Research Article

Assessment of Toxicity and Beneficiary Effects of *Garcinia pedunculata* on the Hematological, Biochemical, and Histological Homeostasis in Rats

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This study was undertaken to investigate the toxicological profile of a methanolic extract of *Garcinia pedunculata* fruit in rats by conducting hematological, biochemical, and histopathological examinations. Long Evans rats were divided into four groups, each with 6 animals, and were treated with three oral doses (250, 500, and 1000 mg/kg) once daily for 21 days. The extract did not cause significant changes in body and relative organ weight, percent water content, or hematological parameters at any administered doses. However, a significant dose-dependent positive effect in serum lipid profile and all atherogenic indices including the cardiac risk ratio, Castelli's risk index-2, and the atherogenic coefficient were observed. Significant increases in the levels of iron and decreases in serum alkaline phosphatase, alanine transaminase, and lactate dehydrogenase activities and the levels of serum glucose were noted when the extract was administered at the highest dose (1000 mg/kg). Histopathological examination of the target tissues further confirmed that the extract was safe and had no observed toxicological features. Our study indicates that *G. pedunculata* fruit is nontoxic, has the potential to be effective against atherosclerosis, and may be used as a hepatoprotectant. The fruit extract is also beneficial to those with iron deficiency and hyperglycemia.

1. Introduction

Garcinia pedunculata (G. pedunculata) is a medicinal indigenous plant belonging to the Clusiaceae family. The fruit of *G. pedunculata* is commonly known as "Taikor" in Bangladesh and "Amlavetasa" in India [1]. The mature *G. pedunculata* fruit is greenish yellow and is consumed as a vegetable; it is also used as an ingredient in cooking different types of meat and chicken [2]. This fruit extract is reported to possess a variety of pharmacological benefits including antioxidant [3, 4], antimicrobial [5], anti-inflammatory [6], hepatoprotective [1], and cardioprotective properties [7].

 G. pedunculata has been traditionally used for cooking and as an antiscorbutic, an astringent, a cardiotonic, and an emollient. It is also utilized in the treatment of chronic catarrh, asthma, cough, bronchitis, dysentery, digestion, and fever [8]. However, the specific chemical compounds responsible for these beneficial effects have not yet been identified. Recently, the phytochemical composition of *G. pedunculata* was investigated and *G. pedunculata* was estimated to contain moisture 88.20%, protein 0.50%, β carotene 45.00 mg/100 g, vitamins (thiamine 0.03, riboflavin 0.02, and ascorbic acid 142.83 mg/100 g, resp.), minerals (sodium 1.80, potassium 106.00, calcium 18.00, magnesium 23.00, iron 0.08, zinc 0.15, copper 0.12, and phosphorus 17.00 mg/100 g), phenolics (19.45 mg gallic acid/100 g), and flavonoids (18.33 mg rutin/g) [2]. The dried fruit rinds and pericarp of *G. pedunculata* have been reported to contain some benzophenones, pedenculol,

hydroxy citric acid, garcinol, and cambogin [9], some of which are strong antioxidants. Previous research suggested that benzophenones and garcinol showed hepatocyte protective and anti-inflammatory effects, respectively [10, 11]. Hence, it is presumed that these chemical compounds and other phytochemicals, as well as polyphenols, present in *G. pedunculata* may contribute to the beneficial pharmacological effects. Despite its diverse uses, there are no studies on the safety and toxicology profile of the fruit. Therefore, the aim of this study is to assess the possible toxic effects of a methanolic extract of *G. pedunculata* in a rat model using biochemical, hematological, and histopathological parameters.

2. Materials and Methods

2.1. Chemicals and Reagents. Methanol was purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA). All chemicals and reagents used in this experiment were of analytical grade.

2.2. Fruit Sample Collection. Mature and fresh *G. pedunculata* fruits were randomly collected from the Sylhet district of Bangladesh in March, 2015. The fruit was authenticated by Professor Nuhu Alam from the Department of Botany, Jahangirnagar University, where a voucher specimen was deposited. Following collection, the fruits were cleaned with sterile water in a laminar blower to remove any possible contamination and were subsequently stored in the refrigerator (4°C) for a day before further processing.

2.3. Extract Preparation. The methanol extract of *G. pedunculata* was prepared according to a method as described by Uddin et al. [12] with slight modifications. Briefly, the cleaned fresh matured fruits were thoroughly rinsed with cold sterile water. The edible portion was carefully removed. Then, the fruits were cut into small pieces using a stainless steel blade and were dried under sunlight. When the samples were free of moisture and had a crunchy appearance, they were separately crushed into fine powders using a blender (Jaipan Commando, Mumbai, India). Crushed samples (100 g) were mixed with a sufficient amount of pure methanol (400 mL) and were kept in a shaker (IKA400i, Germany) at 150 rpm and 30°C for 72 h. The extracts were filtered using Whatman No. 1 filter paper, and the solvent was removed using a rotary evaporator (R-215, BUCHI, Switzerland) under a reduced pressure (100 psi) at a controlled temperature (40°C) to recover the soluble components of the samples. Then, the concentrated (33.51%) extract (mass of extract × 100/mass of powder) was preserved at −20°C for later use.

2.4. Experimental Animals. Long Evans rats at the ages of 16–18 weeks (150–250 g) of both sexes were obtained from the Department of Pharmacology, Bangladesh University of Health Sciences (BUHS), Dhaka, Bangladesh. The animals were maintained in an animal housing facility in the Department of Biochemistry and Molecular Biology, Jahangirnagar University, at a constant temperature of 22 ± 3°C and with humidity ranging from 44% to 56%. The rats were housed in clean plastic cages and received a natural 12 h day-night cycle.

