ORIGINAL ARTICLE

Sub-chronic toxicity of *Garcinia atroviridis* Griff Fruit's ethanol extract on Wistar rats (*Ratus norvegicus*)

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ABSTRACT

Garcinia atroviridis Griff. (GA) is a tropical fruit that commonly used as a traditional medicine to this day. This study was conducted to determine the sub-chronic toxicity effect of GA fruits ethanol extract on body weight, clinical hematology and biochemical parameters, and organ's histopathology. This study is an experimental research by oral treatments for 90 days with completely randomized design. The treatment group consists of five classes. Each class was given the treatment with a dosage of 50 mg/kg BW, 200 mg/kg BW, 800 mg/kg BW and satellite group with 2% PGA, or 800 mg/kgBW. Based on ANOVA and advanced Tukey test results using SPSS, the hematological parameters such as mean corpuscular volume, mean corpuscular hemoglobin concentration, and white blood cell had significant differences with the control group. In the biochemical parameters, the serum glutamic-oxaloacetic transaminase values and triglycerides (in male rats), serum glutamic-pyruvic transaminase and creatinine (in female rats) had significant differences with the control group. In conclusion, GA fruits ethanol extract is safe and non toxic to body weight, clinical hematology and biochemical parameters, and histopathology of ten organs.

Key words: *Garcinia atroviridis* Griff., histopathology, subchronic toxicity

INTRODUCTION

Garcinia atroviridis Griff. (GA) belongs to *Clusiaceae* that grows wild across the peninsular of Malaysia and is being cultivated for its economic value.[1] GA fruits contain phenolic and flavonoid compounds.[2] These fruit's extracts and hydroxycitric acid showed activity as weight loss agent.[3] Garcinia acid is a major compound of GA fruit that plays a role in managing obesity.[4]

The fruits of GA have been proved to be empirically safe, but sub-chronic toxicity tests should be undertaken in relation to their usage as traditional medicine.[5] Sub-chronic toxicity test was conducted to determine the toxic effect on the organs, due to the consumption of these fruit's extract in a long-term period. Toxicity datas were obtained through body weight observation, hematological parameters, blood biochemical parameters, organ index, and organ histopathology of rats in repeated administration for 90 days. Moreover, for the presence or absence of organ recovery , the administration of GA fruits ethanol extract was discontinued for 28–30 days.[6]

MATERIALS AND METHODS

Plant material, extraction, and preliminary phytochemical screening

GA fruits were procured from Deli Serdang in North Sumatra. Fruits were dried, crushed, and extracted by maceration cold extraction using ethanol 70% as a solvent.

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Animals

The animals used in this study were 24 male and female rats of Wistar strain. Each is in healthy condition; 6–8 weeks of age, with weight 120–200 g; were procured form Institute Teknologi Bandung. The present study was carried out after approval from the Health Research Ethics Committee Faculty of Medicine, Universitas Padjadjaran, with ethical approval number of the study 894/UN6.C.10/PN/2017. The animals were acclimatized to environmental conditions for 7 days before conducting this study.

Subchronic toxicity study

The study was conducted on 48 Wistar strain rats (24 male and 24 female) for 90 days and 120 days (satellite). The rats were divided into six groups, namely (i) the control group, (ii) group with dose of 50 mg/kgBW, (iii) group with dose of 200 mg/kgBW, (iv) group with dose of 800 mg/kgBW, two groups of satellites for 120 days, (v) control satellite, and (vi) satellite with dose of 800 mg/kgBW.

These tested animal groups were administered extracts in Arabic gum suspension orally once per day for 90 consecutive days with a volume of 1 ml/100 g and were given Arabic gum suspension of 1 ml/100 g. Blood samples were taken for animal testing in the form of biochemical and hematologic analysis.

Hematology and biochemical analysis

Complete blood count and biochemical analysis were performed after the completion of the study. The analysis was carried out in the Pathology Laboratory of the Hasan Sadikin General Hospital in Bandung.

Histopathology

At the end of the study, all the animals were sacrificed, and then, performed surgery on the organ was being observed. In this study, we also performed a macroscopic observation such as organ size, shape, lesion, and organ weight changes. In microscopic histologically organ, we used hematoxylin and eosin staining.

