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# Potential Curative Effects of Aqueous Extracts of *Cissus quadrangularis* (Vitaceae) and *Jatropha gossypiifolia* (Euphorbiaceae) on Acetaminophen-Induced Liver Injury in Mice

Romeo Joel Guemmogne Temdie, BSc, MSc, Ph.D[1,*], Edwige Ymele Chiogo Vouffo, BSc, MSc, Ph.D[2,3], Borris Rosnay Tietcheu Galani, BSc, MSc, Ph.D[1], Bertrand Dabole Ladane, BSc, MSc[1,4], Josee Bianzoumbe, BSc, MSc[1], Marc Germain Kuum Minoue, BSc, MSc, Ph.D[5], Fidèle Ntchapda, BSc, MSc, Ph.D[1,6], Theophile Dimo, BSc, MSc, Ph.D[3]

[1] *Department of Biological Sciences, Faculty of Science, University of Ngaoundéré, Ngaoundéré, Cameroon*
[2] *Food and Drug Administration, Center for Drug Evaluation and Research, Office of New Drugs, Silver Spring, USA*
[3] *Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon*
[4] *Department of Agriculture, Livestock and Derived Products, National Advanced School of Engineering, University of Maroua, Maroua, Cameroon*
[5] *Department of Psychology, Faculty of Letters and Human Social Sciences, University of Douala, Douala, Cameroon*
[6] *Department of Physiological Sciences and Biochemistry, Faculty of Medicine and Biomedical Sciences, University of Garoua, Garoua, Cameroon*

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ABSTRACT

*Background:* Acetaminophen-induced liver injury remains a significant public health problem because available treatments are limited due to their adverse effects. Medicinal plants, which are an important source of bioactive molecules, could be an alternative treatment for liver disease.

*Objective:* This study was designed to investigate the curative effect of aqueous extracts of *Cissus quadrangularis* (Vitaceae) and *Jatropha gossypiifolia* (Euphorbiaceae) on acetaminophen-induced liver injury in mice.

*Methods:* Mice were divided into groups and treated with distilled water, silymarin (50 mg/kg), a reference hepatoprotective agent, and aqueous extracts of *C quadrangularis* and *J gossypiifolia* (50 and 100 mg/kg, PO, respectively). These substances were given as a single daily dose 4 hours after acetaminophen administration (300 mg/kg, PO) for 2 days. Mice were humanely put to death 24 hours after the last dose and serum alanine aminotransferase and aspartate aminotransferase activities, total bilirubin and protein levels, reduced glutathione, superoxide dismutase, malondialdehyde, catalase, and nitrite tissue levels were assessed. Histology of the livers of the mice was performed by hematoxylin and eosin staining.

*Results:* Acetaminophen administration induced a significant ($P < 0.05$) mean (SEM) body weight loss (−14.45% [5.92%]), a significant elevation of alanine aminotransferase activity (15.08%), total protein and bilirubin levels (25.80%), and a significant ($P < 0.05$) increase in liver superoxide dismutase (67.71%), catalase (63.00%), glutathione (40.29%), malondialdehyde (30.67%), and nitrite levels compared with the control group. In curative treatment, *C quadrangularis* and *J gossypiifolia* (50 and 100 mg/kg) significantly ($P < 0.05$) reduced mean (SEM) body weight loss (16.67% [7.16%] and 1.25% [0.51%], respectively), serum alanine aminotransferase activity (17.62% and 11.14%, respectively), bilirubin level (29.62% and 49.14%, respectively) compared with acetaminophen group, and *J gossypiifolia* normalized serum total protein level. Both extracts significantly ($P < 0.05$) reduced the levels of glutathione and malondialdehyde and normalized that of nitrite, superoxide dismutase, and catalase compared with the acetaminophen group. Hepatocyte necrosis and inflammatory cell infiltration were remarkably reduced by the plant extracts.

*Conclusions:* The results obtained are evidence in favor of the development of a formulation based on the extracts of these plants against liver diseases.



* Address correspondence to: Romeo Joel Guemmogne Temdie, BSc, MSc, Ph.D, Department of Biological Sciences, Faculty of Sciences, PO Box 454, University of Ngaoundere, Ngaoundere Cameroon.
*E-mail address:* temdie2011@gmail.com (R.J. Guemmogne Temdie).

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## Introduction

The liver is a vital organ that plays a key role in the biotransformation and detoxification of xenobiotics. This specific activity is due to the abundance of cytochrome-P450-enzymes, which promote the formation of reactive metabolites from the initial drug during phase I reactions, and then to conjugation enzymes, which increase the hydrophilicity of these metabolites, contributing to their excretion in the urine.[1] However, some hazardous intermediates or the prodrug itself can directly interact with cell macromolecules such as lipids, proteins, or DNA during this process, causing cellular toxicity via oxidative stress pathways.[2] Anesthetics, antibiotics, analgesics, antiretrovirals, antituberculosis agents, cardiac medications, anticancer therapies, and even herbal treatments have all been linked to liver damage.[3] Among pain relievers-induced toxic effects, acetaminophen (APAP) hepatotoxicity comes in the first position.[4,5] In the United States, 46% of acute liver failure cases are due to APAP toxicity.[6] Data on APAP hepatoxicity are scarce in Africa, despite prior findings in Nigeria that found a frequency of 28% in children younger than age of 5 years.[4,5,7]

