Trop J Nat Prod Res, December 2023; 7(12):5453-5459

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Evaluation of Antioxidant, Cytotoxic and Hepato-protective Effect of *Bridelia tomentosa* Fruit Extract

Mohammad A. Rahman[1] Nazmul A.H.M. Hasan[2]*, Milon Mondal[3], Mohi Uddin[4], Tania B. Wahed[1], Khairul K.M. Alam[1]

[1]*Department of Pharmacy, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh*
[2]*Department of Pharmacy, School of Medicine, University of Asia Pacific, 74/A, Green Road, Farmgate, Dhaka-1205, Bangladesh*
[3]*Department of Pharmacy, Life Science Faculty, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj 8100, Bangladesh*
[4]*Department of Pharmacy, Faculty of Biological Sciences, University of Chittagong, Chittagong 4331, Bangladesh*

ARTICLE INFO

Article history:
Received 22 August 2023
Revised 09 November 2023
Accepted 30 November 2023
Published online 01 January 2024

ABSTRACT

Over the past decade, plant derived herbal medicines have become a worldwide concern. This study explored the anti-oxidant, cytotoxic and hepatoprotective potential of the fruit of *Bridelia tomentosa*. The ethanolic extract of *Bridelia tomentosa* fruits was evaluated for *in-vitro* antioxidant, cytotoxic and hepato-protective properties in Sprague-Dawley male rats. Total phenol, flavonoid and antioxidant capacity of the plant extract were extimated as 99.04145 ± 1.195mg/g GAE, 126.5046 ± 3.095mg/g QE and 372.094 ± 3.148 mg/g AAE. The extract showed good inhibitory potential of DPPH free radical (IC_{50} = 30.579 ± 1.895 µg/ml) and exhibited poor cytotoxic potential (LC_{50} = 537.693 ± 70.099 µg/ml). The fruit extract of *Bridelia tomentosa* was found safe at 6000mg/kg BW dose in rats. *Bridelia tomentosa* fruit extract was administered at two different doses (250mg/kg-body weight and 500mg/kg-body weight) in Sprague-Dawley male rats and improved all the hepatic parameters in paracetamol induced hepatic injury and highest effect was observed at 500mg/kg BW dose. At 500mg/kg BW dose, the fruit extract, reduced the ALT, AST and ALP level to 38.250 ± 1.652, 25.500 ± 2.754 and 139.250 ± 10.515 µ/L ($p< 0.05$) respectively. The fruit extract also moderately decreased total bilirubin level. The fruit extract was able to reverse the effect of hepatic damage caused by paracetamol significantly as evidenced from histopathological examination. Fruits of *Bridelia tomentosa* could be a good source of newer antioxidant and hepato-protective agents and isolation of potent bioactive compounds are highly suggested.

Keywords: *Bridelia tomentosa*, Anti-oxidant, Hepato-protective, DPPH, Paracetamol

Introduction

Plants have always abided an inherent part of human civilization throughout the history and human beings have always pursued for remedy into plants for various ailments. Traditional or indigenous medicine treat aliments differently from modern medicine, based on credence and experiences.[1] Medicinal plants are widely available and acceptable to a diverse range of ethnic groups. They propound a convenient and economical health care regime for indigenous rural populations. Therapeutic effects of most plant are because of their secondary metabolities.[2] Plants contain phytochemicals due to their metabolic process, have potential to use against diseases, which are basically served as therapeutic agent as well as important raw materials for pharmaceutical usage.[3]

There are approximately 6500 plant species in Bangladesh, of which over 500 have the potential to be therapeutic. Of these, 250 species are regularly used to make medicines for medical use.[4]. *Bridelia tomentosa* blume is common in Bangladesh.[5]. Reports on the ethnopharmacological investigations of this plant is limited. Bark has an astringent property, leaves are used to treat traumatic injuries, and roots are used to treat influenza and neurasthenia.[6]

*Corresponding author. E mail: nazmul_0155@yahoo.com
Tel: +8801756199375

Citation: Rahman MA, Hasan NAHM, Mondal M, Uddin M, Wahed TB, Alam KKM. Evaluation of Antioxidant, Cytotoxic and Hepato-protective Effect of *Bridelia tomentosa* Fruit Extract. Trop J Nat Prod Res. 2023; 7(12):5453-5459. http://www.doi.org/10.26538/tjnpr/v7i12.14

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Bridelia stipularis (L) Blume bark decoction is used to treat cough, fever, and asthma; leaves are also used to treat jaundice.[7] The purpose of this study was to investigate the cytotoxic, hepatoprotective, and antioxidant properties of *Bridelia tomentosa* fruits.

Methods

Plant Part Collection and Extraction

First, *Bridelia tomentosa* was found in Gazipur district (Latitude: 23.9980797, Longitude: 90.4229848) of Bangladesh. A taxonomist from Botany Department of Jahangirnagar University, Savar, Dhaka, Bangladesh then confirmed the plant (DACB No. 38733). On October 22, 2021, the fruits were collected. Then they were cleaned using water, dried and grounded into powder. 500g of powdered fruit were extracted using a soxhlet extraction at 65°C using 1000 ml of ethanol in succession. When the Soxhlet device began to pull off colorless liquid in cycles, the extraction was deemed to be finished. Following cotton filtration, the extract was dried at 40±2ºC to provide a gummy crude extract. In percentage, the extract yield was 17.16%.[8]

Phytochemical Screening

Various phytoconstituents were detected by following standard procedures.[9]

Determination of Total Phenolics Content

FCR method was used to calculate the total phenolic content of plant extract. Test tubes were filled with 1.0 mL of plant extract or a standard solution at varying concentrations. Next, 5 mL of FCR (diluted ten times) and 4 mL of Na_2CO_3 solution were added. To finish the reaction, the test tubes were incubated at 20^0 C for 30 minutes for the standard and 1 hour for the extract. A spectrophotometer was used

to measure the absorbances of the solutions at 765 nm in comparison to a blank.[10] Plant ethanol extract's total phenolic content was represented in mg/g gallic acid equivalents (GAE).

