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Original Research Article

Methanol Extract of *Citrullus Lanatus* Rind and Silymarin Modulates Liver Function, Haematological Profile, and Liver Antioxidant Status in Thioacetamide-Treated Male Wistar Rats

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ABSTRACT
Thioacetamide (TAA) is known to induce hepatotoxicity and oxidative stress, disrupting liver function and body weight in experimental animals. This study evaluated the hepatoprotective and antioxidant potential of methanolic *Citrullus lanatus* (watermelon) rind extract at 250 mg/kg and 500 mg/kg, as well as silymarin (50 mg/kg), in male Wistar rats treated with TAA (300 mg/kg). Body weight, liver weight, liver function markers, hematological parameters, and antioxidant enzyme activities were assessed. Results showed that TAA significantly decreased body weight and increased liver weight ($p < 0.05$), indicating hepatotoxicity. However, both doses of *C. lanatus* and silymarin significantly mitigated these effects, with the 500 mg/kg dose showing the strongest protective action. Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin, albumin, total protein, and oxidative stress markers (Malondialdehyde, Reduced Glutathione, Superoxide Dismutase, Catalase, Glutathione Peroxidase) were significantly altered in the TAA group but were restored to near-normal levels in rats treated with *C. lanatus* and silymarin. Additionally, hematological indices (Red Blood Cells, Haemoglobin, Packed Cell Volume, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin Concentration, White Blood Cells, and Platelets) were significantly improved by the treatments, with the 500 mg/kg extract and silymarin exhibiting comparable efficacy. These findings suggest that *C. lanatus* rind extract possesses potent antioxidant and hepatoprotective properties, making it a promising therapeutic agent for managing TAA-induced liver damage. Further studies are recommended to explore its long-term safety and clinical applications.

INTRODUCTION

Thioacetamide (TAA) is a well-known hepatotoxic compound commonly used to induce liver injury in experimental models. It exerts its toxic effects by generating reactive oxygen species (ROS), leading to oxidative stress, inflammation, and cellular necrosis (El Hameed, 2023). TAA undergoes metabolic conversion in the liver, forming highly reactive intermediates such as thioacetamide-S-oxide, which causes centrilobular necrosis, fibrosis, and ultimately cirrhosis if left

unchecked (Ezhilarasan, 2023). Due to its ability to mimic clinical liver diseases, TAA-induced hepatotoxicity is widely employed in research to evaluate the therapeutic potential of hepatoprotective agents (Yang et al., 2019; Bashandy et al., 2020). Medicinal plants have long been recognized for their therapeutic potential in treating a variety of ailments, including liver diseases. Increasing interest in natural remedies has led to the exploration of numerous plants for their role in liver protection and the mitigation of oxidative stress. Their bioactive compounds, such as polyphenols, flavonoids, and alkaloids, exhibit strong antioxidant, anti-inflammatory, and hepatoprotective properties (Shawon et al., 2024). *Citrullus lanatus*, commonly known as watermelon, is a member of the Cucurbitaceae family, widely cultivated for its large, juicy fruit. Native to Africa, it is now grown in many tropical and subtropical regions around the world (Paris, 2015). Watermelon is well-known for its refreshing, sweet-tasting flesh, which is composed mostly of water (about 92%), making it a popular choice in hot climates. *Citrullus lanatus* is an annual herbaceous plant with long, sprawling vines that can extend several meters. The plant produces large, deeply lobed leaves and yellow flowers that are either male or female, requiring cross-pollination for fruit development. The fruit of watermelon is large and spherical or oval, with a thick, green rind that may be solid or striped. The flesh is typically red or pink, though some varieties produce yellow or orange flesh. Inside, the fruit contains numerous small seeds, although seedless varieties have been developed (Perkins-Veazie et al., 2012). Watermelon is not only an excellent source of hydration due to its high-water content but also provides essential nutrients. It is rich in vitamins, particularly vitamin C, a potent antioxidant, along with smaller amounts of vitamins A and B_6. It contains key minerals like potassium, which supports heart and muscle function, and magnesium (Leskovar et al., 2004; Reetu & Tomar, 2017). The medicinal properties of Watermelon are primarily due to its rich antioxidant content. For example, lycopene, a carotenoid responsible for its red colour, has been linked to a reduced risk of certain cancers and heart disease due to its ability to combat oxidative stress and neutralize free radicals (Zumuz et al., 2021). Additionally, it provides citrulline, an amino acid that promotes vascular health by increasing nitric oxide production, aiding vasodilation, and improving blood circulation (Smeets et al., 2021; Volino-Souza et al., 2022). *Citrullus lanatus* has been traditionally valued across various cultures for its wide range of health benefits, with the fruit, seeds, and rind all serving different therapeutic purposes. In Sudan, watermelon is traditionally used to treat a variety of ailments, including gastrointestinal disorders, rheumatism, inflammation, and gout (Aderiye et al., 2020; Manivannan et al., 2020; Nkoana et al., 2021). In South Africa, the leaves and fruits of the watermelon plant are commonly used in traditional and alternative medicine to manage hypertension (Nadeem et al., 2022). Watermelon acts as a natural diuretic, promoting fluid excretion and aiding kidney function, which can help manage conditions like hypertension and fluid retention. Both lycopene and citrulline contribute to cardiovascular health, with studies suggesting they can help lower blood pressure and improve arterial function, offering significant heart health benefits (Manivannan et al., 2020; Nadeem et al., 2022). Additionally, roasted watermelon seeds are consumed as an appetite stimulant and to relieve constipation (Biswas et al., 2017).