APAP toxicity is caused by the massive accumulation of its reactive metabolite, N-acetyl-pbenzoquinone imine (NAPQI) after prior activation by CYP 2E1 and following a drug overdose. This causes a depletion of glutathione contents leading to oxidative, nitrosative stress,[8–10] and then to an inflammatory reaction.[11,12] Studies on prevention and protection against liver damage are being conducted to improve the daily life of patients. The development of hepatoprotective formulations based on plant extracts is encouraged.[4]

*Cissus quadrangularis* Linn. (syn: *Vitis quadrangularis* (L) Wall ex Wight) is an edible plant with a thick quadrangular fleshy stem. Widely known in many African and Asian countries,[13–15] this plant has been strongly claimed for its numerous medicinal properties for decades. Several authors have reported that it is used for gout, backache, earache, eyeache, allergies, hemorrhoids, skin diseases, leucorrhoea, and gastrointestinal tract disorders, among others.[13–21] The presence of asymmetric tetracyclic triterpenoids, gamma-amyrin, anabolic steroids, gamma-amyrone, beta-sitosterol, and flavonoids (quercetin) as well as vitamin C has been reported by qualitative phytochemical analyses[14,17,19] and iridoids, carotene, stilbenes, phytoestrogenic steroids, glycerolipids, phytols, linoleic acid, stigmasterol, sterols, and cerebrosides were isolated from the extracts of *C quadrangularis*[21,22] as well as minerals (potassium, zinc, calcium, sodium, iron, copper, lead, cadmium, and magnesium).[15] Anti-inflammatory,[14] antiglucocorticoid, antidiabetic, antiosteoporotic,[13,15,18] antioxidant,[14,18,20] anticonvulsant and sedative,[15] apoptotic and anticancer[15,19] effects of *C quadrangularis* have been demonstrated. Alone or in combination with other plant extracts, *C quadrangularis* normalizes blood levels of cholesterol, glucose, and triglycerides and promotes weight loss.[18] Subchronic and mutagenic toxic effects of *C quadrangularis* were not observed at the doses of 2500 mg/kg body weight/day.[15,18]

Commonly known as black physicnut or bellyache grove, *Jatropha gossypiifolia* L. is a Euphorbiaceae of tropical, subtropical, and dry tropical climates of the semiarid tropical regions of Africa and America.[23] The leaves, roots, and latex of *J gossypiifolia* are used medicinally in both humans and animals. These plant's preparations (decoction, maceration, or infusion) can be used orally or topically for conditions such as hepatitis, inflammation and discomfort, rheumatism, wounds, toothache, swelling,[23–25] stomach ache, colon problems, fever, and cancer.[26] Terpenoids, lignoids, sugars, alkaloids, amino acids, coumarins, steroids, flavonoids, lignans (eg, gossypifan, gossypilin gossypidian, gadain, jatroiden, jatrodian, and arylnaphthalene), proteins, saponins, and tannins were found in *J gossypiifolia* extracts.[23,25] Venkatasin, coumarinolignoid, phenolic acids (eg, gallic, vanilic, syringic, 2,5-dihydroxy benzoic, caffeic, rosmarinic, and p-coumaric) were isolated from this plant.[23,25] Pharmacological studies have demonstrated *in vitro* and in vivo antioxidant, immunomodulatory, anti-inflammatory, analgesic, antipyretic, antianemic, antineoplastic, antimicrobial, antiophidic, hemostatic, antidiabetic, contraceptive, antihemorrhagic, antidiarrheal, neuropharmacological, local anesthetic, anticholinesterase, and antihypertensive activities of *J gossypiifolia*.[23,25]

Aside from the work by Swamy et al,[17] which showed that the methanol extract of *C quadrangularis* stem protects against rifampicin-induced hepatotoxicity owing to antioxidant activities, and although Panda et al[27] established a hepatoprotective antioxidant effect of methanol and water extracts from the aerial portions of *J gossypiifolia* on carbon tetrachloride-induced liver damage, research on these 2 plants' hepatoprotective potential is limited. This study aimed to investigate the curative effect of aqueous extracts of *J. gossypiifolia* and *C. quadrangularis* on APAP-induced liver injury in mice.

## Material and Methods

### *Experimental animals*

The animals were mice from the animal house of the University of Ngaoundere, Cameroon. Their body weight was 22 ±4 g. The mice were adults of both sexes, aged 2 months. They were reared under natural light conditions (12/12 hour light/dark cycle, 25.6 ±8°C temperature, 77% ±10% humidity). They were fed with a standard rodent diet and had access to drinking water. The tests were carried out following laboratory animal care norms and with the permission of Cameroon's National Ethics Committee (FWAIRD 0001954).