Determination of Flavonoid Content
To measure the flavonoid content, the $AlCl_3$ colorimetric method was employed.[11] Sample volume of 1 mL was combined with 3 mL EtOH, 0.2 mL 10% $AlCl_3$, 0.2 mL 1 M CH_3COOK, and 5.6 mL distilled water. The mixture was then incubated for 30 minutes at room temperature. Using a UV/Visible spectrophotometer, the reaction mixture's absorbances were measured at 415 nm. The amount of flavonoids present was stated as milligrams per gram of quercetin equivalent.

Total Antioxidant Capacity
The reduction of Mo (VI) to Mo (V) by the antioxidant component and the development of the phosphate/Mo (V) complex at lower pH served as the foundation of antioxidant capacity estimation. Three milliliters of the reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4, and 4 mM ammonium molybdate) were combined with the extract or standard (0.3 mL). The solution's absorbance was measured at 695 nm in comparison to a blank, and the total antioxidant capacity was calculated using a regression equation created by plotting ascorbic acid content against optical density.[12]

DPPH Free Radical Scavenging Assay
Free radicals like DPPH accept electrons from other substances and cause oxidation of other substances. Antioxidants, on the other hand, donate electrons to other substances and neutralize DPPH by becoming oxidized themselves. Test tubes were filled with 1 ml of either plant extract or standard (ascorbic acid) in varying diluted concentration solutions. A fresh 2 ml of 0.004% DPPH solution was then added to each test tube. After 30 minutes of room temperature incubation, the mixture's absorbance at 517 nm was measured using a UV-VIS spectrophotometer.[9] The extract's and the standard's free radical scavenging activity was assessed as a percentage of inhibition, and the IC_{50} was calculated appropriately.

Reducing Power Capacity Assessment
A compound's ability to reduce can be a useful measure of its possible antioxidant activity. 2.5 ml of 1% $K_3Fe(CN)_6$ solution was added to each test tube, after that 2.0 ml of plant extract or a standard solution of various concentrations was added in each test tube. Following a 10-minute 50^0C incubation period, 2.5 ml of a 10% trichloroacetic acid solution was added to each test tube, and the resulting mixtures were centrifuged for 10 minutes at 3000 rpm. After removing 2.5 ml of the supernatant solution from each mixture and combining it with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride ($FeCl_3$). After that, a spectrophotometer was used to measure the absorbances of the solutions at 700 nm.[13]

Nitric Oxide Scavenging Capacity Assay
Test tubes containing 4.0 ml of plant extract or standard solutions at varying concentrations were filled with 1.0 ml of sodium nitroprusside (5 mM) solution. To finish the reaction, the test tubes were incubated at 30^0C for two hours. A spectrophotometer was used to measure the absorbances of the solutions at 550 nm after 2.0 ml of the solution was taken out of the mixture and mixed with 1.2 ml of Griess reagent. The IC_{50} value of the plant extract was ascertained after the percentage (%) inhibition activity was computed.[14]

Cytotoxic Activity: Brine Shrimp Lethality Bioassay
The cytotoxicity of the plant extracts was assessed using the method described by Akanda and Hasan.[9] Sample solutions were made by dissolving the test materials in a precalculated quantity of dimethyl sulfoxide. Ten nauplii were placed in vials with five milliliters of artificial seawater. Samples with varying concentrations were introduced. After a day, the survivors were counted. Using Microsoft Excel, the percentage of dead shrimps was plotted against the sample concentration's logarithm to determine the test samples' median lethal concentration (LC_{50}) after 24 hours.

Hepato-protective Effect
Male Sprague-Dawley rats, weighing 150-175 grams and aged 16-24 weeks, were kept in a room with a temperature of 25 ± 2 °C and 12-hour light/dark cycles. They were also given regular food and water. The ARRIVE guidelines (Animal Research: Reporting of *In-vivo* Experiments) were followed in this study. Following a week of acclimation, 25 rats in total were split into 5 groups, each with 5 rats, at random. The groups were as follows:
Group-1 (Normal Control) – Animals received water and normal diet for 10 days.
Group-2 (Negative Control) – Animals received paracetamol alone at a dose of 700 mg/kg BW for 10 days
Group-3 (Positive Control-Silymarin) - Animals received Silymarin 100 mg/kg BW with paracetamol 700 mg/kg BW for 10 days with normal diet.
Group-4 (BTF 250+ Paracetamol) - Animals received ethanolic extract of *B. tomentosa* at dose of 250 mg/kg BW and paracetamol 700 mg/kg BW for 10 days.
Group-5 (BTF 500+ Paracetamol) - Animals received ethanolic extract of *B. tomentosa* at dose of 500 mg/kg BW and paracetamol 700 mg/kg BW for 10 days.
The rats in each group were sacrificed at the conclusion of the experiment by being deeply sedated with an injection of ketamine hydrochloride (300 mg/kg BW) and then being dissected. Heparinized syringes (size: 5 mL) were used to draw blood samples (~4 mL) from the inferior vena cava. The liver was then taken out right away and put on an ice bag so that its weight could be determined. The blood samples (about 1 mL) were immediately placed into an EDTA-containing tube for hematological analysis, and they were kept at -20°C. The blood samples were obtained in dry test tubes for biochemical analysis, and they were centrifuged at 2,000 rpm for 10 minutes to separate the serum after they had coagulated for 30 minutes at room temperature.[15] The serum was kept for additional biochemical analysis at -20°C.