Citrullus lanatus is also known for its anti-inflammatory effects, potentially reducing tissue inflammation and supporting overall health. Its rich profile of bioactive compounds has made it a subject of research in managing oxidative stress-related conditions such as liver diseases, cardiovascular disorders, and metabolic syndromes (Manivannan et al., 2020; Poduri et al., 2013; Zumuz et al., 2021).

Silybum marianum, commonly known as milk thistle and belonging to the Asteraceae family, is one of the most ancient and extensively studied plants in herbal medicine (Soleimani et al., 2019). Traditionally, it has been used to treat liver and gallbladder disorders, including jaundice, cirrhosis, and hepatitis (Gillessen & Schmidt, 2020). The primary active component of milk thistle extract is silymarin, a complex of flavonolignans, with silybin being the most potent among them. Silymarin is most renowned for its hepatoprotective properties (Mihailović et al., 2023). Preclinical and clinical studies have demonstrated that silymarin and other flavonolignans exhibit significant antioxidant, anti-inflammatory, and pro-apoptotic activities (Abdel-Moneim et al., 2015; Kim et al., 2019; Adelina, 2022; Iqbal et al., 2022; Shavandi et al., 2022). These properties contribute to a variety of biological and pharmacological effects, including hepatoprotection, neuroprotection, anti-diabetic effects, anti-cancer activity, cardio-protection, photoprotection, and immunomodulation (Wadhwa et al., 2022).

With the growing interest in natural hepatoprotective agents and the limited research on *Citrullus lanatus* in the context of chemically induced liver injury, this study aims to address a critical gap in the literature. The therapeutic potential of *Citrullus lanatus* in thioacetamide (TAA)-induced liver toxicity has not been fully explored, particularly concerning its impact on liver function indices, hematological parameters, and antioxidant enzyme activity. The bioactive compounds present in *Citrullus lanatus*—such as lycopene, phenolics, and citrulline—may help alleviate oxidative stress and inflammation, making it a candidate for further investigation as a natural hepatoprotective agent. This study seeks to evaluate the

hepatoprotective effects of methanolic *Citrullus lanatus* rind extract on liver function, hematological profile, and liver antioxidant status in male Wistar rats subjected to TAA-induced liver injury. Specifically, it investigates whether *Citrullus lanatus* can alleviate the oxidative stress and liver dysfunction caused by TAA, offering valuable insights into its potential as a natural therapeutic agent for liver diseases.

MATERIALS AND METHODS

Chemicals and Reagents

Thioacetamide salt, silymarin, methanol, and chloroform were procured from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Plant Material and Authentication

Fresh fruits of *Citrullus lanatus* (watermelon) were sourced from an urban market in Umuahia, Abia State, Nigeria. The plant was identified and authenticated by a botanist in the Department of Plant Science and Biotechnology at Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, the plant was also cross-checked at https://www.ipni.org and assigned a voucher number: MOUAU/COLNAS/PSB/18/045.

Plant Material and Extraction

Fresh rinds of *Citrullus lanatus* were thoroughly washed with distilled water to remove any surface dirt and pesticides, then cut into smaller pieces to enhance extraction efficiency. A dehydrator was set at approximately 40-50°C to dry the rinds completely, after which they were ground into a coarse powder using a Waring blender. A known quantity (100 g) of the powdered rind was weighed and placed in a clean glass container, followed by the addition of 300 mL of methanol to fully submerge the material. The container was sealed and allowed to macerate at room temperature for 48 hours, with occasional shaking to improve the extraction of bioactive compounds. After maceration, the mixture was filtered through muslin cloth to separate the liquid extract from solid residues. The filtered methanol extract was concentrated using a rotary evaporator at low temperatures (40-50°C), while maintaining vacuum pressure to prevent thermal degradation. The concentrated extract was then freeze-dried, placed in freeze dryer trays, and subjected to a temperature of -40°C under vacuum for 24 hours until all methanol was sublimated, leaving behind the dry extract. The dried methanol extract of *Citrullus lanatus* rind was stored in airtight glass containers, protected from light and moisture to preserve its chemical integrity. The freeze-dried extract was kept at 4°C until needed for biochemical assays.

Preparation and Administration of the Methanol Extract of *Citrullus lanatus* Rind

The dry extract was reconstituted in distilled water to obtain the needed concentration for administration by gavage of the intended dosages of 250, and 500 mg/kg of body weight.

Thioacetamide (TAA) and Silymarin Preparation and Administration

Thioacetamide (TAA) was prepared by dissolving it in normal saline and administered intraperitoneally (i.p.) to the rats at a dose of 300 mg/kg body weight. The standard drug (silymarin) was dissolved in distilled water and administered by gavage at a dose of 50 mg/kg body weight.

Animal Care and Maintenance

Adult Male Wistar Albino Rats, weighing between 150-165 g, were used for this study. The animals were obtained from the animal house of the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. They were housed in metal cages in a well-ventilated room under a 12-hour light/dark cycle, with free access to standard feed and clean drinking water. The rats were acclimatized for one week prior to the commencement of the study.

Throughout the experiment, the rats were maintained in accordance with the guidelines outlined in the *Guide for the Care and Use of Laboratory Animals* (NIH, 2002), ensuring that their care minimized discomfort, distress, and pain.

Determination of Oral LD_{50}

The oral median lethal dose (LD_{50}) of methanol extract of *Citrullus lanatus* rind was estimated according to the method described by Lorke (1983).

Experimental Design and Animal Treatment

This study involved five groups of adult male Wistar rats to evaluate the hepatoprotective effects of methanol extract of *Citrullus lanatus* rind and silymarin against thioacetamide (TAA)-induced liver damage. The groups and corresponding treatments are outlined below:

Group 1 (Control): Rats were administered normal saline (1 mL/kg) orally to serve as the baseline control for comparison.