### *Plant harvesting and conservation*

*C quadrangularis* (Vitaceae) tuberous stem with leaves was obtained during November 2019 in the village of Tchontchi-golombé, department of Mayo-louti, North-Cameroon. *J gossypiifolia* (Euphorbiaceae) leaves were collected in Maroua-Domayo, Cameroon's Far North region, from August 25 to September 15, 2018. Professor Mapongmetsem, a senior botanist of the University of Ngaoundere-Cameroon identified the 2 plants. Samples of the 2 collected plants were cut into small pieces, air-dried in the shade for a week, and ground into powder using an electric grinder. The resulting powders were stored in hermetically sealed bottles for the preparation of the different extracts.

### *Extracts preparation*

*C quadrangularis* leafy stem powder (100 g) or *J gossypiifolia* leaf powder was infused (40°C) in an adiabatic vessel with 1.2 or 0.9 L distilled water, respectively.[28] The preparations were then shaken from time to time for 24 hours. The mixtures were filtered and 10 mL each filtrate was evaporated at 45°C in an oven to determine the extraction yields (18.52% or 8.25%, respectively). The remaining filtrates were stored in a freezer (−20°C) for later use.

### *Pharmacological study*

#### *Allocation and administration of substances*

Forty-two mice, 20 of each sex, were divided into 7 groups of 6 mice each. Group 1 (control group) and group 2 (APAP group) received distilled water; group 3 was given silymarin (50 mg/kg), a widely used hepatoprotective agent for treating liver injuries of various origins[12]; groups 4 and 5 were given an aqueous extract of the leafy stem of *C quadrangularis* (50 and 100 mg/kg, respectively); and groups 6 and 7 were given an aqueous extract of

the leaves of *J gossypiifolia* (50 and 100 mg/kg, respectively). Liver damage was induced in all mice except those in the control group by an intraperitoneal injection of 300 mg/kg APAP (Shijiazhuang No. 4Pharmaceutical Co, Ltd, China) 4 hours before administration of the first dose of plant extracts.[12] Different aqueous plant extracts and the vehicle (distilled water) were administered to the animals as a single daily dose for 2 days. Mice were given free access to food and water 1 hour after induction of liver injury by APAP. Animals were weighed on the first day and the third day of the experiment before they were put to death under anaesthesia (ethyl ether) on the third day of the experiment. Blood was collected in dry test tubes, the liver was dissected immediately, rinsed in cold saline (0.9%), and then weighed. The percentage of body weight growth (%bwg) and the relative liver weight (rlw) were estimated as follows:

$$\%\text{bwg} = \left(\text{final body weight} - \text{initial body weight}\right) \times 100/\text{initial body weight}.$$

$$\text{rlw} = \left(\text{weight of the liver/weight of the mouse}\right) \times 100$$

*Biochemical testing*

Blood was used for serum preparation and liver samples were taken for preparation of 20% homogenate (w/v) in Tris-HCl buffer (50 mM; pH 7.4) and for histopathological analysis. Serum and supernatant collected after centrifugation of blood and homogenate (4000 rpm for 10 minutes) at low temperature were stored at –20°C for biochemical analysis.

*Analysis of liver functional activity of the liver*

Biomarkers of liver function such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, and total bilirubin were determined according to the kit manufacturer's instructions (Biomaghreb and Labcare, India).

For each test, 1.0 mL of the appropriate working reagent was heated for 3 minutes at 37°C. Then 100 µL supernatant was added. After 1, 2, and 3 minutes, absorbance was measured at 340 nm against a blank. The ALT and AST activities were then calculated.

The amount of total bilirubin was determined by mixing 1000 µL total bilirubin reagent with 50 µL distilled water or sodium nitrite (blank). Then, 100 µL supernatant was added and absorbance was read at 550 nm following 3-minute incubation at 37°C.

The amount of total protein was determined by adding 1 mL sodium chloride reagent and 1 mL Biuret reagent to 20 µL supernatant. The mixture was left to rest for 10 minutes at 25°C and the absorbance was read at 550 nm.

*Assessment of oxidative stress*

To quantify oxidative stress in the liver, measurements of malondialdehyde (MDA), nitrite, and reduced glutathione, as well as catalase (CAT) and superoxide dismutase (SOD) activity, have been utilized.[29]

The Griess reagent was prepared by adding sulphanilamide (1%) and naphthylethylenediamide (0.1%) to phosphoric acid (2.5%). A tissue supernatant (100 µL) was added to the Griess reagent (100 µL). The resulting mixture was kept at 25°C for 5 minutes and the absorbance was measured at 570 nm. The nitrite level was determined using a standardized sodium nitrite curve.

Ellman's reagent was made by dissolving 5 mg of 2-dithio bisnitrobenzene acid in phosphate buffer (0.1 M; pH = 6.5) to determine the level of reduced glutathione (GSH) in liver supernatant. Ellman's reagent (3 mL) was mixed with tissue supernatant (20 µL) and stored at 25°C for an hour. The absorbance was read at 412 nm.

The liver supernatant (500 µL) was added to a solution that contained trichloroacetic acid (20%; 250 µL) and thiobarbituric acid (0.67%; 500 µL). The mixture was incubated at 90°C for 1 hour before being cooled and centrifuged. The amount of MDA was evaluated by measuring the absorbance of the supernatant at 530 nm.