Statistical analysis

The collected data are presented in terms of mean ± standard deviation. The diff erence in test between test group and control group using IMB SPSS Statistic 22.0 (Developed Software by International Business Machines (IBM) Corporation in Armonk, New York). $P < 0.05$ was considered as statistically significant.

RESULTS

Animal weight changes

A marked decrease in body weight of male rat observed in small and high dose groups from week 1 onward; but statistically, there are no significant differences. It can be concluded that the GA fruits' extract does not affect the body weight of male and female rats, but the changes in body weight by the tested animals are more influenced by the growth process of rats due day 0 to 91 and 121 along with increasing age or feed intake increases [Supplementary Table 1].

Hematological analysis

Animal treatment groups did not show significant changes in white blood cells (WBCs), red blood cells, hemoglobin, and hematocrit in male and female rats compared to the control group [Supplementary Table 2]. The animal treatment group also did not show significant changes in mean corpuscular volume, mean corpuscular hemoglobin concentration, WBC, and platelets in male and female rat when compared with the control group [Supplementary Table 3]. Supplementary Tables 2 and 3 showed that there was a decrease in the levels of several parameters examined; but statistically, there was no significant difference between the test groups.

Biochemical analysis

In the treatment and satellite groups at a dose of 800 mg/kg, the increase in serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase levels was greater compared to the other treatment groups. It can be seen that in the female control group, increased serum creatinine levels were greater than the treatment group. Whereas in the 800 mg/kg group and satellite control group, the serum creatinine levels were lower than the control group [Supplementary Table 4]. It can be seen that the highest average total cholesterol of level examination occurred in male rats in the satellite control group with a value of 96.50 mg/dl. However, in female rats, the highest value of cholesterol was found in the group with a dose of 50 mg/kg with a value of 90.75 mg/dl. The increase in drug dosage should accelerate the response which is proportional to the increased dose. Hence, increasing doses of increased response will eventually decrease due to the achievement of the dose which can no longer increase the response [Supplementary Table 5].

The increase in SGOT levels of male rats differed significantly between the treatment groups 200 mg/kgBW with the control satellite group and satellite 800 mg/kgBW as shown in Supplementary Table 4. The creatinine levels were significant differences between the control group and the 800 mg/kgBW and control.

Histopathological examination

All animals are subject to necropsy after completion of the 13-week study. In histopathology examination, liver, kidneys, ticker, lungs, spleen, pancreas, stomach, bowel, uterus, and testes were isolated and examined [Figures 1-10].

As shown in Figure 1, the inflammation around blood vessels was occurred by the presence of a solid mass

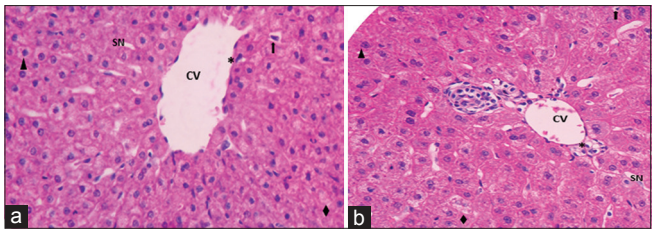

Figure 1: (a and b) Effect of *Garcinia atroviridis* Griff. fruits' extract ethanol on microscopy of the heart. CV: Vena centralis, SN: Sinusoid

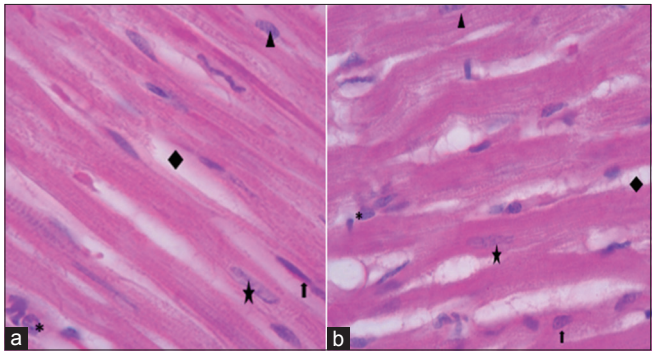

Figure 3: (a and b) Effect of *Garcinia atroviridis* Griff. fruits' extract ethanol on microscopy of ticker