Group 2 (TAA group): Rats received thioacetamide (TAA) at a dose of 300 mg/kg intraperitoneally (i.p.) to induce liver damage.

Group 3 (TAA + 250 mg/kg *Citrullus lanatus* rind extract): Rats were first administered 300 mg/kg of TAA (i.p.) to induce liver injury, followed by oral treatment with 250 mg/kg of methanolic *Citrullus lanatus* extract for 14 consecutive days.

Group 4 (TAA + 500 mg/kg *Citrullus lanatus* rind extract): Rats received 300 mg/kg of TAA (i.p.), were treated with 500 mg/kg of methanolic *Citrullus lanatus* extract orally for 14 days.
Group 5 (TAA + 50 mg/kg silymarin): Following TAA administration (300 mg/kg i.p.), rats were treated orally with 50 mg/kg of silymarin for 14 days.
Silymarin served as the standard hepatoprotective agent. Thioacetamide (TAA) was administered intraperitoneally at 300 mg/kg to induce liver injury at the start of the experiment. Treatment with *Citrullus lanatus* rind extract (250 mg/kg or 500 mg/kg) and silymarin (50 mg/kg) commenced 24 hours later and continued for 14 days. After the treatment period, rats were anesthetized using chloroform, and blood samples were collected for haematological and liver function tests, while the liver was excised for antioxidant analysis.

Biochemical Assays

Blood Sample and Tissue Collection

At the end of the experiment, the animals were fasted overnight and anaesthetized with Ketamine. Blood samples were drawn using a 5 mL syringe via cardiac puncture (Arunachalam & Sasidharan, 2021) into plain sample tubes and centrifuged at 4000 rpm for 10 min to obtain sera used for biochemical assays in this study. The livers were excised and transferred into containers filled with chilled saline to maintain tissue integrity and prevent degradation.

Preparation of Liver Homogenate

A piece (1 g) of liver from each rat was homogenized in 9 mL of cold phosphate buffer (0.05 M, pH 7.0) with a Teflon homogenizer. The homogenate was centrifuged at 4000 rpm for 10 min. The supernatant obtained was stored frozen at -20℃ until required for the analyses of CAT, SOD, GSH, GPx, and MDA levels.

Liver Function Tests

The serum activities of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP), as well as the concentrations of total bilirubin, albumin, and total protein were determined using their respective Randox® kits.

Measurement of Haematological Parameters

Blood samples collected via cardiac puncture were dispensed into EDTA-capped sample tubes with the aid of a 5 ml syringe. The blood samples were then used for evaluating various haematological parameters such as red blood cell (RBC) count, total white blood cell (WBC) count, haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC): (Laposata & McCaffrey, 2022). The blood samples were analysed using an automated cell counter (Coulter Electronics, Luton, Bedfordshire, UK).

Antioxidant Assays

Catalase activity in serum was determined using the modified method described by Cohen *et al*. (1970). Superoxide dismutase (SOD) activity on liver homogenate was determined using the method described by Misra & Fridovich (1972). Reduced glutathione (GSH) level on liver homogenate was determined using the method described by Tietze (1969). Glutathione Peroxidase (GPx) activity on liver homogenate was determined using the method described by Flohe & Guùzler (1984). The concentration of malondialdehyde (MDA) in on liver homogenate was determined using the method described by Ohkawa *et al*. (1979).

Statistical Analysis

Data are presented as mean ± SEM and analyzed using SPSS version 20 (IBM, USA). One-way ANOVA followed by Tukey's post-hoc test for multiple comparisons was used to assess statistical significance, with a P-value < 0.05 considered statistically significant.

RESULTS AND DISCUSSION

Oral LD_{50} of Methanol Extract of *Citrullus lanatus* Rind

The oral LD_{50} of methanol extract of *Citrullus lanatus* rind was greater than 5000 mg/kg body weight as shown in Table 1, indicating that the extract has a high margin of safety, as no deaths were observed in male Wistar rats even at the highest tested dose of 5000 mg/kg body weight.

Table 1: Acute toxicity (LD_{50}) evaluation of Methanol Extract of *Citrullus lanatus* Rind in Male Wistar Rats

Dose (mg/kg b.w.t)	No. of rats	No. of deaths	Survival	Mortality ratio
10	3	0	3	0/3*
100	3	0	3	0/3*
1000	3	0	3	0/3*
1600	1	0	1	0/1*
2900	1	0	1	0/1*
5000	1	0	1	0/1*

*Number of deaths/surviving animals.

Table 2: Effects of Methanol Extract of *Citrullus lanatus* Rind on Body weight of Rats Treated with Thioacetamide (TAA)

Groups	Initial Body Weight (g)	Final Body Weight (g)
CONTROL	156.28 ± 4.11[a]	210.35 ± 5.01[a]
TAA only (300 mg/kg)	162.50 ± 5.43[b]	135.15 ± 3.70[b]
TAA + (250 mg/kg *C. lanatus*)	157.20 ± 4.56[a]	185.40 ± 4.88[c]
TAA + (500 mg/kg *C. lanatus*)	159.30 ± 5.12[a]	195.70 ± 5.46[a]
TAA + 50 mg/kg Silymarin)	158.10 ± 5.00[a]	200.10 ± 4.80[a]

Values are represented as mean ± SEM (n=6). Means with different superscripts are significantly ($p < 0.05$) different while those with the same superscripts are not significantly different.

