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ORIGINAL ARTICLE

DOES METHANOLIC LEAF EXTRACT OF *CASSIA TORA* POSSESS HEPATOPROTECTIVE PROPERTIES IN PARACETAMOL-INDUCED LIVER DAMAGE IN RATS?

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Abstract. *Folklore in Africa holds that Cassia tora L. has numerous health-promoting qualities. We looked at how methanolic leaf extract of Cassia tora (MLECT) affected the hepatotoxicity caused by paracetamol in this case. Six groups of five male rats each, all in good health, were randomly allocated. Paracetamol overdose (2000 mg/kg body weight, peroral, 1 day) was administered to rats in all groups except the normal control group, resulting in liver damage. Following induction, the treatment groups› animals received different dosages of MLECT via gastric gavage (100, 300, and 500 mg/kg b.w./5 days). Following a nighttime fast, the animals were euthanized on the sixth day, and the serum levels of the liver marker enzymes (ALT, AST, and ALP), total bilirubin, total protein, renal function (urea and creatinine), electrolytes (Na+, K-, Cl-, and HCO_3), and antioxidant levels were assayed using standard methods. Terpenoids (81.84 ± 0.01 mg/100 g), flavonoids (17.86 ± 0.16 mg/100 g), saponin (15.72 ± 0.07 mg/100 g), glycosides (14.63 ± 0.03 mg/100 g), steroids (13.23 ± 0.03 mg/100 g), tannins (11.97 ± 0.13 mg/100 g), and phenols (6.62 ± 0.06 mg/100 g) were identified during phytochemical screening of MLECT. Safety was demonstrated at dosages up to 5000 mg/kg b.w. in the LD50 investigation. Based on the findings, an overdose of paracetamol (2000 mg/kg b.w.) caused hepatotoxicity, which was characterized by enhanced liver marker enzyme activity, elevated serum levels of urea and creatinine, decreased total protein, antioxidant enzymes, and skewed electrolyte values. In contrast to the aberrant levels shown in the positive control, treatment with different dosages (100, 300, and 500 mg/kg b.w p.o./5 days) of MLECT dramatically decreased liver marker enzymes and restored normalcy in the levels of antioxidant enzymes and kidney function indices. As a result, the study's findings imply that the methanolic leaf extract of Cassia tora has both antioxidant and hepatoprotective capabilities concerning paracetamol-induced hepatotoxicity.*

Key words: *hepatotoxicity, liver markers, kidney markers, serum electrolytes, antioxidants*

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INTRODUCTION

According to Asrani et al. [1], liver cirrhosis ranks tenth among Western countries' primary causes of death. Chronic hepatitis C is a leading cause of liver cirrhosis and hepatocellular carcinoma [2]. Chronic liver illnesses constitute a significant global health burden. Renal failure is typically the result of advanced liver disease or occurs as a corollary of acute illness involving many organs [3]. Many drug induced liver reactions can lead to hepatotoxicity [4]. The key conditions that still need to be addressed include hepatocellular carcinoma, non-alcoholic fatty liver disease, alcoholic liver disease, and chronic viral hepatitis B and C. An estimated 500 million people worldwide suffer from chronic hepatitis, a severe form of liver disease [5]. This number comes from the World Health Organization. Treatments for common liver illnesses, like cirrhosis, fatty liver, and chronic hepatitis, are frequently ineffective, fraught with side effects, and prohibitively expensive, particularly for those in underdeveloped nations [6]. Effective therapeutics with few adverse effects is needed by doctors and patients. According to Kaiyeto and Oguntibeju [7], herbal medications have the potential to be phytocompounds that are physiologically active and have lower toxicity. One of these phytocompounds are polyphenols, which are strong antioxidants of plant origin exhibiting protective role against the development of several chronic degenerative diseases [8]. The effects of herbs that indigenous healers have long utilized to support liver function and treat liver illnesses have been studied by numerous researchers in recent years. The majority of the time, science has validated conventional knowledge and experience by figuring out these plants' processes and modes of action and by confirming the medicinal efficacy of certain plants or plant extracts in clinical trials [9]. Because herbal medicines are safe, readily available, affordable, and environmentally friendly, they could be a viable treatment for current liver issues [10]. Medicinal plants have gained traction in global healthcare systems due to their well-established and potent medicinal qualities. According to estimates, 80% of people on the planet use medications that contain substances derived from plants [7].