The SOD activity was determined by mixing 134 µL hepatic supernatant and 1666 µL carbonate buffer (0.05 M; pH 10.2). 200 µL 0.3 mM adrenaline solution was added and the absorbance at 480 nm was measured at 20 and 80 seconds after homogenization.

The supernatant (50 µL) was added to 750 µL phosphate buffer (0.1 mM; pH 7.5) and 200 µL hydrogen peroxide (50 mM). The mixture was left to incubate for a minute at 25°C. The mixture was heated at 100°C for 10 minutes after potassium dichromate/glacial acetic acid (2000 µL) was added. The specific activity of catalase was determined by determining its absorbance at 570 nm after cooling.

*Histological investigation*

Liver histology was done on liver samples that were previously fixed in 4% buffered formaldehyde. The blocks were formed by dehydrating and embedding the samples in paraffin (56 ±2 °C). Sections 5-µm thick from each sample were obtained and three sections per mouse were stained with hematoxylin and eosin. Under light microscope (Olympus, GHBS, Japan), hematoxylin and eosin stained sections were evaluated at 100 × magnification.[30]

## Statistical Analysis

The acquired findings were provided as mean (SEM). ANOVA and Dunnett's multiple comparison tests were used to find significant differences using GraphPad InStat 3 software (La Jolla, California). The $P < 0.05$ level of statistical significance was used.

## Results

*Effect of the plant extracts on body weight and relative liver weight of APAP-injured mice*

The administration of APAP resulted in a significant ($P < 0.01$) decrease in the mean (SEM) body weight growth of the animals (–14.45% [5.92%]) compared with the control (16.45% [7.11%]) animals. Treatment of the animals with 50 mg/kg silymarin (2.38% [0.97%]) and both plant extracts improved the body weight growth of the mice only at the doses of 50 mk/kg body weight (16.67% [7.16%]) compared with the APAP group. No significant changes in relative liver weight were observed (Table 1).

*Effect of the plant extracts on biomarkers of liver function of APAP-injured mice*

Administration of APAP resulted in a significant ($P < 0.05$) increase in ALT activity (15.08%) compared with the control. Treatment of animals with silymarin (50 mk/kg body weight) reduced the increase in ALT activity by 4.82% compared with the APAP group. Aqueous leafy stem extract of *C quadrangularis* (50 mg/kg) and aqueous leaves extract of *J gossypiifolia* (50 mg/kg) significantly ($P < 0.05$) inhibited the increase in ALT activity by 17.62% and 11.14%, respectively, compared with the APAP group. Treatment with silymarin and both plant extracts did not significantly modify AST activity (Table 2).

Treatment of animals with silymarin and aqueous leafy stem extract of *C quadrangularis* (50 mg/kg) and aqueous leaf extract of *J gossypiifolia* (100 mg/kg) reduced significantly ($P < 0.05$) the serum total bilirubin level (29.62% and 49.14%, respectively) compared with the APAP group.

**Table 1**
Change in body mass and relative mass of injured mice in response to the treatment with the plant aqueous extracts (N = 6).*

| Treatment | Dose (mg/kg) | Initial body mass (g) | Final body mass (g) | Liver mass (g) | Body mass gain or loss (%) | Liver relative mass (%) |
|---|---|---|---|---|---|---|
| Control | 0 | 19.75 (1.50) | 23.00 (1.41) | 1.40 (0.19) | 16.45 (7.11)‖ | 5.00 (2.04) |
| $H_2O$ + APAP | 0 | 20.75 (3.59) | 17.75 (3.20) | 1.20 (0.29) | −14.45 (5.92)§ | 6.76 (2.77) |
| Sily 50 + APAP | 50 | 20.75 (0.50) | 21.25 (0.95) | 0.96 (0.37) | 2.38 (0.97)§,† | 4.51 (1.85) |
| ALSE Cq 50 + APAP | 50 | 18.00 (0.01) | 21.00 (1.29) | 1.09 (0.09) | 16.67 (7.16)§,‖ | 5.06 (2.07) |
| ALSE Cq 100 + APAP | 100 | 23.20 (2.16) | 20.40 (1.14) | 1.13 (0.31) | −9.93 (4.33)‡ | 5.53 (2.26) |
| ALE Jg 50 + APAP | 50 | 20.00 (5.35) | 20.25 (5.25) | 0.99 (0.43) | 1.25 (0.51)§,† | 5.50 (2.25) |
| ALE Jg 100 + APAP | 100 | 20.75 (1.70) | 18.25 (0.95) | 1.20 (0.51) | −12.04 (4.93)§ | 5.95 (2.43) |

ALE Jg 100 or 50: aqueous leaves extract of *Jatropha gossypiifolia* at the dose of 100 or 50 mg/kg; ALSE Cq 100 or 50 = aqueous leafy stem extract of *Cissus quadrangularis* at the dose of 100 or 50 mg/kg; APAP = acetaminophen at the dose of 300 mg/kg; Sily 50 = silymarin at the dose of 50 mg/kg.
* The values represent the mean (SEM).
† $P < 0.05$, compared with acetaminophen group.
‡ $P < 0.05$, compared with control.
§ $P < 0.01$, compared with control.
‖ $P < 0.01$, compared with APAP group.