Animals were provided with the standard rodent pellet diet and water ad libitum. All of the studies were conducted in accordance with the internationally established principles of the US guidelines (NIH publication #85-23, revised in 1985). The experimental protocol was approved by the Biosafety, Biosecurity & Ethical Committee of Jahangirnagar University.

2.5. Experimental Design. The rats were acclimatized one week prior to the experiment and were randomly divided into four groups of six animals; each group contained an equal number of each sex.

Group A (Normal Control). Animals received normal diets and drinking water ad libitum.

Group B (Treatment 1). Animals received *G. pedunculata* extract (250 mg/kg) dissolved in saline water by oral gavaging for 21 days. (All animals received standard laboratory diet and drinking water ad libitum.)

Group C (Treatment 2). Animals received *G. pedunculata* extract (500 mg/kg) dissolved in saline water by oral gavaging for 21 days. (All animals received standard laboratory diet and drinking water ad libitum.)

Group D (Treatment 3). Animals received *G. pedunculata* extract (1000 mg/kg) dissolved in saline water by oral gavaging for 21 days. (All animals received standard laboratory diet and drinking water ad libitum.)

During the experimental study, the body weights of the rats were recorded weekly. The animals were also monitored for behavioral changes in their feeding and drinking habits to detect any sign of abnormalities. At the end of the experimental period, all animals were fasted for 15 h. Then, all of the rats were anaesthetized by intraperitoneal administration of a ketamine hydrochloride injection (500 mg/kg) [13]. Blood samples (5 mL) were collected from the inferior vena cava and were divided into two portions. The first was transferred into tubes containing ethylene diamine tetra acetic acid (EDTA) for hematological analysis, and the remaining blood was placed into plain tubes for serum separation for subsequent biochemical analysis. Selected organs were immediately removed, weighed, and stored in tubes containing formalin (10%) for histopathological examination.

2.6. Measurements of Body Weight, Relative Organ Weight Profile, and Percentage of Water Content. Immediately after sacrifice, the following tissues were excised and weighed: heart, kidney, lung, liver, spleen, caecum, pancreas, brain, testes, thymus, caput, Cowper's gland, stomach, ovary, and Fallopian tubes. The relative organ weight was calculated by dividing the individual weight of each organ with the final body weight of each rat according to following formula [14]: relative organ weight (%) = wet organ weight/body weight × 100.

The percentage of water content was estimated from the respective weight of each wet organ by subtracting the dry weight of each organ [15].

2.7. Hematological Parameters. Hematological parameters such as white blood cells (WBCs), neutrophils, lymphocytes, monocytes, eosinophils, red blood cells (RBCs), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), red cell distribution width-standard deviation (RDW-SD), red cell distribution width-coefficient of variation (RDW-CV), platelets distribution width (PDW), mean platelets volume (MPV), platelets larger cell ratio (P-LCR), and procalcitonin (PCT) were analyzed using an automated hematology analyzer (8000i, Sysmex, Japan) for the control and treatment groups.

2.8. Biochemical Parameters. The collected sera were used for analysis of biochemical parameters [sodium (Na^+), potassium (K^+), chloride (Cl^-), magnesium (Mg^{2+}), phosphate (PO^{3+}) ions, calcium (Ca^{2+}), total protein (TP), albumin (ALB), albumin/globulin (A/G) ratio, high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglyceride (TG), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyltransferase (GGT), creatinine (Crea), urea, uric acid (UA), iron (Fe), total iron binding capacity (TIBC), glucose (GLU), amylase, and lipase]. The measurements were determined following standard procedures using an automated chemistry analyzer (Dimension EXL with LM Integrated Chemistry System, Siemens Medical Solutions Inc., USA). On the other hand, low-density lipoprotein cholesterol (LDL-C) levels in the serum were estimated based on the following Friedewald formula [16]:

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{\text{TG}}{5}. \tag{1}$$

Atherogenic indices such as cardiac risk ratio (CRR), atherogenic coefficient (AC), atherogenic index of plasma (AIP), and Castelli's risk index-2 (CRI-2) were calculated using the following formulas:

$$\text{CRR} = \frac{\text{TC}}{\text{HDL-C}}, \tag{2}$$

$$\text{AC} = \frac{(\text{TC} - \text{HDL-C})}{\text{HDL-C}}, \tag{3}$$

$$\text{AIP} = \log\left(\frac{\text{TG}}{\text{HDL-C}}\right), \tag{4}$$

$$\text{CRI-2} = \frac{\text{LDL-C}}{\text{HDL-C}}. \tag{5}$$

See [17–20] for equations (2), (3), (4), and (5), respectively.

