce acute liver injury [22]; and the animals were sacrificed 24 hours later; GIII: Lut group which received daily dose of Lut via oral route (50 mg/kg) for 2 weeks [16]; G IV:Lut + TAA group which received oral Lut (50 mg/kg) for the same period, then administrated single dose of TAA IP (500 mg/kg) and sacrificed 24 hours later to investigate the protective effects of Lut against TAA- induced hepatic cell injury; GV: Met + TAA group which received daily IP injection of metformin (250 mg/kg) for 2 weeks [23]; then they received a single dose of TAA IP (500 mg/kg) and were scarified 24 hours later to compare the protective effect of metformin as a standard hepato-protective agent with Lut.

The weight of all rats was recorded at the beginning, and daily all throughout the period of experiment. The animals were observed for any behavioral or clinical abnormalities. Weight gain was calculated according to the following formula (final body weight - initial body weight) / initial body weight X 100). After 24 hours of TAA injections, the animals were sacrificed by cervical dislocation after anaesthetized by diethyl ether anesthesia. The abdomens were opened, and the livers were carefully excised and weighed. The liver index was determined according to the formula of (liver weight/ body weight X100). The tissues from the right and left liver lobes were cut into thin slices. For histological study, some slices were immersed in isotonic buffered formalin fixative; others were rinsed by cold 0.9% normal saline for further homogenization in 50 mM cold potassium phosphate buffer solution (10% w/v). The centrifuge of homogenate was done at 3000x g for ten minutes at 4◦C. The clear supernatants were separated, and stored for the estimation of malondialdehyde (MDA) and reduced glutathione (GSH) levels.

### Liver functions and enzyme assessment

Blood samples (5 ml) were obtained from retro-orbital venous plexus, centrifuged at 2500 rpm for 15 min. The serum was stored at −80 °C till used. The sera levels of aspartate aminotransferase (AST),alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), and alkaline phosphatase (ALP),bilirubin (BI), total proteins (TP), albumin (ALB) and globulin were estimated using commercially available kits.

### Determination of malondialdehyde and reduced glutathione in liver homogenate

The analysis of MDA in liver tissue homogenate was done based on the method used by Gutteridge [24] and Shimizu et al. [25]. GSH was analyzed by spectrophotometer according to Jocelyn [26].

### Histological examination

Pre-fixed liver slices were processed for paraffin blocking in a professional histopathological lab, King Abdul-Aziz University Hospital. Five micron thick sections were stained with hematoxylin-eosin (H&E), photographed by an expert histo-pathologist using light microscopy, and photographed for the assessment.

### Statistical analysis

All of the values were reported as mean +/- standard deviation. The data was recovered and analyzed using SPSS version 21 (SPSS Inc. Chicago, IL, USA). One-

Way ANOVA was used to show the statistical significance with a value *P of* <0.05 which was regarded significant when compared to the control and TAA groups.

## RESULTS

### Body and liver weight changes

The final body weight was significantly decreased in TAA and Lut + TAA groups compared with the control. Weight gain was significantly decreased in TAA, Lut + TAA and Met + TAA groups compared to the control. Liver weight and liver index were significantly increased in TAA group compared with the control, Lut + TAA and Met + TAA groups. Meanwhile, insignificant changes of body weight, weight gain, liver weight and liver index were observed in Lut group compared to the control (Table 1).

### Evaluation of liver markers' enzymes

ALT serum level was significantly increased in TAA, Lut + TAA and Met + TAA groups compared to the control. AST and GGT serum levels were significantly increased in TAA, Lut + TAA and Met + TAA groups versus control. AST and GGT level was significantly decreased in Met + TAA group versus TAA group. ALP level was significantly increased in TAA group compared to control, Lut + TAA and Met + TAA groups. However, ALP serum level was significantly higher in Lut +TAA and Met + TAA groups compared to the control. Bilirubin level was significantly increased in TAA and TAA + Lut groups, but it was significantly decreased in Lut and Met + TAA groups compared with the control. Bilirubin level was significantly high in TAA group compared to Lut and Met + TAA groups. Total protein serum level was significantly decreased in TAA, but it was significantly increased in Lut and Lut + TAA groups compared to the control. Total protein levels were significantly increased in Lut, Lut + TAA and Met + TAA treated groups versus TAA group. Serum albumin level was significantly increased in control, Lut, Lut + TAA and Met + TAA groups compared with TAA group. Insignificant changes in globulin level were observed between studied groups compared with the control and TAA groups (Table2).