The body weights of the experimental male Wistar rats (150–165 g) were monitored throughout the treatment period to evaluate the effects of methanolic *Citrullus lanatus* rind extract at dosages of 250 mg/kg and 500 mg/kg, silymarin (50 mg/kg), and thioacetamide (TAA, 300 mg/kg). As shown in Table 2, rats in the control group exhibited a normal and significant ($p < 0.05$) increase in body weight over the course of the experiment, indicating healthy growth. In contrast, rats treated with TAA alone experienced a significant ($p < 0.05$) decrease in body weight, demonstrating the toxic effects of TAA on overall health and metabolism.

Rats in the TAA + 250 mg/kg *C. lanatus* group showed an increase in body weight, though not as pronounced as the control or 500 mg/kg *C. lanatus* groups. Nevertheless, the final body weight in this group was significantly ($p < 0.05$) higher than that of the TAA group, indicating a protective effect of the extract at this lower dosage.

Rats treated with 500 mg/kg of *C. lanatus* exhibited body weight gains similar to those observed in the control group, suggesting a strong protective effect of the extract against TAA-induced weight loss ($p < 0.05$). Similarly, the TAA + silymarin group demonstrated significant ($p < 0.05$) weight gain, comparable to both the control and high-dose *C. lanatus* groups, further underscoring silymarin's efficacy in protecting against TAA toxicity.

Table 3: Effects of Methanol Extract of *Citrullus lanatus* Rind on Liver weight of Rats Treated with Thioacetamide (TAA)

Groups	Liver Weights (g)
CONTROL	6.25 ± 0.25[a]
TAA only (300 mg/kg)	9.82 ± 0.46[b]
TAA + (250 mg/kg *C. lanatus*)	7.54 ± 0.33[c]
TAA + (500 mg/kg *C. lanatus*)	6.92 ± 0.29[a]
TAA + 50 mg/kg Silymarin)	6.64 ± 0.24[a]

Values are represented as mean ± SEM (n=6). Means with different superscripts are significantly ($p < 0.05$) different while those with the same superscripts are not significantly different.

The liver weights of male Wistar rats treated with 250 mg/kg and 500 mg/kg of methanolic *Citrullus lanatus* rind extract, 50 mg/kg of silymarin, and 300 mg/kg of thioacetamide (TAA) were evaluated at the end of the experiment to assess the impact of these treatments on liver health. In Table 3, the control group displayed normal liver weight values, serving as the baseline for comparison. In contrast, rats administered TAA alone exhibited a significant ($p < 0.05$) increase in liver weight, indicating liver enlargement (hepatomegaly) and injury, which are hallmarks of TAA-induced hepatic toxicity.

Rats treated with 250 mg/kg of *C. lanatus* extract showed a significant ($p < 0.05$) reduction in liver weight compared to the TAA-only group, demonstrating partial protection against TAA-induced liver enlargement. The group treated with 500 mg/kg *C. lanatus* exhibited liver weights comparable to the control group ($p > 0.05$), suggesting that this higher dose provided robust protection against TAA-induced liver damage. Similarly, rats treated with silymarin displayed liver weights close to those of the control group ($p > 0.05$), reflecting its strong hepatoprotective effect, comparable to that of the high-dose *C. lanatus* group.

Both the 500 mg/kg dose of *C. lanatus* and 50 mg/kg silymarin effectively prevented the liver enlargement typically caused by TAA, as evidenced by their liver weights being similar to the control group. The lower dose of *C. lanatus* (250 mg/kg) provided partial protection, significantly reducing liver weight compared to the TAA-only group, though not as effectively as the higher dose or silymarin.

Table 4: Effects of Methanol Extract of *Citrullus lanatus* Rind on Serum ALT, AST, ALP, Total Bilirubin, Albumin, and Total Protein in Rats Treated with Thioacetamide (TAA)

GROUPS	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TOTAL BILIRUBIN (mg/dL)	ALBUMIN (mg/dL)	TOTAL PROTEIN (g/dL)
CONTROL	35.20 ± 2.11[a]	65.50± 3.44[a]	75.40 ± 4.08[a]	0.45 ± 0.05[a]	4.20 ± 0.22[a]	6.80 ± 0.32[a]
TAA only (300 mg/kg)	110.50 ± 5.33[b]	200.41 ± 6.51[b]	250.64 ± 9.52[b]	2.30 ± 0.15[b]	2.00 ± 0.11[b]	4.01 ± 0.20[b]
TAA + (250 mg/kg *C. lanatus*)	65.80 ± 3.24[c]	120.30 ± 4.12[c]	106.20 ± 6.30[c]	1.25 ± 0.07[c]	3.50 ± 0.22[c]	5.50 ± 0.41[c]
TAA + (500 mg/kg *C. lanatus*)	40.30 ± 2.51[a]	85.7 ± 4.03[d]	100.40 ± 5.10[d]	0.80 ± 0.04[d]	4.00 ± 0.20[a]	6.40 ± 0.32[a]
TAA + 50 mg/kg Silymarin)	38.70 ± 1.92[a]	70.21 ± 0.38[a]	85.90 ± 4.62[a]	0.55 ± 0.03[a]	4.10 ± 0.10[a]	6.70 ± 0.40[a]

Alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). Values are represented as mean ± SEM (n=6). Means with different superscripts are significantly ($P < 0.05$) different while those with the same superscripts are not significantly different.

