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Research Article

A comprehensive *in vivo* study of the antihypertensive properties and toxicity of roselle (*Hibiscus sabdariffa* L.)

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Abstract

Background: Roselle (*Hibiscus sabdariffa* L.) calyces have been used in traditional medicine as diuretics, mild laxatives, and antihypertensive agents but to date, a comprehensive study of its pharmacological activity and safety has not been conducted.

Aims of the study: The current study aims to provide a comprehensive evaluation of the antihypertensive efficacy and toxicity profile of Roselle (*H. sabdariffa* L.) calyces extract. Utilizing animal models, the investigation assessed the dose-dependent pharmacological effects and safety of *H. sabdariffa* L.

Results: The findings indicate that the extract exerts a significant antihypertensive effect at a dose of 250 mg/kg body weight (BW), lowering systolic and diastolic blood pressures by 10.12% and 11.63%, respectively. Ethyl acetate fractions administered at 112.5 mg/kg BW demonstrated greater efficacy than *n*-hexane and aqueous fractions, suggesting that the active compounds likely possess semi-polar properties. Acute toxicity testing yielded an LD_{50} of 8.75 g/kg BW for male rats and 7.5 g/kg BW for female rats, classifying the extract as slightly toxic. The sub-chronic toxicity study shows that *H. sabdariffa* L. demonstrates an effect on bodyweight and urea levels in male and female rats, while the change in the blood parameters, creatinine level, and the liver index was only observed in female rats. **Conclusions:** These data suggest that *H. sabdariffa* L. extract exhibits therapeutic promise but should be administered cautiously, preferably at doses lower than 250 mg/kg BW, due to potential toxicity.

Keywords

acute, diastolic, sub-chronic, systolic, toxicity

Introduction

Hypertension is a risk factor for cardiovascular disease which has a high prevalence and mortality (Pattanittum et al. 2010; Stanaway et al. 2018). It is defined as a systolic blood pressure of 140 mmHg or higher and/or diastolic blood pressure of 90 mmHg or higher (Unger et al. 2020). There are various pharmacological approaches to lowering systolic and diastolic blood pressures in adults, such as the administration of antihypertensive drugs such as beta-blockers, thiazide, and thiazide-like diuretics, angiotensin-converting enzyme (ACE) inhibitors, angiotensin

II receptor antagonists, and calcium channel blockers (Pattanittum et al. 2010; Laurent 2017; Williams et al. 2018). However, the use of such classes of antihypertensive drugs is associated with undesirable side effects such as hypokalemia, nausea, dizziness, and dry cough, thus there is a need for alternative medicine (Hopkins et al. 2013; Laurent 2017; Jeffery and Richardson 2021; Salem et al. 2022).

Medicinal plants are of great interest because they are an abundant natural source of novel therapeutic agents for the treatment and prevention of hypertension (Jacob and Narendhirakannan 2019; Ben El Mostafa and Maamri 2020; Jadid et al. 2020; Jeffery and Richardson 2021). For example, Roselle (*Hibiscus sabdariffa*, Malvaceae family) is a medicinal plant which has been widely used to treat hypertension, particularly in Malaysia and Indonesia where it is consumed as a refreshing drink (Pattanittum et al. 2010; Carvajal-Zarrabal et al. 2012; Hopkins et al. 2013; Ali and El-Anany 2017).

Several animal studies have demonstrated that *H. sabdariffa* has several pharmacological activities including antihypertensive, antidiabetic, antibacterial, antioxidant, anticholesterol, and hepatoprotective properties (Aba et al. 2014; Adeyemi et al. 2014; Asgary et al. 2016; Ali and El-Anany 2017; Mohammed Yusof et al. 2017; Adusei 2020; Jeffery and Richardson 2021; Salem et al. 2022). The antioxidant properties of roselle are associated with its anthocyanin content and protect cells from free radical damage caused by exposure to UV light (Nurkhasanah et al. 2016; Rizki et al. 2017; Adusei 2020).