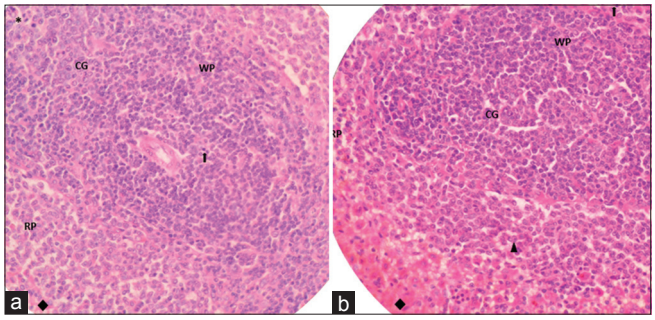

Figure 5: (a and b) Effect of *Garcinia atroviridis* Griff. fruits' extract ethanol on microscopy of the spleen. WP: White pulp, RP: Red pulp, CG: Centro germinativum

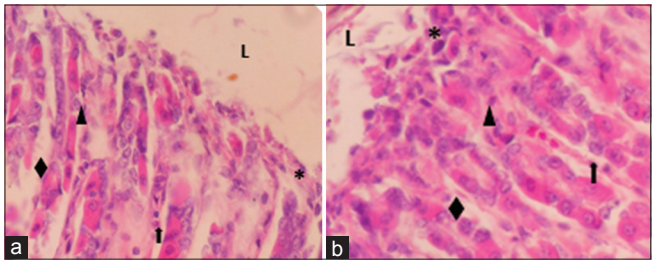

Figure 7: (a and b) Effect of *Garcinia atroviridis* Griff. fruits' extract ethanol on microscopy of the stomach. L: Lumen

around the blood vessel (CV) with a size larger than normal cells due to cell failure to maintain intracellular fluid and electrolyte homeostasis.[7]

Histopathology of the kidney consists of the glomerulus and Bowman capsules that appear to be normal at all levels

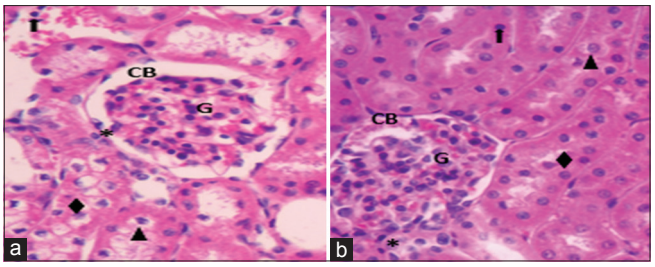

Figure 2: (a and b) Effect of *Garcinia atroviridis* Griff. fruits' extract ethanol fruits on microscopy of the kidney. CB: Capsula Bowman's, G: Glomerulus

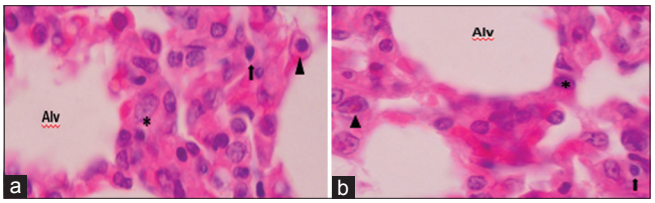

Figure 4: (a and b) Effect of *Garcinia atroviridis* Griff. fruits' extract ethanol on microscopy of lungs. Alv: Alveolus

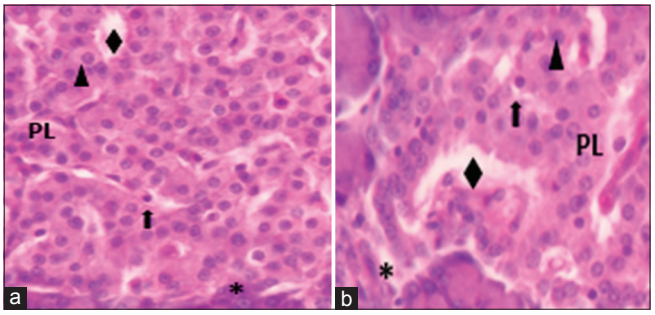

Figure 6: (a and b) Effect of *Garcinia atroviridis* Griff. fruits' extract ethanol on microscopy of pancreas. PL: islets of Langerhans