Biochemical Parameters Analysis
Serum aspartate transaminase (AST), serum alanine transaminase (ALT), serum alkaline phosphatase (ALP), serum albumin, total bilirubin, and serum total protein were the biochemical parameters used to estimate the extent of liver damage. The corresponding commercial kits were used for this purpose.[16]

Histopathological Examination
The animal liver tissues were removed and immediately fixed in 10% formalin for histopathological analysis. Following that, the samples were dehydrated in ethanol solutions ranging from 50% to 100%, cleared in xylene, and embedded in paraffin. Haematoxylin/Eosin dye was used to prepare sections (4–5 μm) for photo microscopic observation (Olymus DP72).[17]

Acute Toxicity Study
Prior to any experiment, all of the animals were kept in standard laboratory conditions, fasting for the entire night and having unlimited access to water. The animals were split up into four groups, with five animals in each group. First group was used as a negative control; second, third, and fourth were the test groups; they were given oral doses of *Bridelia tomentosa* fruit extract (dissolved in normal water) of 2000 mg/kg, 4000 mg/kg, and 6000 mg/kg BW respectively. For the first four hours following the treatment period, the animals were monitored for any toxic effects. The animals were kept under close observation for three days to check for any toxic effects.[9]

Statistical Analysis of Data
The data is displayed as mean ± SEM. One-way ANOVA was used to analyze this dataset, and Dunnet's multiple comparison was then applied. P values were regarded as statistically significant if they were less 0.05 and 0.01.

Ethical Approval
Research proposal was submitted to Biosafety, Biosecurity and Ethical Committee of Faculty of Biological Sciences of Jahangirnagar

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

University and approved in a meeting accordingly (Ref: BBEC,JU/M 2023/02 (16).

Results and Discussion

Phytochemical Screening

Several phytochemical constituents that are listed in Table 1 were found in the ethanolic extract of *Bridelia tomentosa* fruit after preliminary phytochemical screening. The results revealed the presence of carbohydrates, alkaloids, glycosides, saponins, tannin, and flavonoids and the absence of steroids.

In this investigation, we used ethanolic fruit extract. Table 1 summarizes the preliminary phytochemical screening results, which indicate that the pulp is rich in various phytoconstituents. Although the antioxidant qualities of phenols and flavonoids are well known, they also provide protection against tumors, hepatotoxins, microbes, ulcers, allergies, inflammation, and free radicals.[18, 19] For more than 200 years, glycosides have been used as a cardiac failure stimulant.[20] The presence of various phytochemicals in *Bridelia tomentosa* fruit confirms that this species could be a potent source for modern drugs.

Total Phenolic Content

Using the gallic acid calibration curve (y= 0.012x - 0.038; R^2 = 0.999), it was estimated that the plant extract contains 99.04145 ± 1.195 mg/g GAE total phenolic content (Table 2).

Oxidative stress consequences in the pathogenesis of numerous chronic diseases. Free radicals are a normal byproduct of the body's regular metabolic processes in living systems.[21, 22] Antioxidant qualities of naturally occurring substances in plants have long been known, and medicinal plants are receiving more and more attention. Numerous techniques have been developed to assess the effectiveness of pure compounds or plant extracts as natural antioxidants.[23] The current study's findings clearly imply that phenolics are significant elements of the plant extract under investigation. In the assay for determining phenolic content, the ethanol extract of *Bridelia tomentosa* fruits exhibited the highest potency (Table 2). It has been demonstrated that polyphenols inhibit LDL oxidation, lessen the development of atherosclerotic plaques, and lessen arterial stiffness, making arteries more receptive to endogenous vasodilation stimuli.[24, 25, 26] Also, it has been demonstrated that polyphenols have anticarcinogenic properties by modifying the enzyme systems that metabolize procarcinogens or carcinogens by changing them into less reactive substances prior to their reaction with DNA.[26]

Total flavonoid content

Using the quercetin calibration curve (y = 0.009x + 0.074; R^2 = 0.997), the total flavonoid content was determined and expressed as quercetin equivalents (QE) per gram of plant extract. Ethanolic extract of fruits of *Bridelia tomentosa* was found to contain 126.5046 ± 3.095 mg/g QE of flavonoid content (Table-2).

Plants' antioxidant system is significantly influenced by flavonoids. Flavonoids exhibit anti-oxidative characteristics through multiple mechanisms, including the scavenging of free radicals, chelation of metal ions like copper and iron, and inhibition of enzymes that generate free radicals.[27] Given that *Bridelia tomentosa* leaves have been demonstrated to contain a significant amount of flavonoids, it is reasonable to assume that their ethanolic extract can scavenge almost all known reactive oxygen species (ROS).