Numerous elements of these medications are significant. There is a widespread perception that herbal medicines are safe because they are "natural and gentle", making them a risk-free substitute for conventional medication. This idea is reinforced by the claims made about their ability to both treat and prevent diseases [11]. Numerous herbs include components with different bioactivities and mechanisms of action that may be used as pharmaceuticals to treat liver problems. Still, a number of them have been thoroughly investigated for their bioactive constituents and hepatoprotective action mechanisms [12, 13].

The Leguminosae family of plants includes *Cassia tora* Linn., which is found across tropical regions of the world, including Africa and India. Wild wasteland is the habitat of this annual shrub. According to Pawar and D'mello [14], the plant leaves, seeds, and roots are all thought to have therapeutic benefits. As reported by Kumar et al. [15], the plant has been shown to have several therapeutic benefits, including hepatoprotective, hypolipidemic, and antioxidant effects. Yet, not enough research has been done on *Cassia tora's* potential to reduce hepatotoxicity and nephrotoxicity. In this work, rats with liver damage caused by paracetamol will be used to determine whether *Cassia tora* has hepatoprotective and nephroprotective properties.

MATERIALS AND METHODS

Materials

Collection and Authentication of Plant Material

In March 2023, fresh *Cassia tora* leaves were gathered in Orba, in the Udenu Local Government Area of Enugu State, Nigeria. Bio-resources Development and Conservation Program (BDCP) Research Centre, Nsukka, Enugu State, Nigeria; Mr. Alfred Ozioko, botanist, identified and verified the leaves. The specimen was submitted at the BDCP Herbarium after being given the voucher number Intercede/3459Aza.

Chemicals and Reagents

The analytical grade chemicals utilized in this investigation were from Sigma Aldrich, USA, British Drug House (BDH), England, Qualikems, India, Fluka, Germany, May and Baker, England, and Burgoyne, India. Commercial kits from Teco (TC), USA and Randox, USA were used to perform the assays. These consist of 3.5% Na_2CO_3, Chromogen, 10% AlCl3, potassium acetate, n-hexane, ethyl acetate, methanol, alkaline pirate, vanillin reagent, 72% H_2SO_4, 60% H_2SO_4, 0.5% formaldehyde, and folin reagent (1/10 dilution). phosphomolybdenium reagent (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M H_2SO_4), ethanol, 50% m/v potassium, 0.18 mM (0.005%) methanolic solution of DPPH, potassium ferricyanide, sodium phosphate buffer, 4.5% trichloroacetic acid, ferric chloride, and 5% phosphomolybdic acid.

Instrument and Equipment

The Department of Biochemistry at the University of Nigeria, Nsukka, as well as other scientific stores in

Nsukka, Enugu State, Nigeria, provided the equipment, which was used. Conical flasks (Pyrex, England), a water bath (Gallenkamp, England), beakers (Pyrex, England), a weighing balance (Metler HAS, U.S.A.), test tubes (Pyrex, England), a measuring cylinder (Pyrex, England), muslin cloth, a flat bottom flask, a glass funnel (Pyrex, England), micro-pipettes (Perfect, USA), capillary tube, a refrigerator (Thermocool, England), a centrifuge (Vickas Ltd, England), fractionation column, separatory funnel, filter paper, silica gel (200-400 mesh), a 10-liter gallon, and a rotary evaporator were among the items included.

Animals

Male albino rats weighing 70–80 g were used in the investigation. The University of Nigeria, Nsukka's Department of Zoology and Environmental Biology animal holding facility provided the study animals. All of them were housed in standard cages with highly hygienic conditions (relative humidity of 55 ± 5%, temperature of 24 ± 1 °C, and 12/12 hours of light and dark). They were fed high-quality rat chow (Vital feed) and given 14 days to get used to their new surroundings before the experiment began.