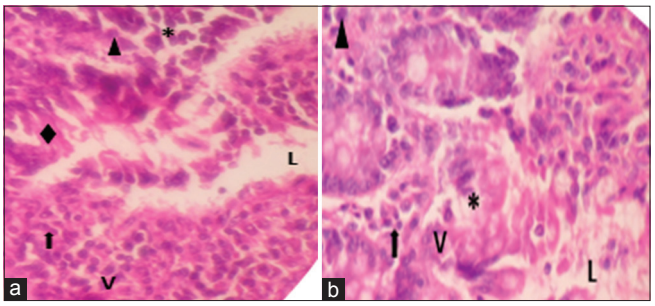

Figure 8: (a and b) Effect of *Garcinia atroviridis* Griff. fruits' extract ethanol on microscopy of the bowel. L; Lumen, V: Villi

of the treatment group and the control group, as shown in Figure 2.

As shown in Figure 3, it shown no significant difference between the control, the control satellite, and the satellite groups as results of statistical analysis.

Enlargement of the alveoli or atelectasis at the dose of 800 mg/kgBW compared to the control indicated that the

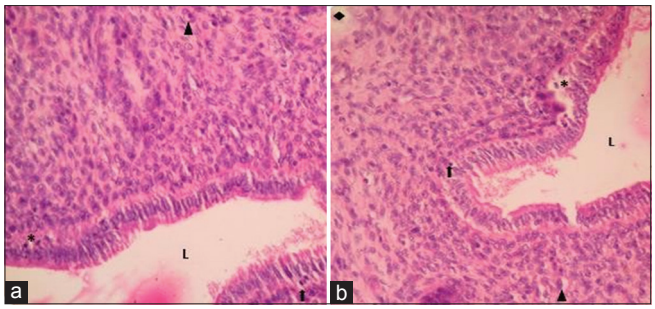

Figure 9: (a and b) Effect of *Garcinia atroviridis* Griff. fruits' extract ethanol on microscopy of the uterus. L: Lumen

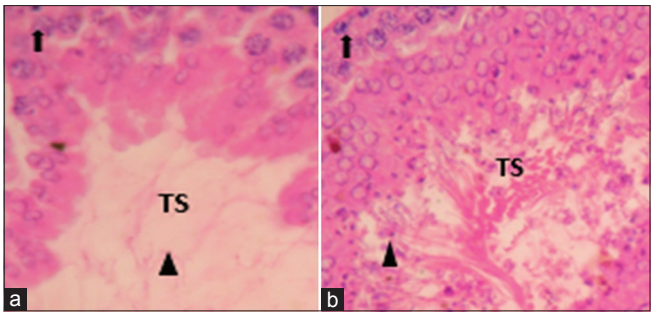

Figure 10: (a and b) Effect of *Garcinia atroviridis* Griff. fruits' extract ethanol on microscopy of testes. TS: Seminiferous tubules

affected part of the lung does not contain air and collapse. In the treatment group that given the GA extract, it was found that the mechanism of inhibition of free radicals in the process of cell wall destruction occurred outside the cell wall has succeeded in maintaining the integrity of the alveolar wall, thus minimizing damage.

Different histological features are shown in Figure 5a; the white pulp consists of nondense lymphocytes because it stretches and dilates blood vessels as a sign of splenic congestion. This microscopic picture of spleen tissue shows a nonspecific acute splenitis. Interestingly, the results are shown in Figure 5b; the spleen tissue of mice in this group showed indication of improvement from acute congestion in the form of a little vasodilation, red pulp and white pulp not stretching, and many macrophage cells, focal fibrosis, and large hematopoietic stem cells.

Based on the results of the microscopic examination shown in Figure 6, islets of Langerhans has more dominant intercell boundary which has a round shape, pink cytoplasm, and a rounded and purple nucleus which can be concluded as normal. In pancreas of the satellite and the satellite control group, cells of islets of Langerhans were arranged tightly, but cell boundaries still can be identified. The pink cytoplasm and nucleus appear to be round purple.

Microscopic appearance as shown in Figure 6b (compared to Figure 6a) indicated the more infiltration of inflammatory cells in the inner regions of plasma lymphocytes gastric submucosal.

In Figure 8a and b, the rat ileum histology appeared under normal conditions. It has been shown intestinal villi arranged very neatly, tightly, as regularly.