### Evaluation of MDA and GSH liver homogenate level

Table (3) shows the activity of MDA and GSH in liver tissue homogenate of different studied groups. MDA level in liver tissue homogenate was significantly increased in TAA, Lut + TAA and Met + TAA groups compared with the control. However, MDA level was significantly decreased in Lut, Lut + TAA and Met + TAA groups compared with TAA group. GSH level in liver homogenate was significantly increased in Lut group compared to the control, but it was significantly decreased in TAA and Lut + TAA groups compared with the control. GSH level was significantly increased in Met + TAA groups compared to TAA group.

### Morphological and histological studies

- **Gross morphology**

Fig (1) shows the gross morphology of both control and treated rat livers. TAA treatment resulted in the shrinkage of liver and the appearance of surface patchy whitish fibrous material (GII). The administration of Lut (G IV) or Metformin (G V) much preserved normal gross morphology of rat liver. Lut alone (GIII) did not alter gross liver appearance.

- **Light microscopic histological structure**

**Histological structure of rat liver of control and Lut groups**.

The control rat liver showed ill-defined lobulation. Hepatocyte cell plates which were separated by liver sinusoids were seen radiating from the central vein peripherally towards the portal areas. Hepatocytes were of normal appearance with rounded vesicular active nuclei. They were arranged as cell plates radiating from the central vein, and separated by thin walled blood sinusoids (Fig2,GI). The administrations of Lut didn't affect the normal structure of liver, but it seemed to activate hepatocyte nuclei which looked larger with dispersed chromatin. The nuclei of phagocytic Kupffer cells lined blood sinusoids looked also more prominent (Fig 2,GIII**)**.

**Histological structure of rat liver of TAA and treated groups.**

After 24 hours of IP administration of TAA, rat liver showed multiple marked centri-lobular necrosis of hepatocytes (around the central vein) where cells became swollen, lost their well-defined outlines, ruptured leaving degenerated nuclei). The necrotic regions were infiltrated by polymorph nuclear inflammatory cells (Fig 3, GII). Prophylactic or protective therapeutic plan was done using Lut. Antioxidant or metformin were given orally for 15 days prior to TAA, provided potential amelioration of TAA acute necrotic effect where histological changes were less compared to non-treated samples (Fig 2, G IV & G V) with superior effect in Lut treated group. Individual variations among animals in the extent of response were also observed.

**Table 1: Comparison of body and liver weights in different studied groups versus control and TAA groups.**

| Parameters | Control group | TAA group | Lut group | Lut + TAA group | Met + TAA group |
|---|---|---|---|---|---|
| **Initial total body weight** (grams) Significance | 185.50±5.50 | 197.00±13.77 $^{1}$P=0.245 | 198.83±23.04 $^{1}$P=0.180; $^{2}$P=0.851 | 199.50±23.80 $^{1}$P=0.160; $^{2}$P=0.798 | 203.50±9.12 $^{1}$P=0.074; $^{2}$P=0.507 |
| **Final total body weight** (grams) Significance | 226.50±9.05 | 200.83±16.68 **$^{1}$P=0.004** | 233.50±22.63 $^{1}$P=0.400;**$^{2}$P=0.001** | 204.50±9.75 **$^{1}$P=0.013**; $^{2}$P=0.658 | 213.67±6.02 $^{1}$P=0.129; $^{2}$P=0.129 |
| **Weight gain** (%) Significance | 22.24±7.00 | 1.91±3.33 **$^{1}$P=0.0001** | 17.79±6.82 $^{1}$P=0.351;**$^{2}$P=0.002** | 3.74±13.59 **$^{1}$P=0.001**; $^{2}$P=0.698 | 5.19±6.06 **$^{1}$P=0.001**; $^{2}$P=0.488 |
| **Liver weight** (grams) Significance | 8.24±0.79 | 10.21±0.87 **$^{1}$P=0.002** | 7.89±1.56 $^{1}$P=0.537;**$^{2}$P=0.0001** | 8.67±0.46 $^{1}$P=0.454;**$^{2}$P=0.011** | 7.17±0.86 $^{1}$P=0.069;**$^{2}$P=0.0001** |
| **Liver weight index** (%) Significance | 3.64±0.30 | 5.13±0.74 **$^{1}$P=0.0001** | 3.29±0.54 $^{1}$P=0.215;**$^{2}$P=0.0001** | 4.35±0.31 **$^{1}$P=0.016;$^{2}$P=0.009** | 3.35±0.31 $^{1}$P=0.301;**$^{2}$P=0.0001** |

Data are expressed as mean +/- standard deviation. $^{1}$P: significance versus control; $^{2}$P: significance versus TAA group using One-way ANOVA (LSD) test.