Methods

Preparation of Plant Material

Cassia tora fresh leaves weighed 1065.15 g and were allowed to air dry at room temperature. After being ground into a fine powder, they were weighed (566.30 g) and kept in a screw-capped container. 3.5 L of 100% methanol was used in a cold maceration process to extract the powdered leaves over the course of 72 hours. Following that, Whatman grade 1 qualitative filter sheets and a muslin cloth were used to filter the sample. Vacuum evaporation was used to concentrate the filtrate, which was then sealed in a sterile screw-capped bottle and kept cold until further examination was required. The weight of the dried, ground seeds before maceration and the weight of the crude extract post concentration were used to calculate the % yield of the methanol extract using the formula below

$$(\%) = \frac{\text{Weight of Crude Extract (g)}}{\text{Weight of Dried Pulverized Sample (g)}} \times 100$$

Quantitative Phytochemical Analysis of the Methanolic Crude Extract of Cassia tora Leaves

Using a conventional protocol, the phytochemical contents of the crude extract of *Cassia tora* leaves were determined using the Harborne method [16].

Acute Toxicity Test of Methanolic Leaf Extract of C. tora

The Lorke's method [17] was used in the acute toxicity study.

Experimental Design and Animal Grouping

A total of twenty-four male albino rats, weighing 70-80 g were used in the study. Animals were consigned into six groups of four rats each, and different treatments were meted on the rats in the various groups as follows:

- Group 1 – Normal Control (no paracetamol, no treatment).
- Group 2 – Positive Control (administered distilled water with 2 g/kg body weight of paracetamol-induced without treatment).
- Group 3 – Standard Control (administered 25 mg/kg body weight of Silymarin with 2 g/kg body weight of paracetamol).
- Group 4 – Low dose (administered 100 mg/kg body weight of methanol extract of *Cassia tora* with 2 g/kg body weight of paracetamol).
- Group 5 – Mid dose (administered 300 mg/kg body weight of methanol extract of *Cassia tora* with 2 g/kg body weight of paracetamol).
- -Group 6 – High dose (administered 500 mg/kg body weight of methanol extract of *Cassia tora* with 2 g/kg body weight of paracetamol).

Blood and Liver Tissues Collection

The rats were allowed to drink water at will throughout the night after the final dose of the various treatments in each group on the fifth day. On the sixth day, through the retrobulbar plexus in the eye, the blood sample was drawn into simple tubes after the cervical dislocation and thoracic cavity evacuation procedures. After allowing the collected blood to coagulate, a bench centrifuge was used to centrifuge it for 5 minutes at 3000 × g. The final serum was kept cold, at -20 °C, until it was required for the investigation.

Biochemical Parameter Determination

Aspartate transaminase (AST) and alanine transaminase (ALT) in serum

Reitman and Frankel's [18] methodology was applied.

Alkaline phosphatase (ALP) in serum

The end-point colorimetric approach was used to measure the amount of alkaline phosphatase in the serum [19].

Total Bilirubin

The Jendrassik and Grof [20] procedure, which is included in Randox assay kits, was followed in this process.

Total Protein in Serum

Using the Lowry et al. [21] approach, this was approximated.

Creatinine Determination

The Randox commercial kit's instructions for determining the creatinine level were followed in Henry's [22] Direct Endpoint method.

Urea Determination

Urease Berthelot, as recommended by Fawcett [23] and included in the commercial kit from Randox, was used to determine the urea level.

Serum Sodium Ion Concentration Measurement

A colorimetric method based on a modified Maruna and Trinders method as reported by Trinder [24] was used to determine the content of sodium in serum.

Measurement of Potassium Ion Concentration in Serum

Following Henry et al.'s [22] instructions, the turbidometric approach was used to measure the serum potassium ion (K^+) concentration.

Measurement of bicarbonate and serum chloride

The Skeggs and Hochstrasser method [25] was used to determine the concentrations of bicarbonate and chloride.

Decreased Glutathione (GSH) Estimation

Using the Exner et al. [26] approach, the reduced glutathione level was ascertained.

SOD (superoxide dismutase) Estimation

Using Arthur and Boyne's technique [27], the SOD activity in the supernatant was measured.

CAT (catalase) Estimation

The Aebi et al. [28] method was used to measure the catalase activity.

Lipid Peroxidation Estimation (MDA)

Using the Thiobarbituric acid reaction method as reported by Walin et al. [29], the degree of lipid peroxidation was measured.