Total antioxidant capacity

Using the ascorbic acid calibration curve (y = 0.003x+ 0.086; R^2 = 0.985), the total antioxidant capacity of the test samples was ascertained. The ability of the *Bridelia tomentosa* fruit extract to reduce the phosphate/Mo (VI) complex to phosphate/Mo (V) was used to estimate the extract's overall antioxidant activity. *Bridelia tomentosa* fruit ethanolic extract was found to have 372.094 ± 3.148 mg/g AAE of total antioxidant capacity (Table 2). The present investigation revealed a significant level of total antioxidant capacity in the *Bridelia tomentosa* fruit extract.[28]

DPPH free radical scavenging assay

Table 2 displays the IC_{50} values for the *Bridelia tomentosa* fruit extract, which were 30.579 ± 1.895 µg/ml, in comparison to the reference standard ascorbic acid (16.253 ± 1.448 µg/ml). This study found that when the concentration of ethanolic extract of *Bridelia tomentosa* fruit increased, the percentage of DPPH radical scavenging is also increased (Figure 1).

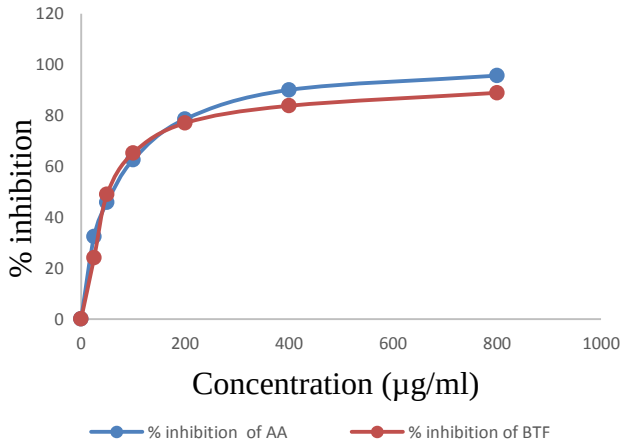


Figure 1: Percent inhibition of DPPH free radical scavenging at different concentration of plant extract and standard

Table 1: Result of phytochemical screening tests of ethanolic extract of *Bridelia tomentosa Fruit*

Phytochemical Groups								
Alkaloid	**Carbohydrate**	**Glycosides**	**Glucoside**	**Flavonoid**	**Saponin**	**Steroid**	**Tannin**	**Terpenoids**
+	+	+	-	+	+	-	+	+

Table 2: *In-vitro* anti-oxidant and cytotoxic activities of crude ethanolic extract of *Bridelia tomentosa* fruits

Extract	Total phenolic content (mg/g GAE) ± SEM	Total flavonoid content (mg/g QE) ± SEM	Total antioxidant capacity (mg/AAE) ± SEM	DPPH free radical scavenging assay (IC_{50}, µg/ml)	NO free radical scavenging assay (IC_{50}, µg/ml)	Cytotoxicity (Brine shrimp lethality assay, LC_{50}, µg/ml)
BTF	99.04145 ± 1.195	126.5046 ± 3.095	372.094 ± 3.148	30.579 ± 1.895	142.344 ± 1.851	537.693 ± 70.099
Ascorbic acid	-	-	-	16.253 ± 1.448	23.159 ± 2.077	-
Vincristine Sulfate	-	-	-	-	-	0.698 ± 0.005

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table-3: Acute toxicity of ethanolic extract of fruits of *Bridelia tomentosa*

Observation	Control group	2000 mg/kg	4000 mg/kg	6000 mg/kg
Body weight	Normal	Not change	Not change	Not change
Temperature	Normal	Normal	Normal	No effect
Food intake	Normal	Normal	Normal	No effect
Urination	Normal	No effect	No effect	No effect
Rate of respiration	Normal	No effect	No effect	No effect
Change in skin	No effect	No effect	No effect	No effect
Drowsiness	Not present	Not present	Not present	Not present
Sedation	No effect	No effect	No effect	No effect
Eye color	No effect	No effect	No effect	No effect
Diarrhea	Not present	Not present	Not present	Not present
General physique	Normal	Normal	Normal	Normal
Coma	Not present	Not present	Not present	Not present
Death	Alive	Alive	Alive	Alive

Table 4: Effects of Paracetamol and *B. tomentosa* fruit extract on ALT, AST, ALP on Normal and Paracetamol treated rats

Enzymes	Normal Control (Mean ± SEM) µ/L	Negative Control (Mean ± SEM) µ/L	Positive Control (Mean ± SEM) µ/L	BTF 1X (Mean ± SEM) µ/L	BTF 2X (Mean ± SEM) µ/L
ALT	32.200 ± 1.497	54.800 ± 5.643^{X}	42.400 ± 1.691^{b}	44.250 ± 3.473	38.250 ± 1.652^{b}
AST	23.200 ± 2.035	49.200 ± 1.934^{X}	28.200 ± 2.728^{a}	45.75 ± 2.016	25.500 ± 2.754^{b}
ALP	156.200 ± 3.426	197.200 ± 10.837^{x}	132.800 ± 6.240^{a}	155.00 ± 10.154	139.250 ± 10.515^{a}

(Note: Values are presented as mean±S.E.M (n=5). One way ANOVA followed by Dunnet's multiple comparison was performed to analyze this dataset. P values: ^{y}< 0.05, ^{x}< 0.01 compared with respective normal control. P values: ^{b}<0.05, ^{a}<0.01 compared with Negative control.)

