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An Assessment of Heptoprotective Activity of Citrullus Lanatus in CCL4-Induced Hepatotoxicity in Rats l with Safety Profile Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Humans have been discovering and incorporating medicinal plants, also known as medicinal herbs, into conventional medical processes since the beginning of time. The researchers investigated the effect of Citrullus lanatus extract on lipid profiles in rats. Throughout the treatment period, rats were given CCl4 and treatment species once a day. Both medium and high doses of the plant extract

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showed statistically significant ($p < 0.05$) changes in Serum Glutamic Pyruvic Transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT) compared to the positive control group. Creatinine levels showed a statistically significant reduction ($p < 0.05$) at low, medium, and high doses. However, at high doses, urea demonstrated a statistically significant effect ($p < 0.05$).

Keywords: Citrullus lanatus; medicinal plant; creatinine; statistical significance; high dose.

1. INTRODUCTION

The liver is a critical and resilient organ that performs vital functions in the body, including detoxification, metabolism, and the preservation of physiological balance. Consequently, it is particularly vulnerable to harm caused by many foreign substances [1]. N-acetylcysteine enhances antioxidant defenses [2]. It may considered as safe drug choice but it have some side effects including nausea, vomiting, diarrhea, discomfort, rash, runny nose [3]. *Citrullus lanatus* (*C. lanatus*) seeds are high in proximate components, vitamins, amino acids, phytochemicals, and macro and micro elements. Differences in the amounts of proximate components, vitamins, amino acids, phytochemicals, macro- and micro elements found in *Citrullus lanatus* compared to previous findings could be attributed to soil type, harvest time, regional differences, genotype, and the geographical and environmental conditions under which *Citrullus lanatus* is grown [4]. Through decreased congestion and necrosis as well as normalized serum AST, ALT, and bilirubin concentrations, *C. lanatus* ameliorates and reverses damage to the rat liver tissues produced by CCl4 [5]. The traditional usage of *C. lanatus* fruit pulp to treat liver damage is supported by the fact that it has antioxidant activity and protects against liver damage in ethanol-induced liver toxicity in rats by boosting cellular glutathione (GSH) and catalase (CAT) enzymes [6]. MeOH or EtOH seed extracts (200–400 mg/kg) demonstrated a dose-dependent liver protective effect by significantly reducing oxidative stress and improving drug metabolizing enzyme activity in the liver [7-8]. About 80% of the world's population lives in underdeveloped nations and relies on the usage of plant-based traditional medicine, which is important to human health [9]. Researchers have found numerous medicinal plants with excellent hepatoprotective properties while approaching this objective which are *Acanthopanax senticosus, Amomum villosum Lour., Amomum kravanh Pierre ex Gagnep., Cimicifuga heracleifolia Kom., Gynura procumbens, Citrullus lanatus* [10]. *Nelumbo nucifera* Gaertn., *Salvia miltiorrhiza Bunge., Artemisia absinthium L. etc.* [11]. Among them *Citrullus lanatus* is an extraordinary herb widely available in deserts of Kalahari in Africa. *Citrullus lanatus*, a fruit of the Citrullus genus and the Cucurbitaceae family, is a member of the Cucurbitales, specifically Citrullus lanatus, a species of the Cucurbitaceae [12]. *Citrullus lanatus* biomass is divided into three major components: meat, seed, and rind. The meat accounts for around 40% of total weight, whereas the rind and seeds account for approximately 60% of total *Citrullus lanatus* fruit weight. Because the seeds and rind are always discarded, this adds to significant agro-waste [13]. The Kalahari Desert in Africa is where the *Citrullus lanatus*, originally known as Tsamma melon, first appeared. It was discovered in Baja, California, thanks to African slaves who migrated to the United States [12]. *Citrullus lanatus* was grown on the African continent as early as 2000 B.C. and was depicted in Egyptian hieroglyphics. It was transported to China in the late 9th century [13]. The fruit is about 90% water and 8 to 12% sugar [14]. The principal pharmacological activities of *Citrullus lanatus* are diabetes, inflammation, cancer, kidney disease, cardiovascular diseases and hypertension [15–17]. In addition, different bioactive molecules such as Lycopene, Citrulline, Vitamin C, β-carotene, flavonoids, and fiber are functional chemical components to successfully treat these sorts of diseases [18–22]. Recent research on the antioxidant properties of this plant suggests it could be used to treat various diseases by boosting antioxidant defenses and protects cells from oxidative stress. As its mechanisms are constrained, it will have the same effect as a medicine that is currently on the market. We will carry out this research because it has been shown in other studies that this plant is hepatoprotective.

2. METHODS AND MATERIALS

2.1 Fruit Collection and Extract Preparation

Initially, *Citrullus lanatus* fruits was procured from the local market. Upon acquisition from the market, the peel was afterwards eliminated. Once fruits had been peeled, their meat and

seeds were dried together. Following the drying process, the substance was extracted using ethanol, with an ethanol concentration ranging from 90%. The extract underwent filtration at regular intervals of three days. The extracted sample was subjected to low temperature and pressure drying using a rotary evaporator. Ultimately, the unrefined residue was employed to conduct the requisite pharmacological examinations. The identification of the specimen was conducted by the Department of Pharmacy, University of Dhaka.