Examination of Statistics

Every data point is shown as the average ± standard error of the mean (SEM). ANOVA, or one-way analysis of variance, was used for statistical analysis.

RESULTS

Percentage Yield

The extraction of known weight (1065.15 g) of pulverized C. tora leaves yielded 566.30 g of crude methanol extract, accounting for 53.17% of the starting material.

Phytochemical Composition of MLECT

Table 1 displays the outcome of the MLECT's quantitative phytochemical composition. Biologically active phytocompounds, including terpenoids (81.84 ± 0.01 mg/100 g), flavonoids (17.86 ± 0.16 mg/100 g), saponin (15.72 ± 0.07 mg/100 g), glycoside (14.63 ± 0.03 mg/100g), steroids (13.23 ± 0.03 mg/100 g), tannins (11.97 ± 0.13 mg/100 g), and other phenols (6.62 ± 0.06 mg/100 g), were detected in different concentrations according to the results. Phenol has the lowest composition (6.62 ± 0.06 mg/100 g), whereas terpenoids have the highest (81.84 ± 0.01 mg/100 g).

Table 1. Quantitative phytochemical analysis of MLECT

Phytochemicals	Composition (mg/100 g)
Flavonoids	17.86 ± 0.16
Tannins	11.97 ± 0.13
Phenols	6.62 ± 0.06
Glycosides	14.63 ± 0.03
Saponins	15.72 ± 0.07
Terpenoids	81.84 ± 0.01
Steroids	13.23 ± 0.03
Alkaloids	18.34 ± 0.12

Note: Results are reported in mean ± SD, n = 3

Acute Toxicity of MLECT

According to the acute toxicity investigation, albino rats treated with MLECT did not exhibit any toxicity-related symptoms or death. Until the conclusion of the research period, no dose was shown to be deadly.

Effect of MLECT on liver function indices in paracetamol-induced hepatotoxic rats

Table 2 below shows the liver function test in albino rats treated with MLECT. ALT activity shows no significant ($p > 0.05$) difference when groups 1 and 3 are compared to group 4. However, the ALT activity of these groups (1 and 3) was found to be significantly ($p > 0.05$) lower when compared to group 2 and significantly ($p > 0.05$) higher when compared to group 5.

AST result revealed that there was no significant ($p > 0.05$) difference when groups 3 and 5 were compared to group 6. However, when compared to group 3, the AST of group 2 was found to be significantly ($p < 0.05$) higher.

There was no significant ($p > 0.05$) difference in ALP activity among groups 3 and 5 when compared with group 6. ALP activity of rats in groups (3 and 5) was found to be significantly ($p < 0.05$) lower when compared to group 2.

There was no significant ($p > 0.05$) difference in the total bilirubin level of groups 3 and 5 when compared to group 1. On the other hand, the total bilirubin level of groups 3 and 5 was found significantly ($p < 0.05$) lower when compared to group 2.

Total protein level revealed that there was no significant ($p > 0.05$) difference when group 1 was compared to group 4. When compared to group 2, the total protein level of groups 1 and 4 was found to be significantly ($p < 0.05$) higher (Tabl. 2).

Effect of MLECTon kidney function indices in paracetamol-induced hepatotoxic rats

Table 3 shows an increase in the serum sodium level of rats administered 500 mg/kg b.w. of extract (group 6) when compared with those in normal control (group 1). There was no significant difference ($p > 0.05$) observed in the serum sodium level of rats given varying doses of MLECT across the groups.

A decrease was observed in the serum potassium level of rats in the normal control (group 1) when compared to those treated with 100 mg//kg b.w. plant extract (group 4). No significant difference ($p > 0.05$) was observed in the serum potassium level across the group.

An increase was observed in the serum chloride level of the tested groups (groups 1, 3, 4, respectively) compared to those in the positive control (group 2). However, no significant difference ($p > 0.05$) was observed in the serum chloride level across the group.

There was an increase observed in the serum bicarbonate level of rats in the standard control (group 3) when compared to the normal control (group 1). However, no significant difference ($p > 0.05$) was obtained in the serum bicarbonate level across the groups.

An increase was observed in the serum urea level of rats in the positive control (group 2) when compared with the standard control (group 3). However, no significant difference ($p > 0.05$) was observed in the serum urea levels of rats administered varying doses of MLECT across the groups.