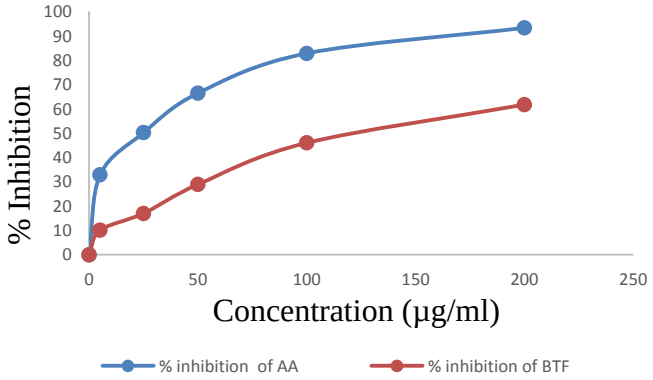


Figure 2: Percent inhibition of NO free radical scavenging at different concentration of plant extract and standard

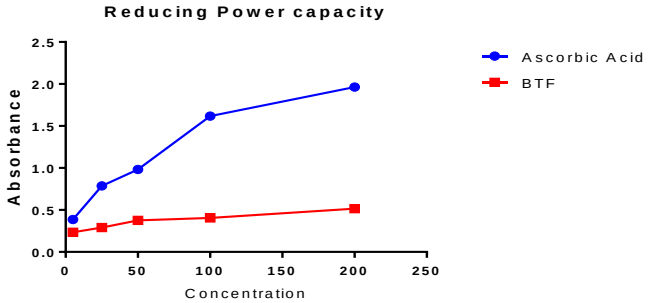


Figure 3: Reducing power capacity assessment of plant extract and standard

The crude extracts of *Bridelia tomentosa* fruits demonstrated dose-dependent DPPH radical scavenging in assays, resembling that of the reference standard ascorbic acid.[29,30, 31] Changes in absorbance can be used to quantify the decolorization of DPPH, which occurs when it accepts an electron given by an antioxidant compound. Thus, fruit extracts from *Bridelia tomentosa* demonstrated the highest potency of electron donation.

Nitric oxide (NO) free radical scavenging assay

Bridelia tomentosa fruit ethanolic extract demonstrated a dose-dependent scavenging of NO comparable to that of the ascorbic acid (Table 2 and Figure 2).

The potential to stop peroxynitrite formation in the cell *in- vivo* is co-related by the scavenging of the NO produced by sodium nitroprusside *in- vitro*.[32] Plant extracts possess the ability to counteract the effects of NO formation and can stop the series of events that result from excessive NO generation.[33] Direct NO scavenging may be partially responsible for the suppression of NO· released; consequently, nitrite produced by the *in -vitro* breakdown of sodium nitroprusside can be scavenged by the plant compounds to help prevent cellular damage.

Reducing power capacity assessment

The reducing power was assessed using the spectrophotometric ferric to ferrous reduction activity, which is obtained from the creation of Perl's Prussian blue color complex.[33] It was found that the extract's reducing power was poor (Figure 3).

Cytotoxicity: Brine Shrimp lethality Assay

There was very poor cytotoxic effect of ethanolic extract of *Bridelia tomentosa* fruits as observed in brine shrimp cytotoxicity compared to standard drug vincristine sulphate (LC_{50} of BTF = 537.693 ± 70.099 µg/ml and vincristine = 0.698 ± 0.005 µg/ml) (Table 2). Brine shrimp lethality assay is a widely used method is performed by identifying the ability of the extract to kill laboratory hatched larvae.[19]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Acute toxicity study

There was no death or abnormal behaviors observed during acute toxicity study even at 6000 mg/kg body weight dose. The result of acute toxicity study is summarized Table 3.

Hepatoprotective effect

Changes in hepatocellular biochemical parameters

When compared to the control group, rats exposed to paracetamol alone showed noticeably higher levels of ALT, AST, and ALP activities. In contrast to the animals co-administered with paracetamol and BTF 1X (an ethanolic extract of *B. tomentosa* fruits at 250 mg/kg BW) and BTF 2X (*B. tomentosa* fruits at 500 mg/kg BW), the hepatic marker enzymes were significantly reduced when compared with only paracetamol group (Table 4 and Figure 4). When comparing the animals from the normal control group with those who received only ethanolic extracts of *B. tomentosa* or silymerin, no discernible variation was seen in the biochemical parameters.

Total Bilirubin

In the case of total bilirubin, administration of paracetamol alone did significant elevation of value where both the treatment groups moderately decreased the values against paracetamol treated rat group (Figure 5).

Effect on serum Albumin, Total protein and Globulin

Data did not show notable elevation for albumin level when treated with groups BTF 1X and BTF 2X which is markedly decreased by the group exposed to paracetamol. However, there was significant effect of BTF 2X on total protein and serum globulin parameter as like as standard drug silymarin compared to paracetamol treated groups (Figure 6)

Histopathological Examination of the livers of rats

Histopathological examination under photo microscope (Figure 7) clearly demonstrated that the damage of liver cells around hepatic portal and central vein of groups treated with paracetamol alone. However, Treatment group BTF 1X and BTF 2X recovered the damage of liver cell comparable to silymarin. This may be possible by scavenging toxic NAPQI molecules during paracetamol toxicity mechanism. So, Polyphenolic compounds in *Bridelia tomentosa* fruit may be used effectively as a natural hepatoprotective agent.