**Table 2: Comparison of serum biochemical parameters in different studied groups versus control and TAA groups.**

| Parameters | Control group | TAA group | Lut group | Lut + TAA group | Met + TAA group |
|---|---|---|---|---|---|
| **ALT** (IU/L) Significance | 22.22±6.66 | 82.70±54.10 **$^{1}$P=0.003** | 21.74±4.97 $^{1}$P=0.979;**$^{2}$P=0.003** | 75.94±39.08 **$^{1}$P=0.007;** $^{2}$P=0.713 | 62.82±20.53 **$^{1}$P=0.035;** $^{2}$P=0.284 |
| **AST** (IU/L) Significance | 15.22±3.39 | 218.57±36.69 **$^{1}$P=0.0001** | 14.35±1.58 $^{1}$P=0.932;**$^{2}$P=0.0001** | 201.84±7.81 **$^{1}$P=0.0001;**$^{2}$P=0.105 | 93.94±8.03 **$^{1}$P=0.0001; $^{2}$P=0.0001** |
| **ALP** (IU/L) Significance | 114.05±4.86 | 286.40±47.03 **$^{1}$P=0.0001** | 108.78±4.50 $^{1}$P=0.684;**$^{2}$P=0.0001** | 234.74±13.00 **$^{1}$P=0.0001;$^{2}$P=0.0001** | 214.47±5.60 **$^{1}$P=0.0001; $^{2}$P=0.0001** |
| **GGT** (IU/L) Significance | 5.04±0.49 | 35.65±1.79 **$^{1}$P=0.0001** | 5.26±0.96 $^{1}$P=0.767;**$^{2}$P=0.0001** | 35.51±1.43 **$^{1}$P=0.0001;** $^{2}$P=0.844 | 19.82±1.14 **$^{1}$P=0.0001; $^{2}$P=0.0001** |
| **Bilirubin** (umol/L) Significance | 5.61±0.58 | 16.40±1.08 **$^{1}$P=0.0001** | 3.30±0.46 **$^{1}$P=0.0001; $^{2}$P=0.0001** | 16.73±1.22 **$^{1}$P=0.0001;**$^{2}$P=0.508 | 4.40±0.65 **$^{1}$P=0.021;$^{2}$P=0.0001** |
| **Total proteins** (mg/dl) Significance | 72.14±2.38 | 50.29±1.32 **$^{1}$P=0.0001** | 79.64±3.17 **$^{1}$P=0.001**;**$^{2}$P=0.0001** | 75.54±2.45 **$^{1}$P=0.015;$^{2}$P=0.0001** | 70.95±1.43 $^{1}$P=0.373;**$^{2}$P=0.0001** |
| **Albumin** (mg/dl) Significance | 45.42±3.01 | 25.91±2.71 **$^{1}$P=0.0001** | 56.47±1.36 **$^{1}$P=0.0001; $^{2}$P=0.0001** | 47.16±1.72 $^{1}$P=0.185**; $^{2}$P=0.0001** | 47.71±1.84 $^{1}$P=0.086**; $^{2}$P=0.0001** |
| **Globulin** (mg/dl) Significance | 27.04±3.45 | 24.39±2.34 $^{1}$P=0.099 | 24.84±2.76 $^{1}$P=0.169; $^{2}$P=0.771 | 27.21±2.56 $^{1}$P=0.914**;** $^{2}$P=0.080 | 24.25±2.10 $^{1}$P=0.084**;** $^{2}$P=0.930 |

ALT: alanine aminotransferase ; AST: aspartate aminotransferase; GGT: gamma glutamyl transferase ; ALP: alkaline phosphatase. Data are expressed as mean +/- standard deviation. $^{1}$P: significance versus control; $^{2}$P: significance versus TAA group using One-way ANOVA (LSD) test.