The main bioactive constituents responsible for the physiological activities of *H. sabdariffa* calyces are organic acids, mainly citric and malic acids, anthocyanins, a myriad of flavonoids and glycosides, and fibre (Carvajal-Zarrabal et al. 2012; Hopkins et al. 2013). A recent phytochemical study of *H. sabdariffa* revealed the presence of phenolic components, anthocyanins, flavonols, and protocatechuic acid (PCA) (Essa et al. 2006). The pharmacological activity of roselle is attributed to the presence of gossypetin, hibiscine glucoside, and anthocyanins. Delphinidin 3-sambubioside and cyanidin 3-sambubioside are two anthocyanin compounds that are generally believed to be the active constituents responsible for the antihypertensive, antioxidant, and hypocholesterolemic effects of *H. sabdariffa* (Hopkins et al. 2013). Additionally, calyces of *H. sabdariffa* contain several amino acids that are very important for improving body health (Da-Costa-Rocha et al. 2014; Jeffery and Richardson 2021).

There are various studies regarding the pharmacological potential of roselle extract, however, a comprehensive study of the antihypertensive effects of roselle calyces extract and active fractions, as well as its safety profiles is limited. Therefore, this study aims to complement and update the information reported previously in the literature.

Materials and methods

Plant materials

Dried roselle (*Hibiscus sabdariffa* L.) calyces were cultivated in the Lembang sub-district, West Java, Indonesia, and identified by the Biology Department Faculty of Mathematics and Science Universitas Padjadjaran with the identification number 207/HB/03/2019.

Animals

Thirty-five healthy Wistar rats (200–250 g) for antihypertensive activity study and twenty-four Sprague Dawley (SD) rats of both genders for sub-chronic toxicity study were obtained from the animal holding facility in the Biological Sciences Center of Bandung Technology Institute. The rats were maintained under standard environmental conditions of temperature, humidity, and light and fed standard rat pellets and water *ad libitum* in The Laboratory of Pharmacology, Faculty of Pharmacy Universitas Padjadjaran. The animals were acclimatised to the laboratory for four weeks before the experiment.

For the acute toxicity study, adult healthy mice (*Mus musculus*) weighting 20–30 g were obtained from the animal holding facility in the Biological Sciences Center of Bandung Technology Institute and kept in the animal house of the Laboratory of Pharmacology, Faculty of Pharmacy Universitas Padjadjaran. The animals were kept in plastic cages in an air-conditioned environment with ten mice per cage at room temperature with relative humidity (60% ± 10%) under 12 h night and light cycle.

The experimental use of animals was approved by the Ethics Committee of Universitas Padjadjaran with the ethical approval number 524/UN6.KEP/EC/2020.

Extraction and fractionation

Dried and pulverised calyces of *H. sabdariffa* (2.000 g) were extracted by the maceration method for 3 × 24 hours using 70% ethanol (3.000 ml × 3) with continuous stirring at room temperature for 24 hours each. The extract was concentrated in a vacuum at 30–40 °C using a vacuum rotary evaporator (IKA RV10 digital).

Antihypertensive activity study

For the antihypertensive activity of *H. sabdariffa* (HS) extract, the animals were randomly assigned into three groups of five rats each: Group I was the positive control group (2.25 mg/kg BW captopril), Group II was the negative control group (2 % of pulvis gum arabic (PGA) suspension), and Group III was the test group for the antihypertensive activity (250 mg/kg BW of HS extract). The systolic and diastole blood pressure was measured using non-invasive blood pressure apparatus and recorded as initial blood pressure. The test sample was then given to the rats orally before the rats were administered 0.25 mg/kg BW of adrenaline intraperitoneally 30 min later to induce blood pressure. After 30 min of induction, the blood pressure was remeasured using non-invasive blood pressure apparatus and recorded as final blood pressure.

Likewise, for the antihypertensive activity of fractions, animals were divided into five groups: Positive control group (captopril 2.25 mg/kg BW), negative control group

(2% of PGA suspension), Hs-I group (112.5 mg/kg BW of *n*-hexane fraction), Hs-II (112.5 mg/kg BW of ethyl acetate fraction), and Hs-III (112.5 mg/kg BW of water fraction). The adrenaline induction method was performed to measure the antihypertensive activity of the extract and the antihypertensive activity of the fractions was tested using the NaCl induction method. The rats were previously induced with 2% NaCl solution for 14 days. Subsequently, NaCl-induced rats were given a single dose fraction orally for a day and their blood pressure was measured at the 60th minute after administration. Systolic and diastolic blood pressure were measured non-invasively using the tail-cuff method through the Coda Invasive Blood Pressure.