The liver is the primary organ in our body and is essential to life due to its ability to perform metabolism and detoxification. There are many different endogenous and exogenous substances that the liver is constantly exposed to. Consequently, an abundance of metabolic intermediates and end products are generated, which have the potential to harm the liver and are the primary cause of liver disorders.[35] Currently, the management of symptoms is the mainstay of treatment for liver disease or damage, and liver transplantation is done in extreme cases.[36] There are currently no medications used to boost the liver's detoxification capacity, although research on and application of botanical hepato-protective agents is rapidly growing. The hepatoprotective effect of the plant extract against hepatic injury induced by paracetamol was assessed in the current study. The current study reports that the liver functions (ALT, AST, total bilirubin, total protein, serum albumin, and globulin) were significantly improved by the administration of the ethanolic extract of the fruit of *Bridelia tomentosa* at two different doses (250 mg/kg body weight = BTF 1X and 500 mg/kg body weight = BTF 2X). A histopathological analysis of the rat's liver revealed that the fruit extract *Bridelia tomentosa* reversed the effects of the paracetamol.

Conclusion

The study's findings show that the plant extract has weak cytotoxic effects, good antioxidant, and hepatoprotective properties. Its stated actions could be attributed to the presence of various secondary metabolites, which could also provide an excellent source of more recent and hepatoprotective antioxidants. In order to establish this plant as a medicinal plant, more research is advised to isolate bioactive compounds and conduct molecular docking studies to comprehend the underlying mechanism of action of observed activities.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

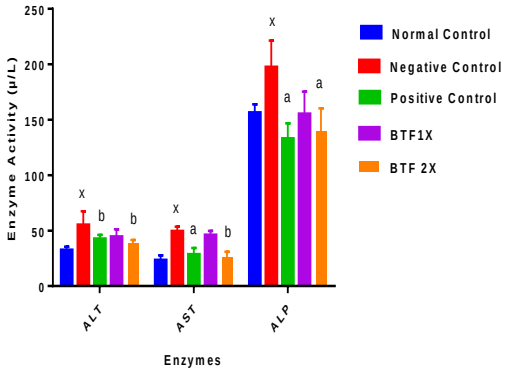


Figure 4: Effects of Paracetamol and BTF on ALT, AST, ALP on Normal and Paracetamol treated rats. One way ANOVA followed by Dunnet's multiple comparison was performed to analyze this dataset. *P* values: ^{y}< 0.05, ^{x}< 0.01 compared with respective normal control. P values: ^{b}<0.05, ^{a}<0.01 compared with Negative control

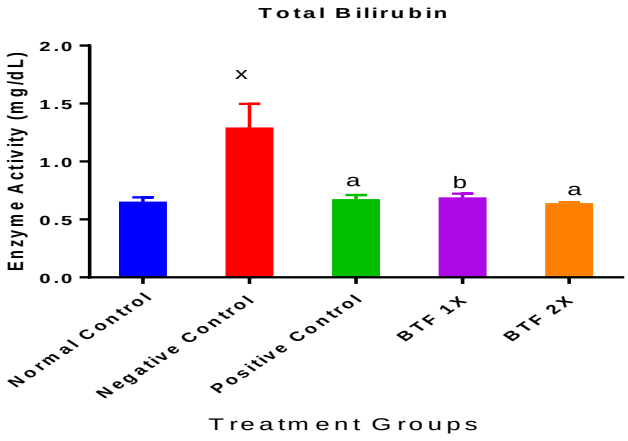


Figure 5: Effects of Paracetamol and BTF on Total Bilirubin on Normal and Paracetamol treated rats. One way ANOVA followed by Dunnet's multiple comparison was performed to analyze this dataset. P values: ^{y}< 0.05, ^{x}< 0.01 compared with respective normal control. P values: ^{b}<0.05, ^{a}<0.01 compared with Negative control

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

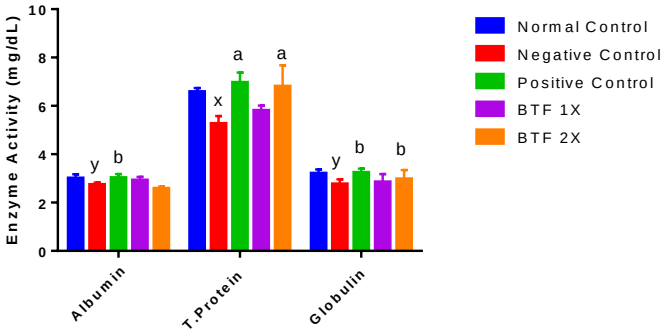
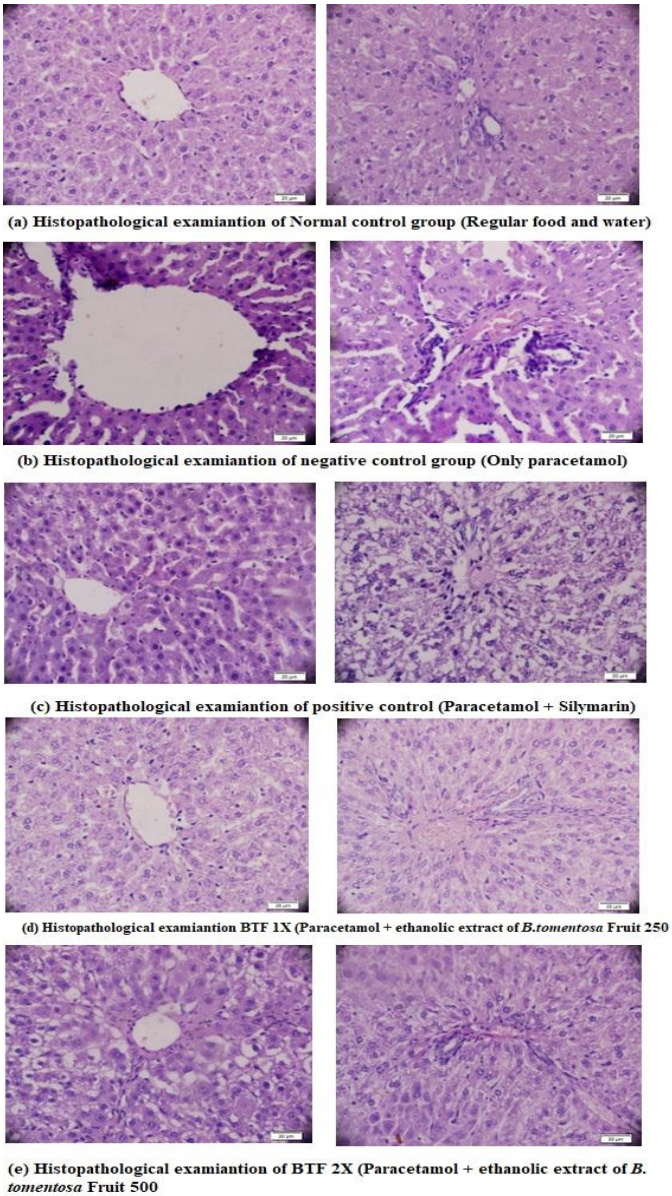