Serum creatinine level revealed that there was no significant ($p > 0.05$) difference when groups 5 and 6 were compared to group 3. The serum creatinine level of these groups (5 and 6) was found to be significantly ($p > 0.05$) higher when compared to group 1 and significantly ($p > 0.05$) lower when compared to group 2 (Tabl. 3).

Table 2. Liver function biomarkers

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TBIL (mg/dL)	TP (g/dL)
Group 1	16.667 ± 1.528^{ab}	15.667 ± 1.528^{a}	25.667 ± 3.512^{a}	0.593 ± 0.117^{b}	5.267 ± 0.513^{b}
Group 2	21.667 ± 5.132^{b}	21.333 ± 2.309^{b}	31.333 ± 0.577^{b}	0.727 ± 0.061^{c}	4.267 ± 0.551^{a}
Group 3	17.000 ± 4.359^{ab}	17.333 ± 1.528^{a}	24.667 ± 0.577^{a}	0.500 ± 0.020^{ab}	4.933 ± 0.208^{ab}
Group 4	16.000 ± 2.000^{ab}	18.667 ± 1.155^{ab}	27.000 ± 4.000^{ab}	0.427 ± 0.042^{a}	5.367 ± 0.306^{b}
Group 5	14.333 ± 3.786^{a}	17.333 ± 1.528^{a}	23.000 ± 4.000^{a}	0.507 ± 0.064^{ab}	4.800 ± 0.100^{ab}
Group 6	14.667 ± 1.528^{a}	16.667 ± 1.155^{a}	24.667 ± 1.528^{a}	0.477 ± 0.085^{ab}	4.967 ± 0.751^{ab}

Notes: ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, AST = Aspartate aminotransferase, TBIL= Total bilirubin, TP = Total protein.
Group 1: Rats not induced (Normal control)
Group 2: Rats induced and not treated (Positive control)
Group 3: Rats induced and treated with standard drug (Standard control)
Group 4: Rats induced and treated with 100 mg/kg b.w. of the extract
Group 5: Rats induced and treated with 300 mg/kg b.w. of the extract
Group 6: Rats induced and treated with 500 mg/kg b.w of the extract
Values are the mean ± SD of 5 animals each in a group, the mean difference is significant at 0.05 level
*Different superscript implies significant difference among group.

Table 3. Serum electrolytes and kidney function biomarkers

Groups	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	HCO_3 (mmol/L)	Urea (mg/dL)	Creatinine (mg/dL)
Group 1	110.333 ± 4.163^{a}	4.367 ± 0.513^{a}	95.000 ± 1.000^{a}	27.000 ± 0.000^{a}	45.333 ± 10.214^{a}	1.300 ± 0.100^{a}
Group 2	127.667 ± 23.502^{a}	3.867 ± 0.651^{a}	94.667 ± 0.577^{a}	27.333 ± 1.155^{a}	51.000 ± 2.646^{a}	1.900 ± 0.265^{b}
Group 3	116.333 ± 25.325^{a}	4.733 ± 0.751^{a}	95.667 ± 0.577^{a}	27.333 ± 0.577^{a}	43.333 ± 2.082^{a}	1.667 ± 0.306^{ab}
Group 4	127.000 ± 25.060^{a}	4.433 ± 0.551^{a}	95.000 ± 1.732^{a}	27.333 ± 1.155^{a}	46.000 ± 2.646^{a}	1.533 ± 0.058^{ab}
Group 5	131.667 ± 9.074^{a}	4.533 ± 0.513^{a}	95.667 ± 0.577^{a}		48.000 ± 2.646^{a}	1.600 ± 0.173^{ab}
Group 6	122.333 ± 20.404^{a}	4.800 ± 0.529^{a}	94.333 ± 0.577^{a}	27.333 ± 0.577^{a}	44.667 ± 4.041^{a}	1.600 ± 0.100^{ab}