2.9. Histopathological Examination. For histopathological examination, the liver, kidney, lung, intestine, brain, stomach,
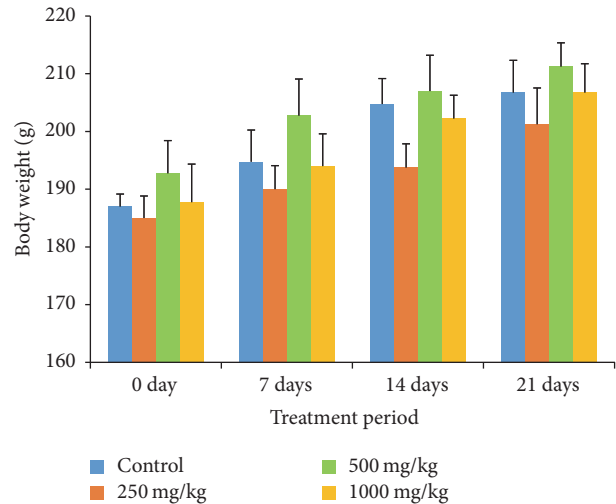

FIGURE 1: Effects of *G. pedunculata* fruit extract on body weight (g) gain. Results are expressed as the mean ± SD ($n = 6$).

pancreas, and heart were fixed in 10% neutral formalin. The fixed organs tissues were then embedded in paraffin. The paraffinized tissue specimens were cut into $5\,\mu$m thick slices and were stained with hematoxylin and eosin (H&E) for histopathological observation using a previously established procedure [21]. Photomicrographs were captured using a normal spectra fluorescent microscope (Olympus DP 72) at 6100x magnification with an attached digital camera (Olympus, Tokyo, Japan).

2.10. Statistical Analysis. All results were presented as the mean values ± standard deviation (SD). Data were analyzed using SPSS (Statistical Packages for Social Science, version 16.0, IBM Corporation, New York, USA) and Microsoft Excel 2007 (Redmond, Washington, USA). All of the data of treatment groups were compared to the control group using one-way ANOVA followed by Dunnett's multiple comparison tests. A p value of <0.05 was considered as statistically significant.

3. Results

3.1. Effects of G. pedunculata Fruit Extract on Body Weight, Relative Organ Weight, and Percent Water Content of Rat's Organs. No significant change in body weight gain was observed among the control and treatment groups. During the experimental period, the body weight gradually increased, but the change was not significantly different from the first to the third week (Figure 1). There was also no significant difference between the control and treatment groups for both the relative organ weight and the percentage of the water content of the organs (Tables 1 and 2).

3.2. Effects of G. pedunculata Fruit Extract on Hematological Parameters. There was no significant difference among the control and treatments groups in terms of the hematological parameters (Table 3).

TABLE 1: Effects of *G. pedunculata* fruit extract on the relative organ weight.

Organ	Control	*G. pedunculata* treatment group		
		250 mg/kg	500 mg/kg	1000 mg/kg
Heart	0.280 ± 0.01	0.283 ± 0.04	0.299 ± 0.02	0.261 ± 0.01
Kidney	0.589 ± 0.03	0.617 ± 0.14	0.663 ± 0.07	0.623 ± 0.03
Lung	0.548 ± 0.08	0.527 ± 0.11	0.596 ± 0.10	0.547 ± 0.02
Liver	3.060 ± 0.29	3.110 ± 0.54	3.080 ± 0.24	3.073 ± 0.28
Spleen	0.352 ± 0.05	0.344 ± 0.03	0.355 ± 0.05	0.353 ± 0.03
Caecum	0.352 ± 0.05	0.367 ± 0.03	0.357 ± 0.05	0.366 ± 0.06
Pancreas	0.165 ± 0.03	0.174 ± 0.03	0.140 ± 0.05	0.189 ± 0.01
Brain	0.811 ± 0.08	0.780 ± 0.01	0.791 ± 0.04	0.787 ± 0.08
Testes	0.072 ± 0.01	0.076 ± 0.01	0.079 ± 0.01	0.073 ± 0.01
Thymus	0.183 ± 0.01	0.204 ± 0.01	0.178 ± 0.02	0.133 ± 0.05
Caput	0.356 ± 0.09	0.371 ± 0.20	0.357 ± 0.12	0.344 ± 0.12
CPG	0.131 ± 0.01	0.137 ± 0.01	0.136 ± 0.01	0.137 ± 0.03
Stomach	0.620 ± 0.13	0.642 ± 0.04	0.625 ± 0.08	0.622 ± 0.09
Ovary	0.065 ± 0.02	0.070 ± 0.01	0.084 ± 0.00	0.063 ± 0.01
FT	0.263 ± 0.00	0.257 ± 0.05	0.252 ± 0.01	0.262 ± 0.03

Results are expressed as the mean ± SD ($n = 6$).
Here, CPG: Cowper's gland; FT: Fallopian tubes.

TABLE 2: Effects of *G. pedunculata* fruit extract on the percentage of water content of different organs.

Organ	Control	*G. pedunculata* treatment group		
		250 mg/kg	500 mg/kg	1000 mg/kg
Heart	75.52 ± 1.55	77.76 ± 4.03	77.71 ± 2.00	75.86 ± 0.42
Kidney	71.82 ± 8.85	74.83 ± 2.03	70.73 ± 4.92	73.07 ± 2.35
Lung	76.33 ± 4.24	76.13 ± 2.96	77.51 ± 1.03	76.76 ± 1.45
Liver	65.38 ± 3.39	67.69 ± 3.29	65.18 ± 4.31	68.56 ± 1.61
Spleen	75.60 ± 1.74	75.10 ± 0.67	75.06 ± 0.71	74.51 ± 1.82
Caecum	72.34 ± 3.48	68.45 ± 2.52	72.69 ± 2.38	73.56 ± 4.29
Pancreas	66.60 ± 5.56	64.36 ± 1.91	69.48 ± 6.94	69.51 ± 5.67
Brain	55.52 ± 2.05	55.44 ± 3.47	59.44 ± 4.14	59.66 ± 5.63
Testes	78.58 ± 5.18	79.38 ± 4.69	77.14 ± 5.13	73.48 ± 9.90
Thymus	71.75 ± 2.17	72.53 ± 2.19	72.74 ± 2.97	74.54 ± 5.33
Caput	73.20 ± 3.39	73.77 ± 3.10	74.84 ± 4.08	74.74 ± 3.96
CPG	74.82 ± 4.04	77.13 ± 5.31	74.74 ± 3.50	72.07 ± 5.91
Stomach	75.33 ± 0.96	77.42 ± 2.50	75.83 ± 1.15	75.13 ± 0.26
Ovary	58.96 ± 3.34	56.69 ± 4.64	61.00 ± 8.48	62.21 ± 2.02
FT	78.74 ± 0.03	74.10 ± 11.96	77.71 ± 6.68	75.51 ± 4.51

Results are expressed as the mean ± SD ($n = 6$).