Figure 6: Effects of Paracetamol and BTF on Albumin, Total protein and Globulin on Normal and Paracetamol treated rats. Values are presented as mean ± S.E.M (n=5). One way ANOVA followed by Dunnet's multiple comparison was performed to analyze this dataset. P values: ^{y}< 0.05, ^{x}< 0.01 compared with respective normal control. P values: ^{b}<0.05, ^{a}<0.01 compared with negative control.



(a) Histopathological examiantion of Normal control group (Regular food and water)

(b) Histopathological examiantion of negative control group (Only paracetamol)

(c) Histopathological examiantion of positive control (Paracetamol + Silymarin)

(d) Histopathological examiantion BTF 1X (Paracetamol + ethanolic extract of *B.tomentosa* Fruit 250

(e) Histopathological examiantion of BTF 2X (Paracetamol + ethanolic extract of *B. tomentosa* Fruit 500

Figure 7: (a, b, c, d, e): Liver histopathology of different treatment group

References

1. Jima TT, Megersa M. Ethnobotanical study of medicinal plants used to treat human diseases in Berbere District, Bale Zone of Oromia Regional State, South East Ethiopia. Evid Based Complement Alternat Med. 2018; 2018: 1-17.
2. Egharevba E, Chukwuemeke-Nwani P, Eboh U, Okoye E, Bolanle IO, Oseghale IO, Imieje VO, Erharuyi O, Falodun A. Antioxidant and hypoglycaemic potentials of the leaf extracts of *Stachytarphyta jamaicensis* (Verbenaceae). Trop J Nat Prod Res. 2019; 3(5):170-174
3. Falodun A, Siraj R, Choudhary MI. GC-MS insecticidal leaf essential oil of *P. staudtii* Hutch and Dalz (Icacinaceae). Trop J. Pharm Res. 2009; 82:139-143.
4. Rahman MH, Roy B, Chowdhury GM, Hasan A. Medicinal plant sources and traditional healthcare practices of forest-dependent communities in and around Chunati Wildlife Sanctuary in Southeastern Bangladesh. Environ Sustainability. 2022; 5: 207-241.
5. Anjum A, Kuddus MR, Sultan MZ, Mansur AA, Hasan CM, Rashid MA. Phytochemical and biological investigation of *Bridelia tomentosa* Blume growing in Bangladesh. Dhaka Univ. J. Pharm. Sci. 2021; 20(2): 213-218.
6. Krishnan KS. The useful plants of India, Reprinted, Publications and Information Directorate, CSIR, New Delhi, India, 1992. pp86-87.
7. Anjum A, Sultan MZ, Sikder MAA, Hasan CM, Al-Mansoor MA, Rashid MA. Bioactive steroid and triterpenoids from *Bridelia stipularis* (L) Blume. Dhaka Univ. J. Pharm. Sci. 2016; 15(2): 221-225.
8. Redfern J, Kinninmonth M, Burdass, D, Verran J. Using soxhlet ethanol extraction to produce and test plant material (Essential Oils) for their antimicrobial properties. J. Microbiol. Biol. Educ. 2014; 15(1): 45-46.
9. Akanda MKM, Hasan AN. Characterization of pharmacological properties of methanolic seed and stem bark extracts of *Ziziphus mauritiana* (BAU Kul) using *in-vitro* and *in-vivo* animal (*Swiss albino* male mice) model. Clin. Phytoscience. 2021; 7(8): 1-13.
10. Thao TTP, Tu PTC, Men TT. A comparative study on polyphenol, flavonoid content, antioxidant and anti-inflammatory capacity of different solvent extract from *Portulaca oleracea* in Carrageenan-induced paw edema in mice. Trop J Nat Prod Res. 2023; 7(10):4152-4159.
11. Trerattanathawan S., Katisart T., Konsue, A. In- vitro antioxidation, α-glucosidase, and α-amylase inhibitory activities of different solvent extracts of Thai traditional diabetic medicine. Trop J Nat Prod Res. 2023; *7*(10): 4146–4151.
12. Prieto P, Pineda M, Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999; 269: 337-341.
13. Okolie NP, Falodun A, Oluseyi D. Evaluation of the antioxidant activity of root extract of pepper fruit (*Dennetia tripetala*), and its potential for the inhibition of lipid peroxidation. Afr J. Trad Compl and Altern Med. 2014; 11(3):221-227.
14. Mohan PK, Adarsh Krishna TP, Senthil Kumar T, Ranjitha Kumari BD. Pharmaco-chemical profiling of *Desmodium gangeticum* (L.) DC. with special reference to soil chemistry. Futur J Pharm Sci. 2021;7(1):210.
15. Kiran PM, Raju AV, Rao BG. Investigation of hepatoprotective activity of *Cyathea gigantea* (Wall. ex. Hook.) leaves against paracetamol-induced hepatotoxicity in rats. Asian Pac. J. Trop. Biomed. 2012; 2(5): 352-356.