2.2 Drugs and Chemicals

The hepatotoxicity-inducing chemical carbon tetrachloride (CCl4), which is widely recognized for its harmful effects on the liver, was purchased from Sigma Company in the United States. The antioxidant medicine silymarin, namely in the form of Livasil 140 mg, was provided as a complimentary offering by Incepta Pharmaceuticals Ltd.

2.3 Experimental Animal Procurement, Nursing, and Grouping

A group of 50 male wistar rats, with individual weights ranging from 120 to 150 grammes, were procured from Jahangirnagar University in Savar, Dhaka. The specimens were each placed in a controlled environment at the Institute of Nutrition & Food Science (INFS), University of Dhaka, where the temperature was maintained at 25±3°C, relative humidity at 55±5%, and a light/dark cycle of 12 hours. The subjects were administered a conventional dietary regimen and had access to purified water. All the animals were housed within this controlled environment to facilitate their adaption, for a minimum period of one week prior to the commencement of the investigation. All experimental protocols were performed according to the guidelines of Institutional Animals Ethics Committee (IEAC).

2.4 Evaluation of Hepato–Protective Activity

In this experimental study, a sample of 50 rats was selected using random sampling techniques. The rats were then divided into ten groups, with an equal number of rats assigned to each group (Table 1).

2.5 Indication of Hepatic Injury

CCl_4 was mixed with Olive Oil at a ratio of 1:1. Then rats were orally treated with this at 3 mL/Kg body weight.

2.6 Assessment of Liver Functioning, Lipid Profile and Kidney Functioning test

All the parameter was measured using respective kit purchased form plasmatec laboratory and the blood parameters were analyzed in Humalyzer 3000.

2.7 Statistical Analysis

The raw data collected and examined in our study can be categorized into several groups based on the numerous parameters gathered. This data was organized and processed using the MS Excel programme. The data collected underwent descriptive statistical analysis, and the findings were reported as the mean value plus or minus the standard deviation. The "One-way ANOVA test" of SPSS 16 software was utilised to analyse the inter-group heterogeneity based on several biological factors and establish its statistical significance. The events are deemed to possess statistical significance, as shown by a 'p' value of less than 0.05 ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 Liver Functioning Test

The researchers investigated the effect of three doses of *Citrullus lanatus* on liver enzymes in the blood of CCl_4-administered rats and the results are given in Table 2.

The study carefully observed the impact of three different doses of ' *Citrullus lanatus* Extract' on liver enzyme levels in blood samples from rats administering CCl_4 (Groups 2–10). Changes in hepatic marker enzymes (SGPT and SGOT) present as indicators of hepatocellular damage, which is associated with CCl_4-induced oxidative stress in the livers of rats.

The Group 2, which induced CCl_4 as treatment, both SGPT and SGOT enzymes showed significantly higher activity post-injection ($p<0.05$) compared to the negative control (Group 1). Administration of *Citrullus lanatus* extract started preventing the abnormal rise in SGPT and SGOT enzyme levels induced by CCl_4, leading to a recovery in Groups 4-6 and demonstrated a gradual reduction in all three groups when compared to Group 2, whereas Groups 5 and 6 reached statistical significance ($p<0.05$). An increase in enzyme levels is correlated with the most pronounced cellular damage. So declining enzyme levels correlated with less cellular damage in this experiment.. Groups 7–10

showed non-significant output when compared with negative control (Group 1), indicating limited side effects or reduced cellular injuries following extract administration.

3.2 Kidney Functioning Test

The effect of three doses of *Citrullus lanatus* on Kidney function markers in CCl_4-induced rats is shown in Table 3.

After a duration of treatment, the consequences of three different doses of *Citrullus lanatus* on the levels of creatinine and urea from rats given with CCl_4 are compared to the positive control (Group 1). Notable changes were observed in creatinine values in groups 4,5, and 6, along with only urea values in group 6, showing statistical significance ($p<0.05$). The remaining groups did not exhibit any significant noteworthy kidney profile parameter alterations. *Citrullus lanatus* extract discloses a dose-dependent ability to alleviate the disrupted pathological state caused by elevated levels of urea and creatinine.

3.3 Cardiac Profile

The effects of *Citrullus lanatus* on the cholesterol levels of rats with liver damage caused by CCl_4 are shown in Table 4.

Table 4 displays the impact of *Citrullus lanatus* extract on lipid profile parameters, encircling serum triglyceride, LDL, HDL, and cholesterol levels, in rats with liver damage induced by CCl_4. Except for HDL, all lipid parameters (Triglyceride, Cholesterol, and LDL) saw an increase following CCl_4 administration.

Noteworthy changes ($p<0.05$) were observed in HDL and Triglyceride values in group 6, demonstrating statistical significance ($p<0.05$). The LDL and Total Cholesterol level were decreased but did not display notable significant alterations. Post-CCl_4 therapy, *Citrullus lanatus* extract has been observed to attribute a dose-dependent effect to alleviate the disrupted pathological condition caused by atypical levels of lipid profiles.