3.3. Effects of G. pedunculata Fruit Extract on the Serum Biochemical Parameters. The effects of oral *G. pedunculata* on liver function are presented in Figure 2 and Table 4. The fruit extract exerted no adverse effect on the parameters of liver function, such as ALT, AST, ALP, GGT, and LDH activities and TB levels. Moreover, ALT, ALP, and GGT levels were significantly decreased when the highest dose (1000 mg/kg) was administered. Nevertheless, TP, ALB, GLB, and A/G, which are important biomarkers for liver function, remained unchanged at the three dose levels (Table 4).

The effects of *G. pedunculata* on renal function were investigated by measuring the serum urea, uric acid, and creatinine levels and the major electrolytes such as Na^+, K^+, Cl^-, Mg^{2+}, PO^{3+}, and Ca^{2+} (Table 5). The methanolic extract of *G. pedunculata* did not significantly affect the renal function biomarkers or alter the body homeostasis markers (electrolytes) when administered at all doses.

Lipid profiles (TC, TG, HDL-C, and LDL-C) and atherogenic indices (CRR, AC, AIP, and CRI-2) are reliable markers for cardiovascular diseases. The methanolic extract of *G. pedunculata* significantly reduced the serum TG and LDL-C levels (Figure 3) and all atherogenic indices (Table 6) when administered at the three different doses. Rats given the 1000 mg/kg dose had significantly reduced TC levels and

TABLE 3: Effects of *G. pedunculata* fruit extract on the hematological parameters of whole blood.

Hematological parameters	Control	*G. pedunculata* treatment group		
		250 mg/kg	500 mg/kg	1000 mg/kg
WBC ($\times 10^9$/L)	5.32 ± 0.70	5.67 ± 1.16	5.71 ± 0.73	5.76 ± 1.70
Neutrophils (%)	34.55 ± 1.56	34.65 ± 6.77	36.87 ± 20.92	34.07 ± 9.92
Lymphocytes (%)	60.90 ± 5.63	61.05 ± 4.70	61.20 ± 21.12	61.8 ± 12.14
Monocytes (%)	1.27 ± 0.17	1.15 ± 0.12	1.15 ± 0.12	1.35 ± 0.31
Eosinophils (%)	1.17 ± 0.53	1.22 ± 0.58	1.05 ± 0.49	0.90 ± 0.21
RBC ($\times 10^{12}$/L)	7.43 ± 0.69	7.83 ± 0.53	7.38 ± 0.90	7.48 ± 1.39
HGB (g/dL)	14.00 ± 2.32	15.10 ± 0.76	14.00 ± 1.57	14.07 ± 2.43
HCT (%)	41.65 ± 3.07	41.55 ± 1.98	40.85 ± 3.58	40.92 ± 0.83
MCV (fL)	52.72 ± 1.51	52.82 ± 2.59	52.00 ± 1.80	52.83 ± 0.89
MCH (pg)	18.92 ± 0.37	19.12 ± 0.38	19.25 ± 0.42	19.12 ± 0.45
MCHC (g/dL)	36.3 ± 0.63	36.92 ± 1.47	36.92 ± 0.38	36.20 ± 0.43
PLT ($\times 10^9$/L)	877 ± 51.26	827 ± 53.67	875.75 ± 52.88	889.25 ± 69.19
RDW-SD (fL)	32.92 ± 3.03	32.60 ± 1.73	31.4 ± 1.34	32.37 ± 2.26
RDW-CV (%)	20.32 ± 2.88	20.65 ± 1.11	20.31 ± 1.42	20.52 ± 2.00
PDW (fL)	10.42 ± 0.32	10.07 ± 0.83	9.52 ± 0.35	9.90 ± 0.74
MPV (fL)	9.30 ± 0.18	9.05 ± 0.44	8.77 ± 0.26	8.95 ± 0.36
P-LCR (%)	19.62 ± 1.38	17.92 ± 3.83	15.37 ± 2.42	16.57 ± 2.71
PCT (%)	0.75 ± 0.12	0.74 ± 0.07	0.72 ± 0.18	0.76 ± 0.04

Results are expressed as the mean ± SD ($n = 6$).
Here, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, PLT: platelets, RDW-SD: red cell distribution width-standard deviation, RDW-CV: red cell distribution width-coefficient of variation, PDW-CV: platelets distribution width, MPV: mean platelets volume, P-LCR: platelets larger cell ratio, and PCT: procalcitonin.
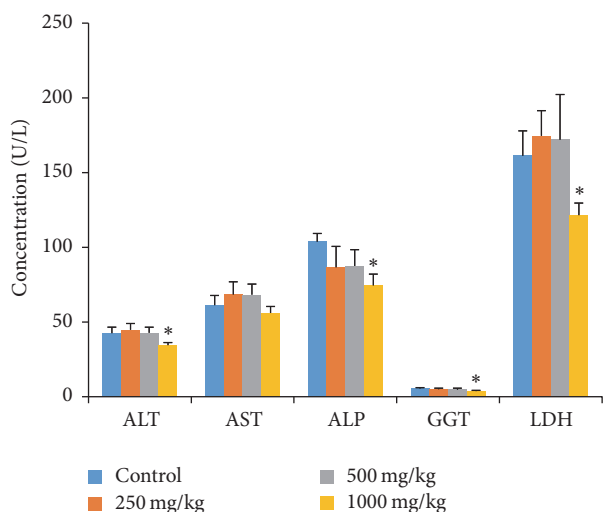