**Table 3: Comparison of enzymatic oxidant / antioxidant activities in liver homogenate in different studied groups versus control and TAA groups.**

| Parameters | Control group | TAA group | Lut group | Lut + TAA group | Met + TAA group |
|---|---|---|---|---|---|
| **MDA** (nmol/mg protein) Significance | 4.74±1.16 | 35.91±7.28 **$^{1}$P=0.0001** | 3.35±0.45 $^{1}$P=0.584;**$^{2}$P=0.0001** | 26.06±5.94 **$^{1}$P=0.0001;$^{2}$P=0.001** | 12.49±1.98 **$^{1}$P=0.005;$^{2}$P=0.0001** |
| **GSH** (umol/ mg protein) Significance | 6.66±0.54 | 5.40±0.34 **$^{1}$P=0.001** | 8.55±0.83 **$^{1}$P=0.0001; $^{2}$P=0.0001** | 5.73±0.19 **$^{1}$P=0.007;**$^{2}$P=0.307 | 6.39±0.60 $^{1}$P=0.407;**$^{2}$P=0.004** |

MDA: malondialdehyde; GSH: reduced glutathione. Data are expressed as mean +/- standard deviation. $^{1}$P: significance versus control; $^{2}$P: significance versus TAA group using One-way ANOVA (LSD) test

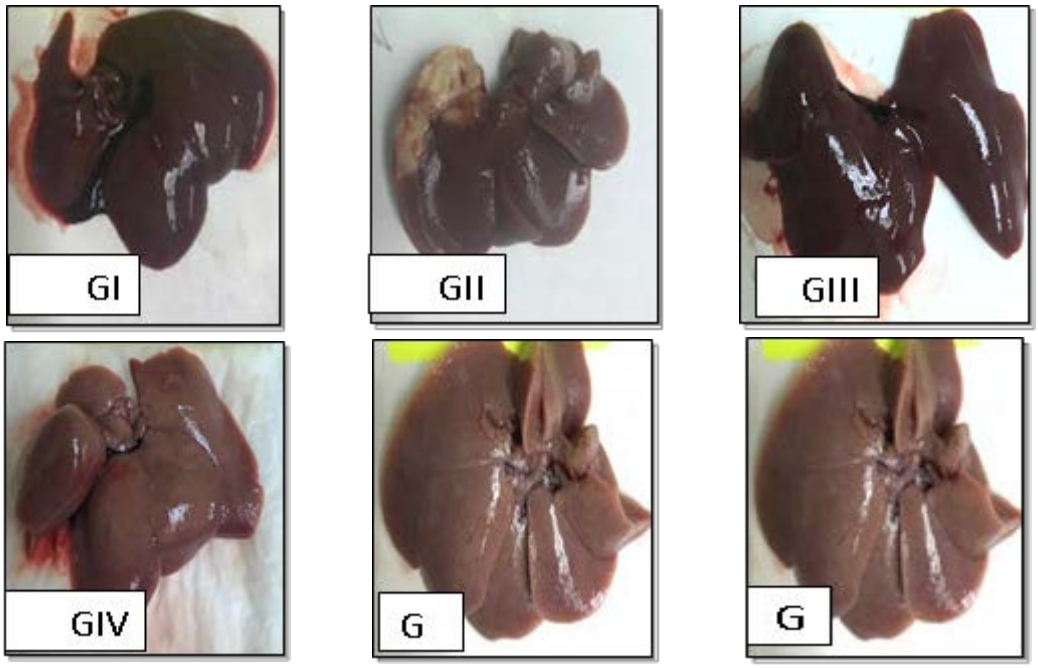


**Fig. 1: Photographs of gross morphology of rat liver in different studied groups**

**GI**: Control group which received daily oral saline (0.9% NaCl) via oral gavage for 2 weeks

**GII**: TAA group which received single dose of TAA (500 mg/kg) via intraperitoneal (IP) route to induce acute liver injury [19]; and animals were sacrificed 24 hours later.

**GIII:** Lut group received daily dose of Lut via oral route (50 mg/kg) for 2 weeks

**G IV:** Lut + TAA group which received oral Lut (50 mg/kg) for the same period then administrated single dose of TAA IP (500 mg/kg) and sacrificed 24 hours later to investigate the protective effects of Lut against TAA- induced hepatic cell injury.

**GV:** Met + TAA group administered daily IP injection of metformin (250 mg/kg) for 2 weeks ,then they received a single dose of TAA IP (500 mg/kg) and scarified 24 hours later to compared the protective effect of metformin as standard hepato-protective agent with Lut.