Table 1. Different groups of the rat with the treatment details

Group Number	Group Specification	Treatment species	Dose Treatment species (mg/kg)	Abbreviation of Groups
1	Negative Control	Physiological Saline	10ml/kg	N
2	CCl4 Control	N/A	N/A	A
3	CCl4 + Silymarin	Silymarin	120	A+S120
4	CCl4 + *Citrullus lanatus*	*Citrullus lanatus*	400	A+CL400
5	CCl4 + *Citrullus lanatus*	*Citrullus lanatus*	700	A+CL700
6	CCl4 + *Citrullus lanatus*	*Citrullus lanatus*	1000	A+CL1000
7	Silymarin	Silymarin	120	S120
8	*Citrullus lanatus*	*Citrullus lanatus*	400	CL400
9	*Citrullus lanatus*	*Citrullus lanatus*	700	CL700
10	*Citrullus lanatus*	*Citrullus lanatus*	1000	CL1000

Table 2. Changes in hepatic enzymes in CCl_4-induced oxidative damage in rat liver

Group No.	Treatment	SGPT	SGOT
1	Negative Control	35.36±2.14	44.24±3.51
2	CCl_4	96.31±6.30	99.39±8.80
3	CCl_4+Drug	52.24±4.14	50.53±6.19
4	CCl_4+ Plant Low Dose	93.19±4.36	96.42±7.45
5	CCL_4+ Plant Medium Dose	89.10±3.21*	91.43±6.32*
6	CCL_4+ Plant High Dose	85.50±2.62*	83.80±5.50*
7	Drug	30.41±3.18	42.30±2.84
8	Plant Low Dose	30.10±2.12	45.39±3.50
9	Plant Medium Dose	30.52±3.57	39.91±4.01
10	Plant High Dose	35.19±4.21	42.42±3.39

**The assay represents the mean ± SEM for 10 groups of rats, with each group indicating a significance level of *p<0.05. Data were compared between the extract-treated groups and the CCl_4 control group*

Table 3. Impact of *Citrullus lanatus* extract on renal function markers in CCl_4-induced rats

Group No.	Treatment	Creatinine	Urea
1	Negative Control	0.60±0.06	30.64±2.43
2	CCl_4	2.3±1.64	85.67±8.37
3	CCl_4+Drug	1.1±0.09	50.32±5.99
4	CCl_4+ Plant Low Dose	2.0±0.08*	83.29±6.74
5	CCL_4+ Plant Medium Dose	1.6±0.02*	80.95±6.82
6	CCL_4+ Plant High Dose	1.3±0.31*	76.41±6.31*
7	Drug	0.5±0.02	32.30±1.45
8	Plant Low Dose	0.8±0.03	28.54±2.63
9	Plant Medium Dose	0.6±0.03	32.35±1.54
10	Plant High Dose	0.5±0.08	30.15±2.02

** The assay represents the mean ± SEM for 10 groups of rats, with each group indicating a significance level of *p<0.05. Data were compared between the extract-treated groups and the CCl_4 control group.*

Table 4. The impacts of *Citrullus lanatus* extract on the cholesterol levels in CCl_4-induced rats

Group No.	Treatment	LDL	HDL	Triglyceride	Cholesterol
1	Negative Control	34.82±4.19	73.74±2.79	45.82±4.12	90.4±2.40
2	CCl_4	88.73±7.73	42.48±8.16	97.39±8.16	144.40±9.30
3	CCl_4+Drug	61.53±5.95	60.17±7.93	60.17±7.93	119.30±7.32
4	CCl_4+ Plant Low Dose	87.42±8.40	43.85±4.55	96.14±6.28	145.34±8.46
5	CCL_4+ Plant Medium Dose	85.90±6.38	46.69±4.78	94.15±7.84	1442.39±7.39
6	CCL_4+ Plant High Dose	84.55±5.28	52.44±3.20*	90.11±6.24*	141.70±6.39
7	Drug	36.15±3.20	74.27±3.83	42.16±3.90	86.40±4.19
8	Plant Low Dose	38.96±4.06	73.21±5.44	46.28±4.53	89.39±5.50
9	Plant Medium Dose	33.14±2.08	71.95±2.67	43.25±2.96	94.15±3.03
10	Plant High Dose	35.14±3.15	76.02±5.13	48.40±3.79	90.19±4.29

** The assay represents the mean ± SEM for 10 groups of rats, with each group indicating a significance level of *p<0.05. Data were compared between the extract-treated groups and the CCl_4 control group*

4. CONCLUSION

Based on our experimental results, it can be concluded that *Citrullus lanatus* undoubtedly has the ability to provide hepatoprotective activity. The concentration of the extract controls the intensity of the pharmacological activity. Therefore, more vigorous studies are needed to speed up the process of developing hepatoprotective medicine from watermelon.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experimental protocols were performed according to the guidelines of Institutional Animals Ethics Committee (IEAC).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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