FIGURE 2: Effects of *G. pedunculata* fruit extract on the serum hepatic marker enzymes. Results are expressed as the mean ± SD ($n = 6$). ∗ denotes a significant difference when compared to the control group.

increased HDL-C levels when compared to those of the other two treatment groups. However, there was no significant difference in the HDL-C levels observed for the other two treatment groups.

The methanolic extract of *G. pedunculata* fruit had a positive effect on the iron profile, as treatment with the 1000 mg/kg dose increased these levels significantly. The other two treatment groups (250 mg/kg and 500 mg/kg) showed nonsignificant increases in iron levels. In addition, all three doses nonsignificantly decreased the TIBC level (Figure 4).

The fruit extract affected neither the pancreatic function (Table 7) nor the blood glucose level (Figure 5). The extract also did not significantly change the serum amylase and lipase levels. The extract had a positive effect on the glycemic status, as rats administered with the highest dose (1000 mg/kg) had significantly reduced blood glucose levels.

3.4. Histopathological Examination. The histopathological investigation of the liver, kidney, lung, intestine, brain, stomach, pancreas, and heart did not show any remarkable morphological and pathological changes following the administration of *G. pedunculata* methanolic extract at the three doses for 21 days when compared with the control (Figure 6).

4. Discussion

To our knowledge, this study is the first to show that *G. pedunculata* extract has the potential to cause significant increases in the levels of iron and high-density lipoprotein cholesterol while decreasing the serum activities of alkaline phosphatase, alanine transaminase, lactate dehydrogenase, and the serum glucose level when administered at a high dose (1000 mg/kg). Because a gradual increase in the body weight

Table 4: Effects of *G. pedunculata* fruit extract on the serum total bilirubin (TB), total protein (TP), and albumin (ALB) levels and the albumin/globulin (A/G) ratio.

Biochemical parameters	Control	*G. pedunculata* treatment group		
		250 mg/kg	500 mg/kg	1000 mg/kg
TB (mg/dL)	0.15 ± 0.05	0.14 ± 0.05	0.15 ± 0.05	0.13 ± 0.04
TP (g/L)	56.00 ± 4.83	54.8 ± 4.20	53.50 ± 1.29	54.5 ± 1.73
ALB (g/L)	34.50 ± 2.64	33.00 ± 3.16	32.75 ± 2.21	31.75 ± 1.70
A/G	1.61 ± 0.10	1.51 ± 0.10	1.58 ± 0.21	1.41 ± 0.24

Results are expressed as the mean ± SD ($n = 6$).

Table 5: Effects of *G. pedunculata* fruit extract on the serum renal markers.

Biochemical parameters	Control	*G. pedunculata* treatment group		
		250 mg/kg	500 mg/kg	1000 mg/kg
Urea (mmol/L)	5.45 ± 0.54	5.32 ± 0.55	6.05 ± 0.12	4.93 ± 0.70
UA (mmol/L)	45.50 ± 4.79	48.20 ± 2.58	44.50 ± 6.75	45.00 ± 3.55
Crea (mmol/L)	39.75 ± 3.09	37.20 ± 4.81	42.00 ± 1.82	42.25 ± 1.25
Na^+ (mmol/L)	141.00 ± 2.58	139.60 ± 6.18	139.50 ± 3.10	142.00 ± 4.39
K^+ (mmol/L)	4.12 ± 0.15	4.12 ± 0.50	4.10 ± 0.33	4.02 ± 0.17
Cl^- (mmol/L)	101.50 ± 2.64	100.8 ± 3.76	100.75 ± 1.70	99.75 ± 1.70
PO^{3+} (mmol/L)	2.30 ± 0.29	2.34 ± 0.26	2.25 ± 0.35	2.17 ± 0.23
Ca^{2+} (mmol/L)	2.12 ± 0.22	2.14 ± 0.27	2.30 ± 0.14	2.27 ± 0.05
Mg^{2+} (mmol/L)	0.82 ± 0.12	0.92 ± 0.13	0.80 ± 0.08	0.85 ± 0.05

Results are expressed as the mean ± SD ($n = 6$).
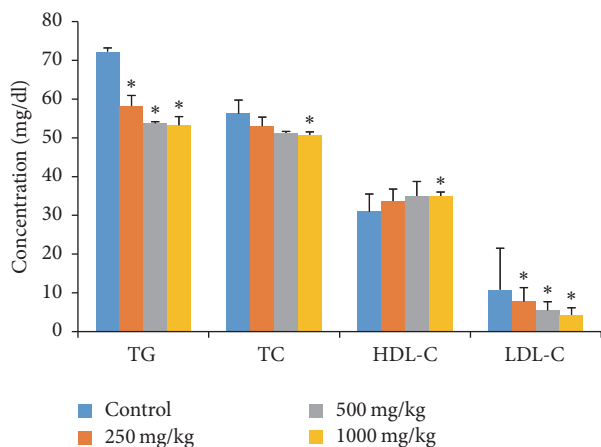

Figure 3: Effects of *G. pedunculata* fruit extract on lipid profiles. Results are expressed as the mean ± SD ($n = 6$). ∗ denotes a significant difference when compared to the control group as determined using one-way ANOVA followed by Dunnett's multiple comparison tests.

was observed in both the treatment and the control groups, it is also concluded that the extract has no negative effects on the normal growth of rats.