16. Boro H., Usha T., Babu D. *et al*. Hepatoprotective activity of the ethanolic extract of *Morus indica* roots from Indian Bodo tribes. SN Appl. Sci. 2022; 4(49): 1-14
17. Middha SK, Usha T, Babu D, Misra AK, Lokesh P, Goyal AK. Evaluation of antioxidative analgesic and anti-inflammatory activities of methanolic extract of *Myrica nagi* leaves-an animal model approach. Symbiosis. 2016; 70(1–3):179–184
18. Barakat MZ., Shahab SK., Darwin N., Zahemy EI. Determination of ascorbic acid from plants. Annals of Biochemtry,1993; 53:225-245.
Mahfuz A, Salam FBA, Deepa FBA, Hasan AN. Characterization of in-vitro antioxidant, cytotoxic, thrombolytic and membrane stabilizing potential of different extracts of *Cheilanthes tenuifolia* and Stigmasterol isolation from n-hexane extract. Clin. Phytoscience. 2019; 5(39): 1-10.
20. Olayinka AO., Onoruvwe O., Lot TY. Cardiovascular effects in rodents of the methanolic extract of the stem bark of *Khaya senegalensis* A. Juss. Phytotherapy Res. 1992; *6*(5): 282-284.
21. Park CH, Yeo HJ, Baskar TB, et al. In Vitro Antioxidant and Antimicrobial Properties of Flower, Leaf, and Stem Extracts of Korean Mint. Antioxidants (Basel). 2019;8(3):75.
22. Mahlo SM, Chauke HR, McGaw L, Eloff J. Antioxidant and antifungal activity of Selected medicinal plant extracts against phytopathogenic fungi. Afr J Tradit Complement Altern Med. 2016;13(4):216-222.
23. Aranda RS, Pérez-López LA, López-Arroyo J, Alanís-Garza BA, de Torres NW. Antimicrobial and antioxidant activities of plants from Northeast of Mexico. Evid Based Complement Alternat Med**.** 2011; 2011: 1-6.
24. Moline J., Bukharovich IF., Wolff MS., Phillips R. Dietary flavonoids and hypertension: is there a link. Med. Hypotheses, 2000; *55*(4): 306-309.
25. Arai Y., Watanabe S., Kimira M., Shimoi K., Mochizuki R., Kinae N. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. J. Nutr. 2000; 130(9): 2243-2250.
26. Duthie GG, Duthie SJ, Kyle JA. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutr. Res. Rev. 2000; 13(1): 79-106.
Stoner GD and Mukhtar H. Polyphenols as cancer chemopreventive agents. J. Cell. Biochem. 1995; *59*(S22):169-180
28. Benavente-García O, Castillo J, Marin FR, Ortuño A, Del Río JA. Uses and properties of *Citrus* flavonoids. J. Agric. Food Chem.1997; 45(12): 4505-4515.
29. Oktay M., Gülçin İ., Küfrevioğlu Öİ. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT-Food Sci. Technol. 2003; *36*(2): 263-271.
30. Mazumder M.E.H., Rahman S. Pharmacological evaluation of Bangladeshi medicinal plants for antioxidant activity. Pharm. Biol. 2008; 46(10-11):704-709.
31. Kaseke T, Opara UL, Fawole OA. Effect of blanching pomegranate seeds on physicochemical attributes, bioactive compounds and antioxidant activity of extracted oil. Molecules. 2020; 25(11): 2554.
32. Mfotie Njoya E, Munvera AM, Mkounga P, Nkengfack AE, McGaw LJ. Phytochemical analysis with free radical scavenging, nitric oxide inhibition and antiproliferative activity of *Sarcocephalus pobeguinii* extracts. BMC Complement Altern Med. 2017;17(1):199.
33. Kumaran A., Karunakaran RJ. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. Food Chem. 2006; 97(1): 109-114.
34. Yildirim A., Mavi A., Oktay M., Kara AA. Comparison of antioxidant and antimicrobial activities of *Tilia*. J. Agric. Food Chem, 2000; 48: 5030-5034.
35. Meharie BG, Amare GG, Belayneh YM. Evaluation of hepatoprotective activity of the crude extract and solvent fractions of *Clutia abyssinica* (*Euphorbiaceae*) leaf against CCl_4-induced hepatotoxicity in mice.J. Exp.Pharmacol. 2020; 12: 137-150.
36. Lampertico P, Agarwal K, Berg T EASL. Clinical practice guidelines on the management of hepatitis B virus infection. J. Hepatol. 2017; 67(2):370–398.