**Table 2**
Variation in biomarkers of liver function of injured mice in response to treatment with the plant aqueous extracts (N = 6).*

| Treatment | Dose (mg/kg) | Liver total bilirubin (mg/dL) | Serum total bilirubin (mg/dL) | ALT (U/mL) | AST (U/mL) | Total protein (mg/mL) |
|---|---|---|---|---|---|---|
| Control | 0 | 4.58 (0.30) | 6.01 (0.20)† | 110.10 (2.67)† | 141.97 (6.55) | 1.59 (0.20)† |
| $H_2O$ + APAP | 0 | 3.64 (0.06) | 8.10 (0.50)‡ | 126.64 (2.15)‡ | 163.44 (7.24) | 2.48 (0.22)‡ |
| Sily 50 + APAP | 50 | 5.38 (0.27)† | 5.81 (1.00)† | 120.53 (5.55) | 154.66 (10.39) | 2.37 (0.24) |
| ALSE Cq 50 + APAP | 50 | 4.68 (0.32) | 5.70 (0.38)† | 104.32 (2.18)§,‖ | 155.30 (4.73) | 2.44 (0.09)‡ |
| ALSE Cq 100 + APAP | 100 | 4.05 (0.33) | 7.62 (0.43) | 117.36 (3.92) | 164.96 (6.85) | 2.50 (0.21)‡ |
| ALE Jg 50 + APAP | 50 | 4.62 (0.83) | 6.90 (0.60) | 112.53 (3.10)† | 147.52 (5.55) | 1.28 (0.14)‖ |
| ALE Jg 100 + APAP | 100 | 5.40 (0.43)† | 4.12 (0.13)‖ | 118.80 (4.08) | 140.64 (6.31) | 1.80 (0.25) |

ALE Jg 100 or 50 = aqueous leaves extract of *Jatropha gossypiifolia* at the dose of 100 or 50 mg/kg; ALSE Cq 100 or 50 = aqueous leafy stem extract of *Cissus quadrangularis* at the dose of 100 or 50 mg/kg; APAP = acetaminophen at the dose of 300 mg/kg.
* The values represent the averages (SEM).
† $P < 0.05$, compared with APAP group.
‡ $P < 0.05$, compared with control.
§ $P < 0.01$, compared with control.
‖ $P < 0.01$, compared with APAP group.

**Table 3**
Evolution of oxidative stress in injured mice in response to treatment with the plant aqueous extracts (N = 6).*

| Treatment | Dose (mg/kg) | Superoxide dismutase (U/mg protein) | Catalase (mM/min/mg protein) | Glutathione (µmol/mg protein) | Nitrite (µmol/mg protein) | Malondialdehyde (µmol/ mg protein) |
|---|---|---|---|---|---|---|
| Control | 0 | 14.04 (3.92)† | 12.61 (3.40)‖ | 63.25 (8.69)† | 0.13 (0 .01)‖ | 34.04 (5.64)† |
| $H_2O$ + APAP | 0 | 43.49 (8.71)‡ | 34.08 (5.71)§ | 102.45 (18.31)‡ | 0.32 (0.02)§ | 48.82 (3.99)‡ |
| Sily 50 + APAP | 50 | 20.87 (6.73) | 31.55 (6.55)‡ | 85.31 (4.09) | 0.15 (0.01)‖ | 47.26 (2.35)†,‡ |
| ALSE Cq 50 + APAP | 50 | 8.73 (3.45)‖ | 21.57 (3.47) | 77.39 (5.64) | 0.19 (0.02)† | 45.29 (4.85) |
| ALSE Cq 100 + APAP | 100 | 24.92 (5.12) | 34.02 (5.57)§ | 84.72 (7.28) | 0.21 (0.05)† | 25.39 (1.70)‖ |
| ALE Jg 50 + APAP | 50 | 23.04 (10.57) | 26.29 (3.10) | 71.57 (4.10) | 0.14 (0.02)‖ | 27.50 (1.40)‖ |
| ALE Jg 100 + APAP | 100 | 13.73 (2.71)† | 11.28 (1.14)‖ | 45.85 (5.11)‖ | 0.14 (0.01)‖ | 21.63 (2.31)‖ |

ALE Jg 100 or 50 = aqueous leaves extract of *Jatropha gossypiifolia* at the dose of 100 or 50 mg/kg; ALSE Cq 100 or 50 = aqueous leafy stem extract of *Cissus quadrangularis* at the dose of 100 or 50 mg/kg; APAP = acetaminophen at the dose of 300 mg/kg; Sily 50 = silymarin at the dose of 50 mg/kg.
* Values are presented as mean (SEM).
† $P < 0.05$, compared with acetaminophen group.
‡ $P < 0.05$, compared with control.
§ $P < 0.01$, compared with control.
‖ $P < 0.01$, compared with acetaminophen group.