In Figure 9, there was a significant difference between the control group and the satellite group 800 mg/kgBW. However, fatty degeneration, hydrophobic degeneration, and inflammatory cell infiltration showed no significant difference between the control group and all treatment groups.

As shown Figure 10, there was no structural change in testicular tissue due to the administration of GA fruits' extract for 90 days in both the control and treatment groups.

DISCUSSION

The inflammation around blood vessels was occurred by the presence of a solid mass around the blood vessel (CV) with a size larger than normal cells due to cell failure to maintain intracellular fluid and electrolyte homeostasis [Figure 1].[7] Liver damage due to medication and chemicals can occur due to loss of regeneration capacity of liver cells.

The results of the metabolism will be carried by the blood flow through the sinusoid to the central vein.[8] If the metabolite is destructive, it will cause damage to the hepatocytes around the central vein.[9]

Fat degeneration resulted from abnormal fat accumulation in the cytoplasm with vacuoles that vary in size can push the nucleus to the edge. Disruption of cell function will occur when fat deposits get excessive and eventually cause changes in fat cells and creates necrosis.[10] Fats will be transported to adipose tissue and muscles that have the lipoprotein lipase enzyme hydrolyzing fat into free fatty acids, where free fatty acids are the main energy source for the myocardium. Flavonoid compounds that have antioxidant activity will experience a process of absorption, distribution, metabolism, and excretion. Similarly, the extract of GA fruits which contain flavonoid compounds will be absorbed by the intestine, next metabolized in the liver, and the metabolic results of the extract will be spread throughout the body including the liver organs.[11]

Widening of the alveoli or atelectasis implies that the affected lung does not contain air and collapse. In the treatment group which was given an extract of GA fruits, it was found that the mechanism of inhibition of free radicals in the cell wall destruction process occurred outside the cell wall has successfully maintained the integrity of the alveolar wall to minimize damage.

The spleen tissue of the Wistar rat in Figure 5b showed the histological features that correspond to the normal spleen, namely the red pulp which consists of macrophages, plasma cells, and blood elements; and white pulp which consists of lymphocytes that are densely arranged in them and central arteries in the middle. Damage that occurred in all organs in both male and female rats was experienced by all groups which indicated the occurrence of pathological changes was not due to the administration of GA extract.

Based on the results of the microscopic examination shown in Figure 6, islets of Langerhans has more dominant intercell boundary which has a round shape, pink cytoplasm, and a rounded and purple nucleus which can be concluded as normal. At the pancreas of the treatment group with a dose of 800 mg/kg exhibits cells arranged tightly in Langerhans Island, but the cell boundaries still can be recognized. The cytoplasm is pink and the nucleus appears purple in round shape. Langerhans island is usually egg shaped and consists of cells that are polygonal or round.[12] Langerhans island appears paler than the exocrine area because it does not have zymogen granules.[13]

Flavonoids can increase prostaglandins in the gastric mucosa and can also prevent the formation of free radicals that make the stomach become ulcerative and erosive.[11] Saponins can activate the gastric mucosal protective membrane.[14] Thus, GA fruits ethanol extract can reduce inflammation, the response to injury to the cell is reduced, and further reduced the infiltration of inflammatory lymphocyte cells and PMN in gastric mucosa.

The results of the histopathological observation of the two treatment groups in this study can be observed in A and B showing the histology of rat ileum under normal conditions. It shows that the arranged intestinal villi are very neat, tight, and regular. The normal ileum can be seen with mucosa with long villi, columnar epithelial cells with goblet cells, submucosal layer, muscularis mucosa layer, muscular layer, and serous layer. Histology in this group can be used as a benchmark for changes and damage that occur in other groups.