The effects of the methanol extract of *Citrullus lanatus* rind on serum ALT, AST, ALP, Total Bilirubin, Albumin, and Total Protein levels in rats treated with thioacetamide (TAA) are presented in Table 4. The TAA-only group showed significantly (P<0.05) elevated levels of ALT, AST, ALP, and Total Bilirubin compared to the control and treated groups, indicating severe liver damage. Treatment with 250 mg/kg of *Citrullus lanatus* significantly (P<0.05) reduced ALT, AST, ALP, and Total Bilirubin levels compared to the TAA group, though not to the extent seen in the control, the higher-dose (500 mg/kg) *Citrullus lanatus*, and the Silymarin groups. TAA treatment also caused significant (P<0.05) reductions in Total Protein and Albumin concentrations, while treatment with the extract and silymarin effectively restored these levels to near-control values, highlighting their protective effects on liver function.

Table 5: Effects of Methanol Extract of *Citrullus lanatus* Rind on RBC, WBC, Hb, PVC, Platelet Count, MCV, MCH, and MCHC in Rats Treated with Thioacetamide (TAA)

Groups	RBC ($\times10^6$/µL)	WBC ($\times10^3$/µL)	Hb (g/dL)	PCV (%)	Platelet Count ($\times10^3$/µL)	MCV (fL)	MCH (pg)	MCHC (g/dL)
CONTROL	8.20 ± 0.25[a]	9.50 ± 0.52[a]	15.30 ± 0.60[a]	45.80 ± 1.90[a]	310 ± 12.50[a]	56.00 ± 1.80[a]	18.70 ± 0.60[a]	33.30 ± 1.20[a]
TAA only (300 mg/kg)	4.12 ± 0.20[b]	14.20 ± 0.70[b]	8.60 ± 0.44[b]	30.50 ± 2.00[b]	145 ± 8.20[b]	74.30 ± 2.50[b]	21.00 ± 0.90[b]	28.20 ± 1.00[b]
TAA + (250 mg/kg *C. lanatus*)	6.30 ± 0.25[c]	11.00 ± 0.60[c]	12.40 ± 0.55[c]	38.00 ± 1.50[c]	210 ± 10.20[c]	60.30 ± 2.00[c]	19.50 ± 0.80[a]	32.30 ± 1.20[a]
TAA + (500 mg/kg *C. lanatus*)	7.80 ± 0.29[a]	9.82 ± 0.55[a]	14.82 ± 0.52[a]	44.50 ± 1.80[a]	300 ± 11.20[a]	57.20 ± 1.90[a]	18.90 ± 0.60[a]	33.10 ± 1.00[a]
TAA + 50 mg/kg Silymarin)	8.00 ± 0.28[a]	9.72 ± 0.52[a]	15.20 ± 0.55[a]	45.20 ± 1.60[a]	305 ± 11.80[a]	56.50 ± 1.70[a]	18.60 ± 0.50[a]	33.00 ± 0.90[a]

Red Blood Cell (RBC), White Blood Cell (WBC), Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC). Values are represented as mean ± SEM (n=6). Means with different superscripts are significantly ($P < 0.05$) different while those with the same superscripts are not significantly different.

The results in Table 5 indicate that the TAA-only group exhibited significantly ($p < 0.05$) lower levels of red blood cells (RBC), hemoglobin (Hb), and packed cell volume (PCV) compared to both the control and treatment groups. Administration of *Citrullus lanatus* at doses of 250 mg/kg and 500 mg/kg, along with 50 mg/kg of silymarin, significantly ($p < 0.05$) improved RBC, Hb, and PCV levels, with the highest dose of *Citrullus lanatus* (500 mg/kg) and silymarin restoring these parameters to levels comparable to the control group.

In terms of white blood cell (WBC) counts, the TAA group showed significantly ($p < 0.05$) elevated levels, indicating an inflammatory response. However, treatment with 250 mg/kg of *Citrullus lanatus* led to a reduction ($p < 0.05$) in

WBC counts, while treatments with 500 mg/kg of *Citrullus lanatus* and silymarin restored WBC levels to near-control values.

Moreover, TAA administration resulted in a significant ($p < 0.05$) decrease in platelet count. In contrast, treatment with 250 mg/kg of *Citrullus lanatus* significantly ($p < 0.05$) increased platelet count compared to the TAA group, and both 500 mg/kg of *Citrullus lanatus* and silymarin fully restored the platelet count.

Additionally, TAA administration caused significant ($p < 0.05$) increases in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) while reducing mean corpuscular hemoglobin concentration (MCHC), which are indicative of macrocytic anemia. Treatments with *Citrullus lanatus* and silymarin restored MCV values closer to those of the control group.

Table 6: Effects of Methanol Extract of *Citrullus lanatus* Rind on Liver Homogenate Supernatant SOD, CAT, GPx, GSH, and MDA in Rats Treated with Thioacetamide (TAA)

Groups	SOD (u/mL)	CAT (u/mL)	GPx (u/mL)	GSH (u/mL)	MDA x 10^{-3} mmole/mL
CONTROL	12.52 ± 0.50^{a}	15.30 ± 0.60^{a}	8.80 ± 0.40^{a}	6.50 ± 0.32^{a}	2.11 ± 0.10^{a}
TAA only (300 mg/kg)	4.20 ± 0.35^{b}	5.30 ± 0.32^{b}	3.10 ± 0.25^{b}	2.50 ± 0.24^{b}	6.82 ± 0.35^{b}
TAA + (250 mg/kg *C. lanatus*)	8.52 ± 0.40^{c}	10.40 ± 0.45^{c}	6.22 ± 0.30^{c}	4.75 ± 0.25^{c}	4.02 ± 0.20^{c}
TAA + (500 mg/kg *C. lanatus*)	11.70 ± 0.45^{a}	14.74 ± 0.60^{a}	8.22 ± 0.35^{a}	6.30 ± 0.28^{a}	2.60 ± 0.15^{a}
TAA + 50 mg/kg Silymarin)	12.04 ± 0.52^{a}	15.10 ± 0.55^{a}	8.70 ± 0.40^{a}	6.40 ± 0.30^{a}	2.30 ± 0.12^{a}

Superoxide Dismutase (SOD), Catalase (CAT) Glutathione Peroxidase (GPx), Reduced Glutathione (GSH), Malondialdehyde (MDA). Values are represented as mean ± SEM (n=6). Means with different superscripts are significantly ($P < 0.05$) different while those with the same superscripts are not significantly different.