Notes: Na = Sodium, K = Potassium, Cl = Chloride, HCO_3 = Bicarbonate.
Group 1: Rats not induced (Normal control)
Group 2: Rats induced and not treated (Positive control)
Group 3: Rats induced and treated with standard drug (Standard control)
Group 4: Rats induced and treated with 100 mg/kg b.w. of the extract
Group 5: Rats induced and treated with 300 mg/kg b.w. of the extract
Group 6: Rats induced and treated with 500 mg/kg b.w of the extract
Values are the mean ± SD of 5 animals each in a group, the mean difference is significant at 0.05 level.
*Different superscript implies significant difference among group

Effects of MLECT on antioxidant enzymes and lipid peroxidation

Table 4 below shows that a significant increase ($p < 0.05$) was observed in GSH levels of rats in group 5 administered 300mg/kg body weight of MLECT compared with the positive control group (group 2). However, no significant difference ($p < 0.05$) was observed in the GSH levels of rats in groups 3, 4 and 6 (standard, 100 mg/kg body weight and 500 mg/kg body weight) compared with group 2.

A significant increase ($p < 0.05$) was observed in GPx levels of rats administered varying doses of MLECT and the standard group compared with the positive control group.

However, no significant difference ($p < 0.05$) was observed across the groups in CAT levels of rats administered varying doses of MLECT compared with both the normal control (group 1) and the positive control (group 2).

A significant decrease ($p < 0.05$) was observed in MDA levels of rats administered varying doses of MLECT and the standard group compared with group 2 (positive control).

DISCUSSION

Throughout the world, medicinal herbs have gained a lot of attention recently as a potential remedy for the perceived drawbacks of traditional pharmacotherapy. These plants include substances that affect human bodies in a particular physiological way [11]. In African folklore, *Cassia tora* L. is widely recognized for its health-promoting qualities. African traditional treatments make use of the plant's leaves, seeds, and bark; however, research has not completely substantiated these claims. The current study examined the impact of *Cassia tora* leaf extract in methanol on the liver and kidney function markers during paracetamol-induced hepatotoxicity.

The LD50 of the MLECT exhibited a non-toxic nature of up to 5000 mg/kg bw. This depicts the safety of MLECT for human and animal usage. However, a previous study by Cholendra et al. [30] observed no signs of toxicity up to 3200 mg/kg b.w., but reported mortality of experimental animals at higher doses.

The liver was ascertained by measuring the activity of serum ALP, AST, and ALT enzymes, which are normally found in the cytoplasm, mitochondria, or microsomes at higher quantities. Depending on the extent of the injury, alterations in cell membrane permeability, and enhanced or decreased aminotransferase catabolism, these enzymes leak into the bloodstream in response to liver injury [31]. According to the study findings, compared to the normal control, giving 2000 mg/kg b.w. paracetamol caused a remarkable rise in the levels of ALP, AST, and ALT. The considerable liver impairment caused by paracetamol is consistent with the elevated activity of liver marker enzymes in the untreated and paracetamol-induced groups (positive control). These results corroborate earlier research showing that rat serum levels of ALP, AST, and ALT were significantly elevated after taking paracetamol [32]. After rats in the treatment groups were given varying doses of C. tora methanolic leaf extract (100, 300, and 500 mg/kg bw), these enzyme activities were considerably lowered. The presence of polyphenolic compounds (flavonoids, tannins, and other phenols), terpenoids, and alkaloids – which have been demonstrated to have the ability to inhibit in-

Table 4. Antioxidant activities and malondialdehyde levels

Groups	GSH (mg/dL)	GPx (U/mg)	SOD (U/mg)	CAT (U/mg)	MDA(mg/ml)
Group 1	3.693 ± 0.575^{ab}	85.393 ± 5.914^{b}	11.477 ± 0.110^{b}	1.957 ± 0.428^{a}	1.353 ± 0.465^{ab}
Group 2	3.047 ± 0.222^{a}	50.630 ± 6.942^{a}	10.8437 ± 0.489^{a}	1.633 ± 0.520^{a}	2.020 ± 0.675^{b}
Group 3	4.020 ± 0.925^{ab}	80.470 ± 14.439^{b}	11.1107 ± 0.294^{ab}	1.767 ± 0.489^{a}	0.923 ± 0.453^{a}
Group 4	3.510 ± 0.171^{ab}	85.233 ± 13.155^{b}	11.4977 ± 0.051^{b}	2.003 ± 0.460^{a}	0.563 ± 0.214^{a}
Group 5	4.387 ± 0.693^{b}	70.950 ± 9.628^{b}	11.4407 ± 0.115^{b}	1.947 ± 0.386^{a}	0.600 ± 0.397^{a}
Group 6	3.667 ± 0.811^{ab}	87.300 ± 6.393^{b}	11.4237 ± 0.025^{b}	1.937 ± 0.341^{a}	0.967 ± 0.107^{a}