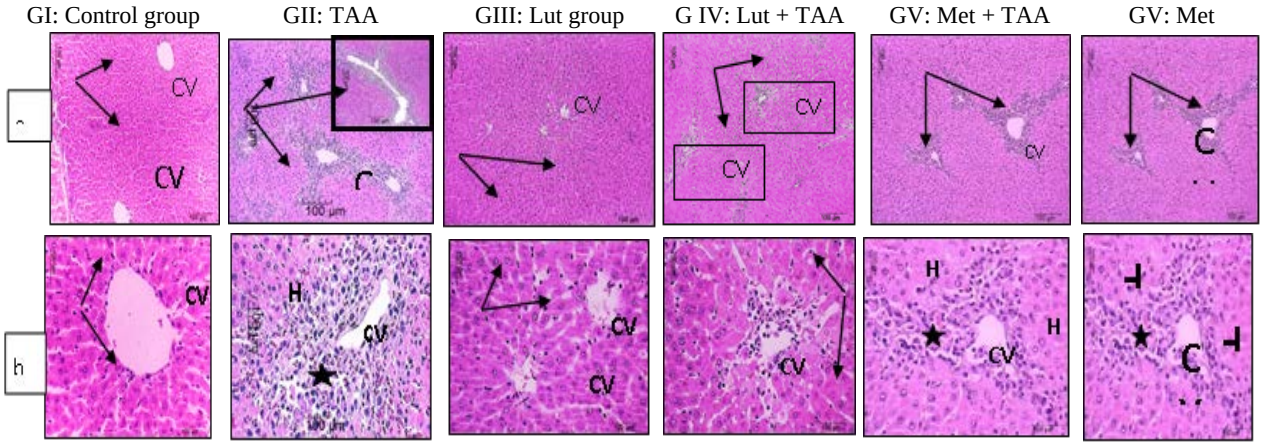


**Fig. 2: Sections of rat liver of control and Luteolin groups.**

**GI**: Control showed a. Normal hepatic lobulation, central veins (CV) and hepatocytes (arrows). b. Hepatocyte cell plates (black arrows) radiating from central vein (CV). They have rounded nuclei and separated by normal blood sinusoids (white arrows).

**G II**: TAA With Multiple Centri-lobular Hepatocyte Necrosis (Arrows In A. Low Power & Star In B. High Power). Notice The Peri1pheral Normal Hepatocyte (H).

**GIII**: Luteolin alone showed a. Normal healthy hepatic tissue, central veins (CV) and hepatic tissue (arrows). b. Hepatocytes cell plates with active large nuclei (arrows) and prominent sinusoidal Kupffer cells (white arrows). (H &E stain x100 &400(

**GIV:** Lut+Taa; Showing potential decrease In the area of hepato-necrosis (Arrows) with decrease of inflammatory cells (a & b G Iv).

**GV:** Met +Taa; Showing marked decrease In the area of hepato-necrosis. Most hepatocytes (H) looked healthy (a & b Gv). (H &E Stain X100 &40

## DISCUSSION

The regulation of metabolic balance in the body and detoxification of harmful chemicals and drugs were among the important functions of the liver [2]. TAA was reported as a potent hepatotoxic [11]. TAA-induced liver damage was assumed to be linked to the oxidative stress. Literature pointed to the beneficial effects of using antioxidant supplements as an anti-fibrotic agent through inhibiting ROS damaging effects or hepatic stellate cells' activation [27].

The present work revealed the decrease in the final body weight and weight gain in TAA treated group. The increase in rat body weight in Lut treated group provided that this supplement is a safe natural herbal flavonoid. Meanwhile, the weight gain was still significantly decreased in Lut + TAA and Metformin + TAA groups which indicated that Lut and Metformin have potential protective effects against the hepatotoxic effects of TAA. In this respect, Al-Attar et al. [8] reported that TAA administration for more than 6 -12 weeks to male albino mice led to a significant decrease of body weight. Similar observations were also detected in many experimental studies using TAA to induce hepatotoxicity [4-6, 28]. Anabolic effects of overwhelming catabolic processes were suggested to be the underlying mechanism of weight gain. Both direct and indirect toxic effects of TAA resulting in liver damage could be the cause of decreasing body weight in TAA treated groups [29, 30].

Determination of liver index could be considered an accurate procedure to determine the alteration of liver weight among different experimental groups. In the present study, Lut as well as Metformin resulted in significant decrease in liver index compared to the non-treated TAA group pointing to their protective antioxidant effect against hepatic necrosis which was confirmed in the present study by both biochemical and histological studies. In this respect, Yamada and Fausto [31] reported that the supplementation of Lut and Metformin for 2 weeks before the induction of TAA hepatotoxicity resulted in hepatocyte regeneration that led to an increase in the liver weight.