The uterus has a lining in the endometrium, followed by a myometrial layer and an outer perimetrium layer. The seminiferous tubules of testes each contain sperm cells at various stages of development of spermatogenesis. Each seminiferous tubule is separated by interstitial tissue containing Leydig cells.[15] The results of the histopathological observation of the two treatment groups in this study can be observed in A and B showing a normal uterine and testicular histology both in the control group and in the dose group of 800 mg/kgBW. The acute toxicity test described the adverse effects that occurred within 14 days of the administration of the substances. Lethal Dose 50 (LD_{50}) are frequently used as a general indicator of a substance's acute toxicity. The value of LD_{50} for a subtance's is the dose required to death half the members of a tested population after a specified test duration.[16]

The LD_{50} of GA fruits ethanol extract in rats was estimated to be at ≥ 2000 mg/kg BW due to acute toxicity test. Because the treatment 2000 mg/kg BW did not cause acute toxicity effect. Thus, subchronic toxicity testing was carried out for complementing the GA fruit extract safety data in long-term use as traditional medicine.

However, this study has limitations due to the *in vitro* subchronic toxicity approach that most cell systems represent only one cell type when compared to all animal experiments where hundreds of tissues interact with each other physiologically. Cell degeneration due to continuous depletion of nutrients, accumulation of waste products, and inadequate oxygen supply resulting in anaerobic culture conditions often occur in several *in vitro* conditions.[17]

CONCLUSION

We can conclude that the GA fruit extract is not toxic in all doses studied here and does not produce toxic signs or obvious symptoms of subchronic toxicity. This study provides valuable data about the subchronic toxicity profile of GA, which can be useful in chronic toxicity studies and clinical studies of this valuable medicinal plant.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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Supplementary Table 1: Effect of ethanol extract of *Garcinia atroviridis* fruit on body weight of male and female rats (G/rats)

Group	Mean±SD	
	Male	Female
Control	171.77±13.14	154.82±14.89
Dose 50 mg/kg	188.79±23.32	152.84±17.74
Dose 200 mg/kg	158.66±14.45	158.35±21.19
Dose 800 mg/kg	146.85±15.40	142.87±13.84
Control satellite	169.45±15.24	152.96±18.63
Satellite 800 mg/kg	162.11±15.80	154.05±17.38

SD: Standard deviation

Supplementary Table 2: Effect of ethanol extract of *Garcinia atroviridis* fruit on hematological parameters

Parameters	Gender	Group						
		Control	50 mg/kg	200 mg/kg	800 mg/kg	Control satellite	Satellite 800 mg/kg	Significant
MCV (fl)	Male	61.32±7.04	59.70±5.37	57.42±3.47	60.47±7.22	59.45±1.81	57.93±3.65	0.902
	Female	64.72±2.62	64.32±1.92	64.52±2.28	60.85±2.35	57.67±2.27	57.45±1.34	0.001*
MCH (pg)	Male	17.62±0.75	18.15±1.50	17.47±0.38	17.67±2.0	18.42±0.49	17.9±1.15	0.870
	Female	18.37±0.56	18.37±0.59	18.82±0.43	18.02±0.35	18.07±0.91	17.75±0.63	0.343
MCHC (g/dL)	Male	28.95±2.14	30.40±0.40	30.52±1.20	29.30±0.28	30.97±0.34	30.86±0.15	0.078
	Female	28.40±0.62	28.55±0.83	29.15±0.66	29.67±1.20	31.32±0.34	30.95±0.35	0.000*
Trombosit ($10^3/\mu L$)	Male	1034.50±435.25	958.75±131.14	844.75±224.89	1121.25±246.12	1155.25±100.43	1020.0±226.04	0.570
	Female	910.50±141.35	831.75±280.48	933.75±120.90	938.50±316.91	1094.0±75.69	1294.0±31.11	0.170

*Significantly different compared to the control group (Tukey's HSD test, $P<0.05$). MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, HSD: Honestly significant difference

Supplementary Table 3: Effect of ethanol extract of *Garcinia atroviridis* fruit on hematological parameters