In Table 6, the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were significantly ($p < 0.05$) decreased in the thioacetamide (TAA) group compared to the control and treatment groups. In contrast, treatment with varying doses of the extract and silymarin led to significant increases in the activities of these antioxidant enzymes. Notably, the higher dose of the extract (500 mg/kg) and silymarin effectively restored their activities to levels comparable to those in the control group.

Similarly, glutathione (GSH) levels were significantly ($p < 0.05$) reduced in the TAA group compared to the control and treatment groups. However, treatment with the extract and silymarin significantly restored GSH levels, with the 500 mg/kg dose of the extract and silymarin demonstrating particularly strong efficacy, resulting in near-complete restoration of GSH levels.

Additionally, malondialdehyde (MDA) concentrations were elevated ($p < 0.05$) in the TAA group compared to both the control and treatment groups, indicating increased lipid peroxidation. The administration of the extract and silymarin significantly reduced MDA levels, with higher doses exhibiting the most pronounced effects.

Discussion

The liver, a key metabolic organ, is highly susceptible to the toxic effects of thioacetamide (TAA). TAA is a commonly used chemical for inducing liver injury in experimental animals, with chronic exposure leading to liver cirrhosis (Abood et al., 2020; Gratte et al., 2021). This study began with an oral acute toxicity assessment of the methanol extract from *Citrullus lanatus* rind in experimental animals, following Lorke's method. The extract, with an LD_{50} exceeding 5000 mg/kg, indicates low acute toxicity. This means that even at high doses, it does not cause significant toxic effects, indicating its safety for potential consumption or therapeutic use when administered orally. According to the Organization for Economic Co-operation and Development (OECD, 2008) guidelines, substances with an LD_{50} above 5000 mg/kg are classified as "relatively non-toxic," highlighting a broad safety margin for pharmacological applications. The low toxicity may be due to the bioactive constituents of the extract, including flavonoids, phenolic compounds, saponins, and terpenoids, which are known for their antioxidant and anti-inflammatory properties ((Manivannan et al., 2020; Zamuz et al., 2021). These compounds support cellular health and do not interfere with vital processes, even at high doses. As a result, the extract can be safely explored for therapeutic purposes, particularly for managing oxidative stress and liver protection, without significant risk of acute toxicity. However, while acute toxicity is minimal, long-term studies are needed to assess potential chronic toxicity, bioaccumulation, and any adverse effects from extended use. Further research into the extract's molecular mechanisms could shed light on its therapeutic potential for conditions like liver diseases, inflammation, or oxidative stress-related disorders. Its historical medicinal use and the high LD_{50} further support its safety and efficacy for traditional applications. The findings of this study align with those of Ebhohon et al. (2019) and Belemkar & Shendge (2021).

The reduction in body weight observed in Wistar rats administered 300 mg/kg of thioacetamide (TAA) can be attributed to several potential mechanisms related to the toxic effects of TAA on the liver and overall metabolism. Thioacetamide (TAA) causes severe liver damage by inducing necrosis, inflammation, and fibrosis, disrupting the liver's vital role in regulating proteins, fats, and carbohydrates (El-Hameed et al., 2023). This damage impairs nutrient metabolism and reduces protein production, such as albumin, contributing to weight loss (Katayama, 2020; Espina et al., 2023). TAA-induced liver injury leads to increased catabolism, where body tissues, especially muscle, are broken down for energy due to the liver's inability to regulate energy from food intake. Additionally, TAA toxicity may reduce appetite, further exacerbating weight loss. Impaired protein synthesis and muscle wasting also play a significant role in the observed body weight reduction (Zhang & Xu, 2024). The improved weight gain in TAA-induced male Wistar rats treated with *Citrullus lanatus* (250 and 500 mg/kg) and silymarin (50 mg/kg) may be attributed to their hepatoprotective and antioxidant properties (Mushtaq et al., 2015; Ebhohon et al., 2019; Ferraz et al., 2021). These treatments may have neutralized oxidative stress caused by TAA-induced liver damage, preserving liver function and improving metabolic activity. They may have also protected the liver from necrosis and fibrosis, aiding in nutrient regulation and energy balance. By restoring protein synthesis, particularly albumin, the treatments may have helped maintain muscle mass and reduced catabolism. Additionally, alleviating TAA's systemic toxicity improves appetite and nutrient absorption, further promoting weight gain. Therefore, the combined antioxidant, hepatoprotective, and regenerative properties of *Citrullus lanatus* and silymarin may have helped to reverse the damaging effects of TAA on the liver, leading to improved nutrient metabolism, reduced muscle wasting, and overall weight gain in treated rats. The results of this study are in agreement with the findings reported by Chinnala et al. (2018), Abood et al. (2020), El-Deberky et al. (2021), Shareef et al. (2021), Abdelghfar et al. (2022), and Alamri (2024).