TAA administration in the present work resulted in an increase in the serum levels of liver enzymes as ALT, AST, ALP, GGT and total bilirubinin rats, since necrosis of hepatocytes usually result in the release of these enzymes into circulation, and this was confirmed in the present study by the histological observation that showed marked hepatocytes necrosis aroundthe central veins [32, 33]. A lot of research studies reported what were reported herein [4-6, 8, 28, 34]. In cases of liver damage, ALT was more accurate and selective for liver damage compared to AST [35]. The increase in the AST was observed to be associated with the concomitant increase in ALT levels, which played a vital role in the conversion of amino acids to keto acids [30]. The administration of Lut and Metformin prior to TAA was found to partially attenuate hepatotoxicity in rats, as demonstrated by the partial decrease of elevated serum AST activity. In this respect, Domitrovic et al. [36] reported that microscopic examination revealed that the marked hepatic necrosis induced by carbon tetrachloride ($CCl_4$) was attenuated by the administration of Lut given in a dose of 50 mg/kg for 2 days. Hepato-protective effects of Lut were mostly related to its antioxidant and scavenging activity of ROS and its anti-inflammatory effects. The elevated serum activities of ALP and GGT observed in this study indicated cholestasis and bile duct necrosis [37]. Likewise, their partial suppression in Lut and Metformin treated rats suggested that Lut and Metformin can stabilize biliary dysfunction.

In the present study, an increase in the level of serum bilirubin was observed in TAA group. However, its level was decreased in animals receiving Lut or Metformin prior to TAA administration. Such evaluation was attributed to be induced by the inflammatory reaction and fibrosis that may result in biliary obstruction and increased bilirubin in the serum [38]. Anker and Smilkstein [39] reported that the estimation of serum total bilirubin was clinically important to determine the severity of hepatic necrosis and this was confirmed by the histological examination of hepatic tissues. Following the hepatic cellular damage, protein synthesis was decreased with the consequence of the reduction of serum total protein [40]. Alkiyumi et al. [41] reported a decrease in the total protein and albumin levels in the serum of TAA hepatotoxic animals.

The current study demonstrated that TAA-treated rats exhibited an elevation in hepatic TBARs values with a low level of reduced GSH content as compared to the control rats, suggesting the impairment of the hepatic antioxidant capabilities. Treatment with Lut and Metformin for two weeks led to a decrease in MDA and an increase in GSH liver homogenate levels, but these levels did not reach the significant level. Previous studies had showed that Lut inhibits liver $CCl_4$- induced microsomal lipid peroxidation [42]. Domitrovic et al. [36] reported that Lut significantly reduced the oxidative stress in the liver of $CCl_4$- treated mice in time- and dose-dependent manner. Alkiyumi et al. [41] reported that a rise in MDA level due to TAA toxicity was reduced significantly by Lut administration. MDA level estimation was considered as an indirect index of tissue injury result from lipid peroxidation [43, 44]. The destruction of free radicals was known to be the most

common function of nutritional antioxidants including flavonoids [45]. They had protective effect against TAA induced liver toxicity [46]. The decrease in GSH observed in the present study was most probably the underlying cause of tissue damage due to lipid peroxidation and subsequent elevation of MDA. Similar mechanism was suggested by the others [47, 48].

In the present study, the histological examination of liver tissue went hand in hand with what was recorded by the biochemical analysis. Acute hepatocyte necrosis seen in TAA group explained the increase in liver enzymes. Similar results were reported in most research studies using TAA to induce acute or chronic hepatic necrosis as mentioned before. The alteration in liver functions were partially or potentially improved upon using Metformin or Lut, and this was also observed by the histological studies, although, the regions of necrosis were less in frequency and sizes they were still found in both treatments explaining the incomplete return of the liver function tests to normal levels.

## CONCLUSIONS

Both biochemical and histological findings of the present study can provide preliminary evidence that Lut has partial potential hepato-protective effects against TAA-induced liver toxicity. Accordingly, using higher doses of Lut and/ or increased duration of administration may increase its efficacy as a hepato-protective therapy. Further work is needed to confirm such hypothesis.

### Declaration of conflicting interest

The Author declared that there was/were not any conflict(s) of interest.

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