The administration of APAP resulted in a significant ($P < 0.05$) increase in total protein level (55.97%) compared with the control. Treatment with aqueous leafy stem extract of *C quadrangularis* (50 or 100 mg/kg) did not significantly influence the total protein level compared with the APAP group. Aqueous leaves extract of *J gossypiifolia* (50 mg/kg) inhibited the rise in plasma protein levels by 48.39% compared with the APAP group.

### *Effect of plant extracts on oxidative stress in APAP-injured mice*

Administration of APAP resulted in a significant increase in SOD and CAT activity (67.71% and 63.00%, respectively) in the animals compared with control. Treatment with silymarin, or aqueous leafy stem extract of *C quadrangularis* and aqueous leaves extract of *J gossypiifolia* maintained SOD and CAT activity at levels comparable to those of the control animals.

The results showed that the treatment of the animals with silymarin and the different plant extracts reduced the levels of glutathione and nitrites and kept them more or less close to the levels recorded in the control animals (Table 3).

The administration of APAP resulted in a significant ($P < 0.01$) increase in MDA levels (43.37%) in mice compared with the control group. Treatment with aqueous extracts of the leaves of *J gossypiifolia* (50 or 100 mg/kg) and leafy stem of *C quadrangularis* (100 mg/kg) resulted in a significant ($P < 0.01$) decrease in the level of MDA compared with the APAP group, with a maximum percentage of 55.69%.