Parameters	Gender	Group						
		Control	50 mg/kg	200 mg/kg	800 mg/kg	Control satellite	Satellite 800 mg/kg	Significant
WBC ($10^3/\mu L$)	Male	6.38±0.65	6.02±0.52	5.99±1.44	3.02±0.64	7.82±1.46	9.94±1.04	0.000*
	Female	5.12±2.76	5.15±1.29	6.02±0.59	6.15±1.07	11.17±2.51	11.12±0.97	0.000*
RBC ($10^6/\mu L$)	Male	9.16±2.68	9.11±1.02	8.74±0.63	8.77±1.42	9.81±0.76	9.19±1.02	0.915
	Female	9.15±0.65	8.42±0.49	8.43±0.65	8.74±0.24	9.41±0.58	9.42±0.10	0.062
Hb (g/dL)	Male	16.02±3.96	16.42±0.85	15.27±0.86	15.35±1.46	18.05±0.92	16.40±1.40	0.408
	Female	16.80±0.78	15.45±0.47	15.85±1.21	15.77±0.68	17.0±1.0	16.75±0.77	0.113
HCT (%)	Male	55.0±10.31	54.0±2.27	50.07±2.14	52.37±4.45	58.27±2.79	53.13±4.24	0.396
	Female	59.15±2.16	54.12±2.23	54.40±4.50	53.17±1.56	54.27±3.07	54.15±1.90	0.101

*Significantly different compared to the control group (Tukey's HSD test, $P<0.05$). WBC: White blood cell, RBC: Red blood cell, Hb: Hemoglobin, HCT: Hematocrit, HSD: Honestly significant difference

Supplementary Table 4: Effect of ethanol extract of *Garcinia atroviridis* fruit on biochemical parameters

Parameters	Gender	Group						
		Control	50 mg/kg	200 mg/kg	800 mg/kg	Control satellite	Satellite 800 mg/kg	Significant
SGOT (U/L)	Male	162.50±14.27	139.50±22.39	116.75±9.77	146.50±27.38	194.25±38.58	193.33±39.80	0.006*
	Female	178.75±16.58	155.25±17.23	144.50±20.48	160.75±41.93	160.75±13.22	197.0±24.04	0.193
SGPT (U/L)	Male	74.75±8.38	80.50±14.88	73.75±18.73	65.0±22.73	102.0±22.52	92.0±16.52	0.108
	Female	80.75±13.27	72.75±12.91	76.0±12.51	76.50±5.80	99.75±19.31	114.50±30.40	0.028*
Ureum (mg/dL)	Male	31.25±4.57	34.50±5.97	34.25±6.99	32.50±5.0	30.0±2.94	30.33±6.42	0.781
	Female	39.75±8.18	35.75±2.98	32.25±6.55	31.50±6.85	29.25±3.20	39.50±2.12	0.135
Kreatinin (mg/dL)	Male	0.56±0.112	0.46±0.042	0.45±0.063	0.62±0.128	0.39±0.046	0.52±0.170	0.051
	Female	0.83±0.040	0.62±0.142	0.64±0.036	0.59±0.165	0.44±0.055	0.61±0.035	0.003*

*Significantly different compared to the control group (Tukey's HSD test, $P<0.05$). SGOT: Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic-pyruvic transaminase, HSD: Honestly significant difference

Supplementary Table 5: Effect of ethanol extract of *Garcinia atroviridis* fruit on biochemical parameters

Parameters	Gender	Group						
		Control	50 mg/kg	200 mg/kg	800 mg/kg	Control satellite	Satellite 800 mg/kg	Significant
Total cholesterol (mg/dL)	Male	71.0±21.05	63.50±8.58	79.25±20.56	57.0±6.48	96.50±14.84	80.0±24.02	0.053
	Female	83.0±7.11	90.75±16.85	77.25±8.84	83.75±10.81	90.0±11.91	66.50±4.94	0.181
Trigliserida (mg/dL)	Male	43.0±4.86	45.0±11.97	74.25±13.81	27.75±9.91	128.0±23.0	98.66±51.03	0.000*
	Female	85.75±24.28	66.75±27.87	114.75±53.85	84.0±53.55	142.75±69.23	129.0±22.62	0.271
Glukosa (mg/dL)	Male	57.25±9.39	61.50±23.07	61.75±9.94	57.50±7.93	55.75±11.11	41.66±7.02	0.416
	Female	108.0±44.87	81.75±6.9	68.75±27.47	65.0±17.90	59.50±9.88	75.00±15.55	0.145
Bilirubin (mg/dL)	Male	0.15±0.062	0.11±0.066	0.14±0.015	0.16±0.066	0.21±0.042	0.15±0.124	0.420
	Female	0.20±0.14	0.12±0.016	0.20±0.026	0.17±0.076	0.24±0.050	0.09±0.020	0.185

*Significantly different compared to the control group (Tukey's HSD test, $P<0.05$). HSD: Honestly significant difference