Acute toxicity study

The acute toxicity study was conducted according to the Organization of Economic Co-operation and Development (OECD) guidelines for testing chemicals. One hundred and fifty mice were randomly divided into six groups of male mice and six groups of female mice, with one group of male and female mice serving as the control group (10 mice per group). All mice were fasted overnight before the experiment with free access to water. The control groups received 0.3 ml of 2% gum arabic suspension orally and the female test groups were treated with 5.00, 6.25, 7.50, 8.75, and 10.00 g/kg BW doses of HS extract dissolved in 2% gum arabic suspension orally, while the male mice group received 7.50, 8.75, 10.00, 11.25 and 12.00 mg/kg BW doses. Signs of toxicity (convulsion, hypoactivity, weakness, ataxia, and salivation) and mortality were assessed for 24 hours after extract administration and observation for signs of toxicity was performed daily for 14 days. The data are presented in tabular form and analysed statistically. Observational data in the form of the emergence of toxic symptoms were analysed using Friedman's two-way variance.

Sub-chronic toxicity

The animals were weighed and divided into three groups of four animals. After overnight fasting, the control group received a dose of 2% of PGA suspension orally once a day for 90 days while the animals in the test and satellite groups were given a dose of 250 mg/kg of the extract orally once a day for 90 days. The animals were weighed and observed daily for the manifestation of toxicity and mortality until day 90th for the test group and 120th for the satellite group.

Biochemical analysis

At the end of the observation periods, the animals were fasted overnight in the sub-chronic toxicity studies. Anesthesia was administered successively to the animals in a jar saturated with dichloromethane vapour. Blood samples were collected via ocular puncture into EDTA-coated bottles for the determination of haematological parameters and heparinised bottles for biochemical parameters. The serum was collected by centrifugation for biological parameters.

Histopathology

The rats were sacrificed by decapitation with scissors and the vital organs (kidney and liver) were harvested for histology. The organs were observed macroscopically and their weight was recorded. The ratio of organ weight to body weight was calculated to obtain the organ index (per cent). The condition of the gastric mucosa was examined macroscopically and observed under a magnifying glass for the presence of ulcers. The number and width of ulcers were recorded to calculate the ulcer index as follows:

$$\text{Ulcer Index} = \text{UN} + \text{US} + 0.1\ \text{UP}$$

where, UN = Average score number of ulcers per animal, US = Average number of severity score, and UP = Percentage of animals with ulcers.

Results

Extraction and fractionation

Solvent elimination under reduced pressure resulting 620 g (33.17% extract yield) of a dark red, concentrated extract of *H. sabdariffa* (HS). The HS was then divided into two parts, one part was used for an antihypertension study and the rest was partitioned with *n*-hexane and ethyl acetate (EtOAc) yielding *n*-hexane fraction 17.143 g (5.53%), ethyl acetate fraction 62.062 g (20.02%), and water fraction 147.653 g (47.63%).

Antihypertensive activity of roselle extract and fractions

The blood pressure decrease in each test group was calculated based on the mean decrease in blood pressure (mmHg) and inhibition (%) to compare the percentage reduction in systolic and diastolic blood pressure for each group at 1 hr (Table 1). The ANOVA revealed that the test group had a p-value < 0.01, indicating that the HS extract significantly lowers blood pressure in rats.

The antihypertensive activity of *n*-hexane, ethyl acetate, and water fractions of the HS extract were then investigated to determine which fraction exhibited the highest antihypertensive activity (Table 2).

The differences in the blood pressure reduction between groups were compared using ANOVA (Table 3), showing that there was no significant difference between the positive control group and Hs-II, meaning that the Hs-II group (ethyl acetate fraction 112.5 mg/kg BW) was equally effective as the positive control (Captopril 2.25 mg/kg BW).