Toxicological agents or substances cause abnormal metabolic reactions in the body that may affect major organs such as the liver, heart, lung, spleen, brain, kidney, and reproductive organs including the testes, caput, and Cowper's gland in males and the ovary and Fallopian tubes in females [22]. Therefore, the body weight, relative organ weight profile, and percentage water content are important parameters for the pathological and physiological status of both human and animals. Relative organ weight profiles are essential for interpreting the effect of a drug and reduce the variability of the data and increase the chances of detecting treatment effects. Loss of organ weight is a common parameter in the assessment of a drug's toxicity [23, 24]. In the present study, no significant changes in relative organ weight were observed between the three treatment groups and the control group, indicating that the methanolic extract of *G. pedunculata* did not have any toxic effects on the rats.

The percentage of water content is another important factor in assessing the toxic effects of a drug in animals. In this study, we also found no significant changes in the percent water content in the different organs of rats after *G. pedunculata* administration, indicating that the fruit has no toxic effect on normal body homeostasis.

Analysis of the hematological parameters is very important in assessing the toxic effects of any foreign compound or plant extract, as well as in determining the physiological and pathological status of the body [25]. Various hematological tests were performed in this study for the diagnosis and monitoring of various diseases and conditions, including anemia, leukemia, reactions to inflammation and infections, hydration and dehydration states, and polycythemia, as well as hemolysis. Our study indicated that the administration of *G. pedunculata* fruit causes no abnormal changes in any of

TABLE 6: Effects of *G. pedunculata* fruit extract on the atherogenic indices.

Biochemical parameters	Control	*G. pedunculata* treatment group		
		250 mg/kg	500 mg/kg	1000 mg/kg
CRR	1.81 ± 0.07	1.58 ± 0.05*	1.47 ± 0.08*	1.42 ± 0.11*
ACC	0.81 ± 0.07	0.58 ± 0.05*	0.47 ± 0.08*	0.42 ± 0.11*
AIP	0.36 ± 0.02	0.23 ± 0.03*	0.18 ± 0.06*	0.16 ± 0.10*
CRI-2	0.34 ± 0.07	0.23 ± 0.04*	0.16 ± 0.04*	0.12 ± 0.05*

Values are expressed as the mean ± SD ($n = 6$).
* denotes a significant difference when compared to the control group.

TABLE 7: Effects of *G. pedunculata* fruit extract on pancreatic function.

Biochemical parameters	Control	*G. pedunculata* treatment group		
		250 mg/kg	500 mg/kg	1000 mg/kg
Amylase (U/L)	730.50 ± 30.96	753.60 ± 49.57	734.50 ± 20.55	725.75 ± 84.45
Lipase (U/L)	32.25 ± 3.40	29.40 ± 1.67	29.50 ± 1.73	29.00 ± 0.81

Results are expressed as the mean ± SD ($n = 6$).

the hematological parameters investigated with no observed anemia, leukemia, inflammation, infections, polycythemia, and/or thalassemia.

WBCs of all types (neutrophils, lymphocytes, eosinophils, monocytes, and basophils) play important role in the immune system to combat infections. Excess production of leukocytes is a biomarker for inflammation and stress disorders [26]. Red blood cell and hematocrit counts as well as the hemoglobin concentration are essential to determine the effect of chemical substances on the hematopoietic system [27]. Previous studies reported that the levels of hemoglobin and hematocrit increase with dehydration, renal cell carcinoma, hypoxia, pulmonary fibrosis, and/or polycythemia vera and decrease with anemia, bleeding, bone marrow failure, hemolysis, leukemia, malnutrition, and overhydration where the body is intoxicated by foreign toxic substances [28, 29]. Moreover, RBC indices (MCV, MCH, and MCHC) are important biomarkers for the early diagnosis of anemia. Platelets indices (MPV and PDW) and P-LCR are also key markers for the diagnosis of atherosclerosis, thromboembolic and ischemic heart disease, which increases during platelet activation [30–32]. Nevertheless, the administration of *G. pedunculata* to the rats yielded no significant changes in these investigated parameters, indicating that it is nontoxic and does not affect the blood composition.

The liver is the largest and most vital organ in animals, as it is involved in the detoxification and elimination of foreign substances [33]. Increased activities of ALT, AST, GGT, ALP, and LDH in the serum are utilized to determine hepatic dysfunction and are directly implicated in the extent of hepatic damage and toxicity [33, 34]. ALT is a hepatocyte cytoplasmic enzyme, and ALP is mostly synthesized by liver. Elevation of ALT and ALP activities in the serum indicates liver dysfunction or, more specifically, cholestasis [35, 36]. On the other hand, increased activities of AST and LDH in the serum indicate not only myocardial but also hepatocellular injuries [37]. Our study revealed that rats treated with
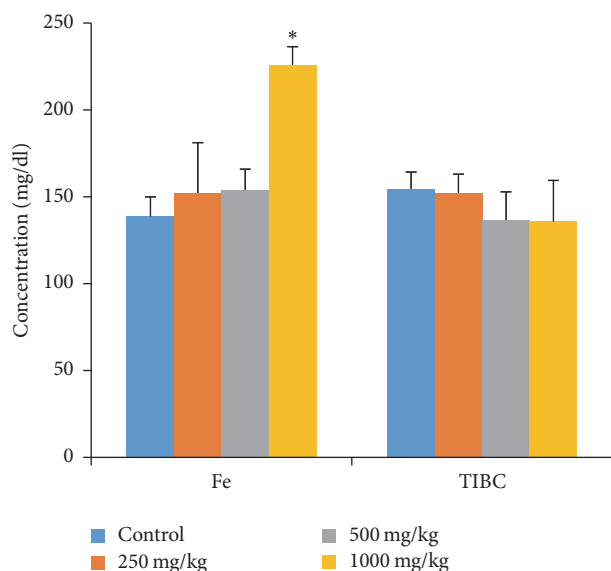