Notes: GSH = Glutathione, GPx = Glutathione peroxidase, MDA = Malondialdehyde, SOD = superoxide dismutase, CAT = Catalase.
Group 1: Rats not induced (Normal control)
Group 2: Rats induced and not treated (Positive control)
Group 3: Rats induced and treated with standard drug (Standard control)
Group 4: Rats induced and treated with 100 mg/kg b.w. of the extract
Group 5: Rats induced and treated with 300 mg/kg b.w. of the extract
Group 6: Rats induced and treated with 500 mg/kg b.w of the extract

Values are the mean ± SD of 5 animals each in a group, the mean difference is significant at 0.05 level

*Different superscript implies significant difference among group.

tracellular enzyme leakage into the serum as well as exert membrane-stabilizing activities – is what allows the methanolic leaf extract of C. tora to restore these serum enzyme levels of paracetamol-induced liver injury to near normalcy [32]. Our findings concur with those of Shehu et al. [33], who found that in rats given paracetamol, the elevated activity of liver marker enzymes (ALP, AST, and ALT) was lowered by Cassia italic, a closely similar plant. Similar to this, a prior investigation by Tiwari et al. [34] similarly proved the hepatoprotective benefits of *Cassia tora* as a complete plant. Additionally, 200 mg/kg b.w. *Cassia tora* significantly decreased ALT and AST as well as blood urea nitrogen levels in a study by Chen et al. [35] to determine the anticancer potential of *Cassia tora* on Balb/c sarcoma 180 injected mice. Its extensive phytochemistry may be the basis for the methanolic leaf extract of C. tora's shown ameliorative potential on liver marker enzymes. Furthermore, flavonoids, which were found in the extract in a significant concentration, have a well-established protective effect against oxidative stress-induced hepatotoxicity by lowering the synthesis of inducible nitric oxide synthase (iNOS), increasing the levels of superoxide dismutase, catalase, oxygenase-1, nuclear factor, and total antioxidant capacity in peroxidation. The reduction of AST and ALT in the blood may also have increased the Bcl2/Bax ratio, suppressed caspase proteins, and subsequently released inflammatory cytokines that prevented hepatocyte apoptosis [36].

Serum electrolyte levels are another important indicator of kidney and osmoregulation health. Potassium ion concentration affects renal function, and potassium levels rise as renal function declines. Sodium gives the organism access to its osmotic and hydration states. In both the electrolyte balance of humans and rats, chloride and bicarbonate ions access the acid-base condition. According to Yamada and Inaba [37], an excess reduction in these ions in the blood serum indicates acidosis, whereas a rise in these ions shows alkalinity. Comparing our results to the positive control, we found that treatment with C. tora methanolic leaf extract resulted in a significant ($p > 0.05$) increase in potassium and a non-significant ($p > 0.05$) fall in blood sodium levels toward normalcy. Our study findings also revealed that the chloride ion in the positive control decreased non-significantly ($p > 0.05$) when compared to the normal control and that the methanolic leaf extract of C. tora increased non-significantly ($p > 0.05$) in groups treated with different doses when compared to the positive control and was comparable to the standard control. In a similar vein, there were no appreciable differences in the groups' serum bicarbonate ion levels ($p > 0.05$). Comparing our results to the positive control, we also observed a significant improvement in serum total protein in rats given different dosages of C. tora's methanolic leaf extract; this improvement was comparable to that seen with standard medication (silymarin). Our findings are consistent with a recent work by Yohanna and Yakubu [38], which showed that an extract from C. tora might help hepatotoxic rats regain normal electrolyte levels.