The increased liver weight observed in rats treated with thioacetamide (TAA) is likely due to TAA-induced liver injury, characterized by hepatocyte necrosis, inflammation, and fibrosis (El-Hameed et al., 2023). These pathological changes lead to liver enlargement (hepatomegaly), as the liver swells due to inflammatory cell infiltration, accumulation of fibrotic tissue, and regenerative responses. TAA causes oxidative stress and lipid peroxidation, further contributing to liver damage and swelling (Li et al., 2015; Ezhilarasan, 2023). Additionally, the proliferation of bile duct cells (ductular reaction) and accumulation of extracellular matrix components as part of fibrosis contribute to increased liver weight (Arriazu, et al., 2014).

The improvement in liver weight to near control values in rats treated with the rind of *Citrullus lanatus* methanol extract (250 and 500 mg/kg) and silymarin (50 mg/kg) may be due to their hepatoprotective and antioxidant effects ((Mushtaq et al., 2015; Ebhohon et al., 2019; Ferraz et al., 2021). Both treatments are rich in bioactive compounds (such as phenolic compounds, flavonoids, and silybin) that reduce oxidative stress, inflammation, and fibrosis, thereby preventing further liver damage and promoting the regeneration of healthy hepatocytes (Saha et al., 2019; Abdelghfar et al., 2022; Jaffar et al., 2024). *Citrullus lanatus* and silymarin may have also stabilized liver cell membranes, reduce fibrosis, and support tissue repair, allowing the liver to recover its normal size and function. These treatments also counteract the liver swelling caused by TAA, helping to restore liver weight closer to control values through mechanisms such as enhancing antioxidant defenses, reducing inflammation, and stimulating hepatocyte regeneration. The findings of this study align with those of Chinnala et al. (2018), Abood et al. (2020), El-Deberky et al. (2021), Shareef et al. (2021), Abdelghfar et al. (2022), and Alamri (2024).

ALT and AST are liver enzymes released into the bloodstream when liver cells are damaged (Lala et al., 2024). ALP, which is present in the liver, bile ducts, and bones, increases in conditions involving liver or bile duct obstruction as well as certain bone disorders (Lowe et al., 2024). Bilirubin, a byproduct of red blood cell breakdown, is processed by the liver, and elevated levels suggest liver dysfunction or bile duct blockage (Guerra Ruiz et al., 2021; Kalakonda et al., 2024). Albumin, produced by the liver, helps maintain blood pressure and transport substances (such as steroid hormones, fatty acids, bilirubin, and drugs), with low levels indicating liver disease or malnutrition (Soeters et al., 2019). Total protein, which includes albumin and globulins, is essential for immune function and osmotic pressure, and low levels often point to liver or kidney disease (Busher, 1990; Belinskaia et al., 2021). The biochemical alterations observed in the TAA-only group and the subsequent improvements in the treatment groups can be attributed to several molecular mechanisms involved in liver injury (Ezhilarasan, 2023) and the protective effects of the extract and silymarin (Masruk et al., 2023).

Thioacetamide (TAA) is metabolized in the liver into toxic metabolites (primarily thioacetamide-S-oxide (TASO) and thioacetamide-S, S-dioxide ($TASO_2$) that induce oxidative stress, leading to hepatocyte necrosis (Ezhilarasan, 2023). This damage results in the release of liver enzymes such as ALT, AST, and ALP into the bloodstream, signaling liver injury. The oxidative stress caused by TAA increases reactive oxygen species (ROS), which damage cellular

components, including lipids, proteins, and DNA, ultimately contributing to hepatocyte death and dysfunction (Allameh et al., 2023). Elevated ALP levels in the TAA group may indicate impaired bile flow, possibly due to hepatocyte damage or cholestasis, which can further elevate bilirubin levels and contribute to jaundice (Pollock & Minuk, 2017). Additionally, TAA-induced liver injury compromises protein synthesis, resulting in significantly reduced total protein and albumin levels, reflecting impaired liver function (Zargar et al., 2017).

Both extract and silymarin are rich in antioxidants (such as phenolic compounds, flavonoids, vitamin C, and silybin) that neutralize ROS, thereby reducing oxidative stress (Manivannan et al., 2020; Zumuz et al., 2021; Aghemo et al., 2022). This protective effect of the extract and silymarin may help safeguard hepatocytes from injury, leading to decreased serum levels of ALT, AST, and ALP, as silymarin has been shown to stabilize liver cell membranes and prevent enzyme leakage into the bloodstream, thereby preserving hepatocyte integrity and further reducing enzyme levels associated with liver damage (Calderon Martinez et al., 2023). Furthermore, both treatments may enhance hepatocyte regeneration, with silymarin particularly known for stimulating liver regeneration and restoring normal liver architecture and function (Karimi et al., 2011). The administration of the extract and silymarin supports the restoration of liver functions, including the synthesis of essential proteins, which in turn increases total protein and albumin concentrations in serum, indicative of improved hepatic function. Additionally, both compounds may exert anti-inflammatory effects, reducing the inflammatory response associated with TAA-induced liver injury and providing further protection and support for hepatocyte recovery. The findings of this study are consistent with those reported by Abood et al. (2020), El-Deberky et al. (2021), and Shareef et al. (2021).

Hematological parameters are widely utilized for diagnosing various diseases and pathologies caused by toxicants, environmental pollutants, and drugs in both humans and animals (Al-Attar, 2022). The hematological changes observed in thioacetamide (TAA)-treated rats, along with the improvements from treatments with the extract and silymarin, can be attributed to several biochemical mechanisms. TAA induces liver injury, impairing the liver's ability to produce extrarenal erythropoietin (EPO), a hormone essential for red blood cell (RBC) production (Fried,1972; Yasuoka et al., 2020). Reduced EPO levels lead to decreased RBC counts, hemoglobin (Hb), and packed cell volume (PCV) (Bhoopalan et al., 2020). TAA metabolism also generates reactive oxygen species (ROS), causing oxidative damage to RBC membranes and resulting in hemolysis, further decreasing RBC and Hb levels ((Türkmen et al., 2022; Alamri, 2024).