FIGURE 4: Effects of *G. pedunculata* fruit extract on iron profiles. Results are expressed as the mean ± SD ($n = 6$). * denotes a significant difference when compared to the control group.

G. pedunculata had almost unchanged serum activities of ALT, ALP, AST, GGT, and LDH, except for the rats that received the highest (1000 mg/kg) dose. Because the animals treated with *G. pedunculata* extract at 1000 mg/kg showed significantly decreased levels of the marker enzymes in the serum compared to those of the control, it is plausible that the extract can restrict the leakage of hepatic enzymes into the circulation (as also confirmed by the preservation of cells based on the histopathological findings) via some unknown mechanism. Our findings are in agreement with a previous study where *G. pedunculata* was shown to confer some hepatoprotective effects [1] when administered at
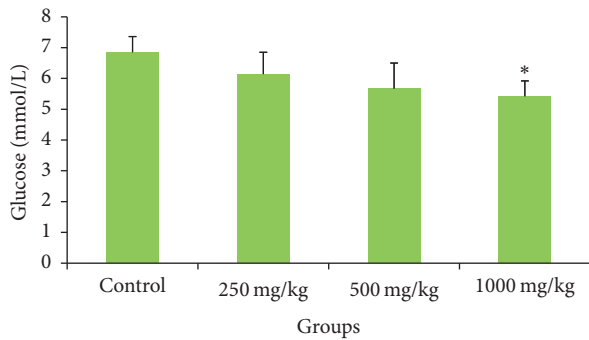
FIGURE 5: Effects of *G. pedunculata* fruit extract on blood glucose levels. Values are expressed as the mean ± SD ($n = 6$). ∗ denotes a significant difference when compared to the control group.

400 mg/kg. Furthermore, the microscopic examinations of rat liver treated with 1000 mg/kg of the fruit extract lacked any cellular necrosis or centrilobular degenerative changes, infiltration of any inflammatory cells, and changes in cell sizes or architecture, further confirming the safety of the fruit extract shown by the biochemical findings.

The TB, TP, ALB, GLB, and albumin levels and the globulin (A/G) ratio are also important biomarkers for liver toxicity [37]. The rise in the TB level is an important clinical indicator for the severity of necrosis, whereas its accumulation is the measure of the binding, conjugation, and excretory capacity of the hepatic cells [38]. In addition, serum TP and albumin levels can provide information about the severity of the necrosis and the capacity for protein synthesis [34]. In this study, there were no significant differences in the levels of TB, TP, ALB, GLB, or albumin or the globulin (A/G) ratio among the treatment groups. These findings indicate that the extracts of the *G. pedunculata* fruit contain no toxic constituents, thus allowing normal hepatic excretory and synthetic functions.

Our research also investigated the effects of *G. pedunculata* on the kidney by measuring the serum level of urea, uric acid, and creatinine, which are specific indicators and highly sensitive biomarkers of kidney function. An incremental change in these molecules tends to be observed when marked damage to functional nephrons occurs [39, 40]. In our study, no significant changes were observed in these renal biomarkers with the administration of all doses, indicating that the fruit extract is not toxic to the kidneys. These findings were further confirmed by the relative organ weight, the percent water content, and the histopathological observations of the kidney tissues.

Electrolytes are minerals found in the blood and other bodily fluids that carry an electrical charge and are essential for the maintenance of bodily functions including heartbeat, nerve transmission, muscle contraction, energy production, pH balance, and fluid balance [41]. The kidneys play an important role in keeping the electrolyte balance constant, even while bodily changes occur. Significantly imbalanced electrolytes may contribute to nervous system disorders, muscle spasms, weakness, convulsion, and changes in blood pressure [42, 43]. In our study, the measured electrolytes,

including Na^+, K^+, Cl^-, Mg^{2+}, PO^{3+}, and Ca^{2+}, remained almost unchanged in all of the treatment groups even when *G. pedunculata* was administered at the highest dose of 1000 mg/kg. This finding indicates that the fruit extract of *G. pedunculata* does not alter the homeostasis of the body.

The lipid profile is often quantitatively measured to diagnose primary and secondary hyperlipoproteinemia, triglyceridemia, liver obstruction, fatty liver disease [39, 44], and pancreatitis [45]. High plasma triglycerides and cholesterol levels are linked to atherosclerosis and predispose an individual to cardiovascular disease. They also contribute to hypertension, obesity, and diabetes mellitus [39, 46, 47]. LDL-C (also called "bad cholesterol") can accumulate in the walls of arteries to cause cardiovascular diseases. Therefore, it is considered a major risk factor in the occurrence of coronary artery disease [48, 49], hypertension, and obesity [50]. In this work, the fruit extract showed dose-dependent reductions in the serum levels of TG, TC, and LDL-C and an incremental change in the HDL-C levels. Rats treated with 1000 mg/kg of extract had significantly reduced serum TC levels and significantly increased HDL-C levels, whereas there were nonsignificant increases in HDL-C levels for the other two doses. This finding confirms that *G. pedunculata* fruit may have a protective effect against cardiovascular-related disorders. The presence of high HDL-C levels in the plasma tends to exert a protective effect by reversing cholesterol transport because HDL-C scavenges excess cholesterol from the peripheral tissues of the body [39].