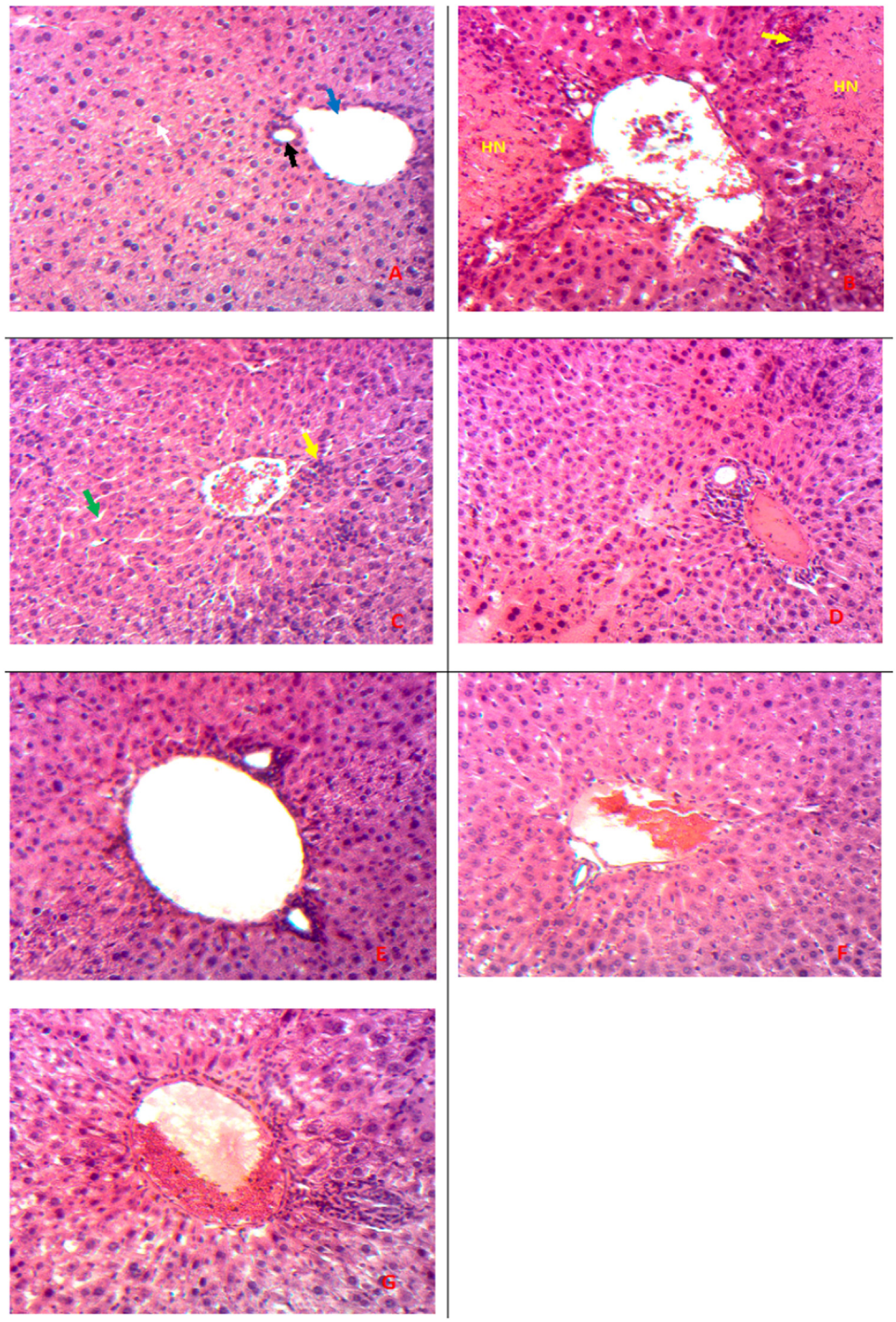


**Figure.** Microphotography of liver tissue of acetaminophen-injured mice treated with plant aqueous extracts. Histological sections stained with hematoxylin and eosin (× 100). (A) Histological section of mice treated with distilled water. (B) Histological section of acetaminophen-injured mice. (C, D, E, F and G) Histological liver sections of acetaminophen-injured mice treated with aqueous leafy stem extract of *Cissus quadrangularis* (100 and 50 mg/kg), aqueous leaves extract of *Jatropha gossypiifolia* (100 and 50 mg/kg), or silymarin (50 mg/kg), respectively. HN = hepatocytes necrosis. White arrow indicates hepatocyte, blue arrow indicates the hepatic portal vein, green arrow indicates the hepatic sinusoid, black arrow indicates the hepatic artery, and yellow arrow indicates infiltration of inflammatory cells.

*Effect of the plant extracts on the liver inflammatory lesions of APAP-injured mice*

The cross-section of the liver from healthy mice showed a classical architecture with normal cells uniform in size and staining appearance. The portal space showed ordinary hepatic vessels (see panel A of the Figure). Microphotography of the livers of APAP-treated mice showed an irregular liver parenchyma that is partially destroyed due to cellular necrosis and the entire tissue is invaded by inflammatory cells (see panel B of the Figure). Treatment of APAP-injured mice with aqueous extract leafy stem of *C quadrangularis*, aqueous leaves extract of *J gossypiifolia* (100 or 50 mg/kg) and silymarin (50 mg/kg) reduced APAP-induced hepatocellular necrosis, they reduced mobilization of inflammatory cells, and attempted to preserved the classical architecture of liver tissue (See panels C–G of the Figure).

## Discussion

We have provided evidence that curative administration of aqueous leafy stem extract of *C quadrangularis* and aqueous leaf extract of *J gossypiifolia* significantly attenuated APAP-induced liver injury. The main objective of this study was to evaluate the potential curative effect of aqueous leafy stem extract of *C quadrangularis* and aqueous leaf extract of *J gossypiifolia* on APAP-induced liver injury in mice. We evaluated the effect of these plant extracts on different aspects of APAP-induced liver injury, including hepatotoxicity, oxidative stress, and inflammation. We have demonstrated that the curative protective effect of *C quadrangularis* and *J gossypiifolia* extracts consists in inhibiting the pathological mechanisms following APAP overdose by reducing hepatocyte necrosis, decreasing oxidative stress, and inflammatory processes.

APAP, a popular and widely used conventional analgesic, is a product that is primarily metabolized by the liver. Thus, <2% of this product is eliminated unchanged. A fraction of about 90% undergoes conjugation reactions to be eliminated, whereas the remainder of APAP (about 10%) undergoes phase I oxidation reactions, catalysed by cytochromes P450, to produce NAPQI, a highly reactive metabolite.[31]

The hepatotoxicity of APAP results from the accumulation in the body of large quantities of NAPQI, a harmful metabolite responsible for many dysfunctions in the body. APAP-induced liver injury is currently a popular pathophysiological model for the evaluation of phytotherapeutic preparations and other formulations with hepatoprotective potential.[12]

The assessment of the severity of pathologies and the normal functioning of an organism can be done by examining the variation in body weight. Normal body weight growth is also an indication of the absence of physiological dysfunction.[32] A significant decrease in body mass was recorded in the APAP group of mice in this study compared with the control group. This reduction in body weight growth is indicative of a proven dysfunctional state caused by APAP administration. The aqueous extracts of *C quadrangularis* and *J gossypiifolia* resulted in a significant improvement in weight growth compared with the APAP group, suggesting a beneficial effect of the extract against APAP-induced liver injury in mice.

APAP hepatotoxicity results in liver failure due to hepatocyte death by apoptosis and necrosis. The pathophysiology leading to this severe liver dysfunction involves the pro-oxidative (oxidative and nitrosative stress) and proinflammatory processes triggered by NAPQI.[12] Regarding the proinflammatory process, it has been shown that the recruitment of inflammatory cells has been observed in animals after induction of liver injury by APAP.[12,33] These inflammatory cells invade the liver parenchyma where they perform several activities such as increasing the expression of inflammatory markers (proinflammatory cytokines such as tumor necrosis factor alpha, interleukin 1B, and adhesion molecules), clearing of debris, recruitment of activated monocytes (which starts 6 hours after APAP administration and continues as long as tissue damage exists), and the massive production of reactive species following the activation of inducible nitric oxide synthase in macrophages and infiltrating neutrophils. Inducible nitric oxide synthase is responsible for the excessive production of nitric oxide.[12] The most significant increase in the expression of inflammatory markers correlates with the onset of necrotic lesions and their extent. Necrotic cells lead to sterile inflammation through the release of lesion-associated molecular patterns. Decreased cell necrosis leads to reduced release of lesion-associated molecular patterns and therefore prevents the production of proinflammatory cytokines and leukocyte infiltration.[12] The results of this study show that aqueous extracts of *C quadrangularis* and *J gossypiifolia* drastically reduced APAP-induced liver lesions. Liver sections from mice treated with the plant extracts showed less necrosis and almost no leukocyte infiltration compared with those from the APAP group, suggesting anti-inflammatory effects of these plant extracts. Indeed, if the inflammatory response is maintained then hepatocyte necrosis will be more extensive, as observed in APAP mice. The results also show a significant decrease in the activity of ALT, an intracellular enzyme whose elevated serum activity is a sign of cellular and specifically hepatocyte necrosis. Krithika and Verma,[34] reported that serum transaminase levels, especially that of ALT, is an appropriate way to estimate the extent of hepatocyte lysis. These observations corroborate those on weight growth and the state of hepatic parenchyma.