Following the administration of paracetamol, there was a notable rise in serum urea and creatinine levels, suggesting nephrotoxicity. Nonetheless, different doses of C. tora's methanolic leaf extract administered showed varying degrees of reversal effects on serum creatinine and urea concentrations. In rats given different dosages of C. tora methanolic leaf extract, our results likewise demonstrated a significant ($p > 0.05$) decrease in total bilirubin levels, which was similar to the standard group. These outcomes are consistent with those of Park et al. [39], who demonstrated that treatment with C. tora extract significantly decreased the concentrations of urea and creatinine in hepatotoxic rats. Yohanna and Yakubu [38] also showed that C. tora extract could have a positive effect on renal indices (urea, creatinine, and total protein). This implies that the kidney's integrity can be preserved by the extract.

When the liver is harmed, there is a lack of normal physiological or biochemical processes, which can then impact the biochemical functions of other secondary organs like the kidneys. By reabsorbing vital nutrients and eliminating waste, the kidney contributes to the preservation of bodily homeostasis [40]. The liver is the primary organ in the synthesis of creatinine, which is subsequently carried by blood to the brain, muscles, and other organs. The kidneys use glomerular filtration to help remove creatinine from the blood. A spike in concentration indicates a deficiency in renal filtration [41]. Urea is the main byproduct of the breakdown of proteins. Amino acid deamination and the transformation of ammonia into urea, which is subsequently eliminated by urine, both take place in the liver. Urea concentrations in the blood increased as a result of renal disorders that lower the glomerular filtration rate [42]. By reducing sulphydryl groups and attaching to proteins in the rat renal cortex and medulla, paracetamol produces various free radicals that lead to renal failure. The generated free radicals harm the lipid and protein molecules in the membranes through oxidative stress, which ultimately impairs the renal cells' ability to form and function. Following the administration of paracetamol, there

was a notable rise in serum urea and creatinine levels, suggesting nephrotoxicity [43].

The study's hepatoprotective effects might be explained by the existence of significant classes of phytochemicals, notably terpenoids, flavonoids, saponin, glycoside, steroids, tannins, and other phenols, which were found in high concentration in the methanolic leaf extract of C. tora. According to Gutiérrez-del-Río et al. [44], terpenoids, flavonoids, and phenolics have been found to exhibit antioxidant qualities and interact with the majority of regulatory proteins, potentially contributing to the extract hepatoprotective and hepatocurative potential. It has been demonstrated that flavonoids exhibit antimicrobial properties against viruses, cancers, liver toxins, and other microorganisms [45].

When compared to the positive control group, the effect of the *Cassia tora* methanolic extract demonstrates an increase in the levels of antioxidant biomarkers (GSH, CAT, SOD, and GPx), suggesting that the leaves may have the ability to balance reactive oxygen species that could cause damage. In line with this study, another investigation was conducted on the effects of two different diets on oxidative stress in male triathletes over 14 days. The results showed an increase in SOD activity but not GPx activity, indicating a potential decrease in damage caused by protein oxidation [46]. In contrast to our findings, ageing naturally is associated with a decrease in antioxidant biomarkers, which raises the possibility of an imbalance between the pro-oxidant and antioxidant mechanisms, increasing the risk of cell damage and the intensity of free radical activity [47]. Increased amounts of GPx and GSH are linked with improved immune system performance, according to research on the connection between the glutathione cycle and several peripheral blood leukocyte functions in ageing [48].

Additionally, this study demonstrates a significant ($p < 0.05$) reduction in the test groups' levels of lipid peroxidation biomarkers (MDAs) when compared to the positive control, which is consistent with research conducted by Akrami [49]. In this trial, 60 patients with metabolic syndrome participated in a randomized controlled clinical trial in which they were given sunflower and flaxseed oils in different groups. It was observed that the flaxseed oil group experienced a decrease in MDA levels only. In a different study, 51 adult male patients who were candidates for liver transplantation with liver cirrhosis showed a negative correlation between their body mass index (BMI) and MDA levels. Furthermore, it was noted that the patient's consumption of antioxidants was inadequate, indicating that a dietary deficiency could potentially be a factor in the elevation of MDA [50].

CONCLUSION

It is recommended that people consume therapeutic herbs as food instead of overly depending on synthetic medications, which can have fatal side effects. According to the study, C. tora's methanolic leaf extract has hepatoprotective properties and may be used as a hepatoprotectant because it brought important liver function indices back to normal and reduced oxidative stress indicators to baseline levels.

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