Acute toxicity

The acute toxicity test was conducted by observing the mortality and weight of mice for 14 days and observing the behaviour of mice for 24 hours as a result of the administration of an ethanol extract of roselle calyces (BPOM 2014).

Table 1. The antihypertensive effects of the HS extract.

Group	Average Blood Pressure (mmHg)						Blood pressure reduction (%)	
	BP1		BP2		Blood pressure reduction			
	S	D	S	D	S	D	S	D
Positive Control	168 ± 9	132 ± 3.00	137 ± 4.58	109 ± 3.00	31	23	18.45	17.42
HS extract	159 ± 9	115±8.66	143 ± 10.53	101.67 ± 8.50	16	13.33	10.06	11.59

BP1: initial blood pressure; BP2: blood pressure at 60th minute; S: systole; D: diastole

Table 2. Antihypertensive activity of the *n*-hexane, ethyl acetate, and water fractions of the HS extract.

Group	Average blood pressure (mmHg)						Blood pressure reduction (%)	
	BP1		BP2		Blood pressure reduction			
	S	D	S	D	S	D	S	D
Positive Control	134.92 ± 3.98	104.1 ± 7.99	104.5 ± 4.09	82 ± 1.63	30.34	22.16	22.48	21.27
Hs-I	134.67 ± 2.53	96.75 ± 3.91	130 ± 1.89	93.4± 3.09	4.67	3.34	3.47	3.45
Hs-II	**133.92 ± 5.06**	**95.75 ± 4.77**	**106.1± 5.88**	**77.41± 1.23**	**27.76**	**18.34**	**20.72**	**19.15**
Hs-III	138.33± 10.04	102.2 ± 3.97	128.0 ± 10.07	92.25 ± 0.50	10.25	9.75	7.41	9.53

BP1: initial blood pressure; BP2: blood pressure at 60th minute; S: systole; D: diastole; Hs-I: *n*-hexane fraction; Hs-II: ethyl acetate fraction; Hs-III: water fraction.

Table 3. The ANOVA results of the antihypertensive activity.

Source	Type III sum of squares	Df	Mean square	F	Sig.
Corrected Model	1862.710[a]	11	169.337	22.413	0.000
Intercept	1913.841	1	1913.41	253.306	0.000
Treatment	664.716	3	221.572	29.326	0.000
Time	866.165	2	433.082	57.321	0.000
Treatment * time	331.828	6	55.305	7.320	0.000
Error	181.331	24	7.555	–	–
Total	3957.881	36	–	–	–

Mortality

The observed mortality in male (Fig. 1A) and female (Fig. 1B) mice for 14 days after the administration of the extract compared to the control indicates that it is dose-dependent, with 100% mortality at a dose of 12.5 g/kg BW for male mice and 10.0 g/kg BW for female mice. The mortality data probit log graph illustrates the relationship between dose and the per cent mortality showing that there is a difference in the LD_{50} dose between female and male mice (7.5 g/kg BW vs. 8.75 g/kg BW). This result is possibly because the body resistance of male mice is stronger than that of female mice.

Pharmacology screening

The mice were observed for 24 hours after administration of the HS extract and the observations included:

a. Central nervous system: motoric, sedative, convulsions, tremor, pineal, breathing, giddiness, reestablishment, flexion, Hafner, Straub, and catalepsy.
b. Autonomic nervous system: piloerection, salivation, lacrimation, urination, and diarrhoea.

The general symptoms that appeared 24 hours after mice were given the extract are presented in Table 4, showing that the movement activity of mice decreased in all groups except the control after the HS extract administration. The mice tended to be quiet and not engage in any activity but the motoric activity began to return to normal after 24 hrs. The coordination of the locomotion of some mice also decreased at 4 hours after extract administration except in the control group. For example, the mice were unable to walk on a wire and respond to treatment such as clamping the ear.

Sub-chronic toxicity

Body weight

The average body weight of male and female rats from each group was significantly different, with the test groups weighing less than the control group (Table 5). The average body weight of male and female mice in the satellite group was higher significantly than the control and test groups (Fig. 2).

Urine

The urine pH and specific gravity of the test groups were higher but not significantly different to the control group. The satellite group's urine pH was higher (but still normal) and the specific gravity was lower (remaining normal) than the control group and test. However, the increase in urine pH and specific gravity were within normal limits, therefore there was no significant kidney damage.