Increased mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), alongside decreased mean corpuscular hemoglobin concentration (MCHC), indicate macrocytic anemia (Ho et al., 2018; Yang et al., 2018). TAA-induced liver damage may disrupt the synthesis of vital nutrients (vitamin B_{12} and folate,) needed for proper RBC maturation, leading to larger, immature RBCs with lower hemoglobin concentrations (Koury & Ponka, 2004). Furthermore, TAA can trigger an inflammatory response, increasing white blood cell (WBC) counts, while chronic inflammation suppresses bone marrow function, resulting in low platelet counts (thrombocytopenia) (Foy et al., 2021).

In contrast, *Citrullus lanatus* and silymarin may possess antioxidant properties (like flavonoids, and phenolic compounds) that neutralize ROS, reducing oxidative stress on hepatocytes and enhancing liver function (Manivannan et al., 2020; Zumuz et al., 2021; Aghemo et al., 2022). This may have helped to improve EPO production and facilitate the recovery of RBC, Hb, and PCV levels. Both treatments may have also stabilized liver cell membranes and promote hepatocyte regeneration, enhancing the synthesis of proteins and hormones crucial for RBC production and normalizing blood parameters. Their anti-inflammatory effects of the extract and silymarin may have also mitigated the inflammatory response associated with TAA, promoting healthier bone marrow function, which might have contributed to the improved platelet counts and normalization of WBC levels. Additionally, these treatments may have also enhanced nutrient absorption and utilization, providing essential vitamins (vitamin B_{12} and folate) and minerals (iron and zinc) necessary for erythropoiesis and overall blood health, further normalizing RBC indices (MCV, MCH, MCHC). Silymarin is known to stimulate liver regeneration, which may also boost the production of hematopoietic stem cells in the bone marrow, leading to increased RBC and platelet production (Abdel-Moneim et al., 2015). The findings of this study are consistent with those reported by Ebhohon et al. (2023) and Alamri (2024).

The alterations in oxidative stress markers and antioxidant enzyme activities in rats treated with thioacetamide (TAA) can be attributed to TAA's metabolism in the liver, where it is converted into reactive metabolites that produce reactive oxygen species-ROS (Türkmen et al., 2022). This process induces oxidative stress, resulting in damage to cellular components such as lipids, proteins, and nucleic acids (Allameh et al., 2023). This oxidative stress impairs the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), reducing the liver's capacity to detoxify ROS (Ighodaro & Akinloye, 2018). Additionally, TAA-induced oxidative stress depletes glutathione (GSH), a key intracellular antioxidant, compromising the liver's

detoxification ability and contributing to increased oxidative damage (Vairetti et al., 2021).

The oxidative stress also initiates lipid peroxidation, resulting in elevated levels of malondialdehyde (MDA), a marker of significant cellular damage (Mas-Bargues et al., 2021). Conversely, *Citrullus lanatus* rind extract and silymarin, rich in antioxidant compounds, can scavenge ROS, reducing oxidative stress and enhancing the liver's antioxidant capacity (Manivannan et al., 2020; Zumuz et al., 2021; Aghemo et al., 2022). These treatments may have stimulated the expression and activities of SOD, CAT, and GPx, helping restore the liver's ability to neutralize ROS. The treatments may have also promoted GSH regeneration, which is crucial for maintaining redox balance and protecting against oxidative damage. Additionally, the antioxidant properties of these treatments might have lowered MDA levels by preventing lipid peroxidation, thus protecting cellular membranes and liver function. Silymarin further stabilizes liver cell membranes and supports hepatocyte regeneration, contributing to reduced oxidative stress and improved liver recovery (Calderon Martinez et al., 2023). The findings of this study are in alignment with those of Chinnala et al. (2018), Abood et al. (2020), El-Deberky et al. (2021), Shareef et al. (2021), Abdelghfar et al. (2022), and Alamri (2024).

CONCLUSION

In summary, this study demonstrates that the methanol extract of *Citrullus lanatus* rind, along with silymarin, exerts substantial protective effects against thioacetamide (TAA)-induced liver toxicity in male Wistar rats. The extract, showing low acute toxicity with an LD_{50} >5000 mg/kg, is classified as relatively non-toxic and holds a promising safety profile for pharmacological applications. Treatment with *Citrullus lanatus* extract and silymarin improved liver function and overall metabolism, evidenced by normalized liver enzymes, albumin, and total protein levels, which were significantly elevated in the TAA-only group. The protective effect is likely due to the extract's bioactive compounds, including flavonoids and phenolics, which reduced oxidative stress by scavenging reactive oxygen species (ROS), enhancing antioxidant enzyme activity, and decreasing lipid peroxidation. Additionally, both treatments mitigated hematological alterations, supported nutrient regulation, and facilitated tissue repair and regeneration, reflected by improved liver weight, red blood cell indices, and platelet counts. The combined antioxidant, anti-inflammatory, and hepatoprotective properties of *Citrullus lanatus* and silymarin effectively attenuated the TAA-induced hepatic and systemic toxicities, underscoring their potential for managing liver injuries, oxidative stress, and related metabolic dysfunctions. Further research is recommended to investigate the molecular pathways underlying these protective effects and to assess the safety of long-term therapeutic use.

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