Atherogenic indices are derived from the lipid profiles to predict the risk of cardiovascular disease. When these indices increase, the risk of cardiovascular disease increases [47, 51, 52]. The administration of fruit extract to rats at three doses significantly reduced all of the atherogenic indices. Therefore, these findings provide strong support in favor of the cardioprotective effect of the fruit.

When interpreted together, the serum iron and TIBC can help to confirm the diagnosis of iron deficiency; low iron levels and high TIBC are associated with iron deficiency anemia or hemochromatosis [53]. In addition, a high level of iron is also associated with liver and kidney disease and is also a risk factor for cancer. Low TIBC is associated with liver and ischemic heart disease [54–56]. Our findings suggest that *G. pedunculata* significantly ameliorated iron levels when administered at the highest dose. This finding further supports the use of *G. pedunculata* fruit for preventive effects against iron deficiency-related disorders.

Serum amylase and lipase activities are two major indicators of pancreatic function. According to the guidelines from the American College of Gastroenterology (ACG), diagnostic values of serum amylase and/or lipase in excess of three to five times upper limit of normal are characteristic of acute pancreatitis and are usually not reported to occur in other conditions [39, 57]. However, research shows that serum lipase activity is a more reliable diagnostic marker for acute pancreatitis [58, 59]. In our study, no significant effects on pancreatic function, amylase and lipase levels, were observed in the treated rats.

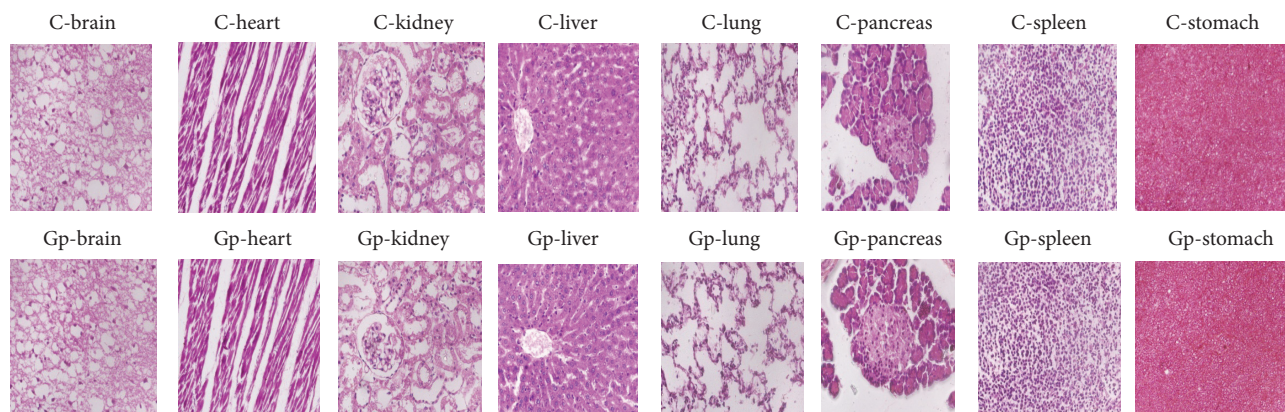Blood glucose level measurements are used for the early diagnosis of diabetes mellitus [60]. Traditional medicinal

FIGURE 6: Histopathological photomicrographs of the control and treatment groups (1000 mg/kg) (6100x magnification, scale bar: 20 μm). No morphological or pathological changes were observed between the control and *G. pedunculata* administered groups. Here, C: control; Gp: *G. pedunculata* (1000 mg/kg) treatment group.

plants are widely used for the prevention of diabetes mellitus based on their ability to restore pancreatic function by increasing insulin secretion, inhibiting intestinal glucose absorption, reducing oxidative stress, and facilitating metabolites in insulin dependent processes [61]. The extract of the *G. pedunculata* fruit has a positive effect on the glycemic status. We observed that the rats administered with 1000 mg/kg dose had significantly reduced blood glucose levels, indicating that *G. pedunculata* fruit may be effective against hyperglycemia. The histomicrograph of the pancreas and spleen further confirmed the biochemical findings. The investigated possible effects of the fruit extract on some other vital organs including the brain, lung, and stomach revealed no adverse sign of damage to these tissues, as confirmed by histopathological examination of the microphotograph. The overall study of *G. pedunculata* fruit suggests that the consumption of this fruit at the investigated dose levels is nontoxic, safe, and beneficial to some extent.

5. Conclusion

The biochemical measurements of liver function, lipid profile, atherogenic indices, and blood glucose levels indicate that *G. pedunculata* fruit has significant hepatoprotective, cardioprotective, and hypoglycemic activities and ameliorates iron levels. The extract is also safe when used at up to 1000 mg/kg; it showed no undesirable effects on body weight, relative organ weight, or the percent water content of vital organs. In addition, no major changes were observed in any other hematological, biochemical, and histopathological parameters.

Disclosure

Sudip Paul and Md. Yousuf Ali are joint first authors.

Competing Interests

The authors declare that there is no conflict of interests.

Acknowledgments

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