Several pro-oxidative mechanisms are known and all contribute to the creation of a state of oxidative and nitrosative stress, through the massive production of highly reactive by-products and the inhibition of the body's antioxidant defense. GSH depletion is the initial step in APAP hepatotoxicity as it is GSH that neutralizes NAPQI, which is a highly reactive compound responsible for the toxic effects of APAP. NAPQI forms covalent bonds with sulfhydryl groups of cysteine, lysine of GSH, or other cellular proteins to form APAP protein adducts.[10] Once GSH is depleted, any NAPQI formed then reacts with other targets. NAPQI interacts with certain cellular proteins such as mitochondrial proteins and induces the formation of free radicals that cause oxidative stress. Another mechanism of hepatotoxicity is the reaction of superoxide anion and nitric oxide to form peroxynitrite, which plays a critical role in the mechanisms of APAP-induced hepatotoxicity. Peroxynitrite forms nitrotyrosine adducts within the mitochondria that cause its dysfunction, leading to the shutdown of ATP synthesis and subsequent cell death.[12] Oxidative stress associated with lysosomal iron uptake into mitochondria leads to mitochondrial membrane dysfunction, similarly, the release of mitochondrial apoptosis-inducing factor and endonuclease G, following mitochondrial lysis, leads to cellular DNA fragmentation. Both mechanisms result in cellular necrosis.[35,36] The protein adducts formed, particularly those of mitochondrial proteins, lead to oxidative damage and hepatocellular necrosis.[12] Overall, these data indicate that the organs, especially the liver, of APAP-treated animals are highly exposed to reactive nitrogen and oxygen species, resulting in nitrosative and oxidative stress. Experimental data indicate that oxidative and nitrosative stress results from an imbalance between antioxidants and oxidants.[10,12,33] Proteins, DNA, and lipids are the cellular targets affected by reactive nitrogen and oxygen species.[37] Reactive free radicals interact with cellular lipids to form MDA, the end product of lipid peroxidation.[10,38] Our results showed that the levels of SOD, GSH, and CAT in mice treated with aqueous leafy stem extract of *C quadrangularis*, aqueous leaf extract of *J gossypiifolia* remained significantly close to those of the controls, whereas those of mice treated with APAP differed significantly. These results suggest that APAP treatment does not appear to affect mice treated with plant extracts. Induction of liver injury by APAP caused a significant elevation of nitrite levels in the liver compared with the control. However, administration of the aqueous extract of the leafy stem of *C quadrangularis* and the aqueous extract of the leaves of *J gossypiifolia* to the mice kept their levels similar to that of the control. In an oxidative environment, high levels of nitric oxide react with superoxide to form peroxynitrite, which plays a crucial role in the mechanisms of APAP-induced liver injury.[12] These results suggest that the aqueous extract leafy stem of *C quadrangularis*, and aqueous leaves extract of *J gossypiifolia* would offer tissues better protection by neutralizing the mechanism allowing nitrosative stress. Furthermore, we observed a significant increase in MDA levels in the liver at the end of the study. However, this increase was significantly less pronounced in the mice treated with the plant extracts and somehow reflects the intensity of lipid peroxidation. Overall, these results support the hypothesis that in mice treated with plant extracts, fewer reactive nitrogen and oxygen species are produced to

react with biomolecules and cause damage. Furthermore, Papackova et al[12] showed that lipids are not the more important targets of oxidative stress than proteins and DNA in APAP-induced liver injury. This constitutes a weakness of this work. We then plan to evaluate the effects of extracts from these plants on proteins and DNA.

Phenolic compounds (ie, polyphenols, flavonoids, coumarins, tannins, and anthraquinones), terpenes (especially saponins), and nitrogenous compounds such as alkaloids have been reported in aqueous extracts of *C quadragularis* and *J gossypiifolia* (data not shown). These effects are attributable to the bioactive metabolites of these extracts because saponins have immunomodulatory properties.[39] Terpenes have anti-inflammatory and neuroprotective properties,[40] and sometimes could exhibit hepatotoxic effects.[41] Phenolic compounds have antioxidant properties.[42–44]

## Conclusions

In our study, we demonstrated that aqueous extracts of *J gossypiifolia* and *C quadrangularis* significantly reduced APAP-induced liver injury in mice. These extracts were found to be effective in limiting oxidative and nitrosative stress, and inflammation due to the toxic metabolite of APAP that causes liver damage. These results provide a basis for further work to evaluate the potential use of these plants in the management of APAP-induced toxic hepatitis.

## Declaration of Competing Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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