Haematology

Haemoglobin and hematocrit levels were higher (above normal) in the test group than in the control group, while haemoglobin and hematocrit levels were lower in the satellite group than in the test group although slightly above normal values (Table 6). The number of leukocytes in all groups was lower than normal, while the number of leukocytes in the satellite group was higher than in the test group (Table 6) . However, there was no significant differ-

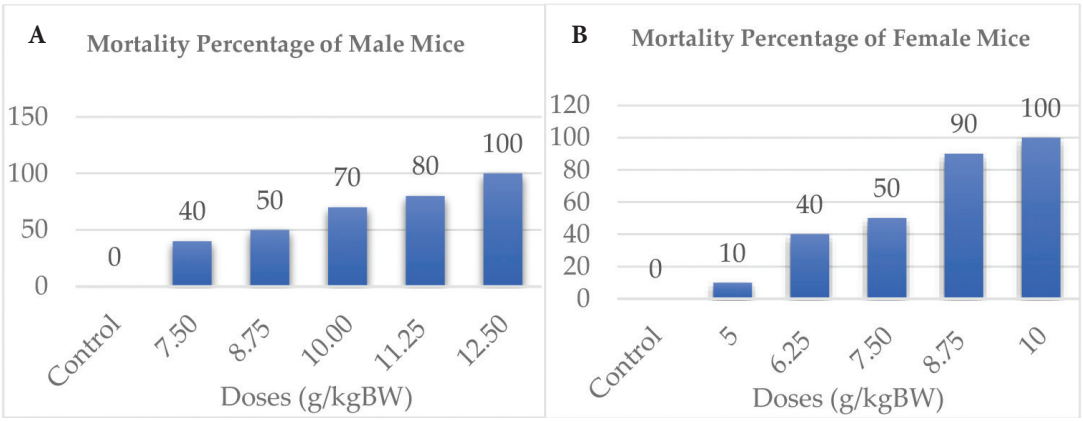

Figure 1. Percentage cumulative mortality of male mice (**A**) and female (**B**) after administration of the extract.

Table 4. The acute oral toxicity test results of the HS extract.

Observations	Control group			Treatment groups														
				I			II			III			IV			V		
	+	-	N/A	+	-	N/A	+	-	N/A	+	-	N/A	+	-	N/A	+	-	N/A
Male																		
Central nervous system																		
Motoric effect	10	0	0	5	1	4	5	0	5	2	2	6	2	0	8	0	0	10
Sedative, Tremor, Straub, convulsion, and catalepsy	0	0	10	2	4	4	5	0	5	1	3	6	2	0	8	0	0	10
Reestablishment effect	10	0	0	6	0	4	5	0	5	4	0	6	2	0	8	0	0	10
Flexi effect	10	0	0	6	0	4	5	0	5	4	0	6	1	1	8	0	0	10
Hafner effect	10	0	0	6	0	4	5	0	5	4	0	6	1	1	8	0	0	10
Pineal effect	10	0	0	6	0	4	5	0	5	4	0	6	1	1	8	0	0	10
Effect on breathing	10	0	0	5	1	4	5	0	5	4	0	6	1	1	8	0	0	10
Autonomic nervous system																		
Abnormal piloerection, salivation, lacrimation, urination, diarrhoea	10	0	0	6	0	4	4	1	5	4	0	6	1	1	8	0	0	10
Female																		
Central nervous system																		
Motoric effect	10	0	0	9	0	1	6	0	4	5	2	3	1	0	9	0	0	10
Sedative, Tremor, Straub, convulsion, and catalepsy	0	0	10	8	1	1	4	2	4	7	0	3	0	1	9	0	0	10
Reestablishment effect	10	0	0	6	3	1	4	2	4	2	5	3	1	0	9	0	0	10
Flexi effect	10	0	0	9	0	1	6	0	4	7	0	3	1	0	9	0	0	10
Hafner effect	10	0	0	9	0	1	6	0	4	7	0	3	1	0	9	0	0	10
Pineal effect	10	0	0	9	0	1	6	0	4	6	1	3	1	0	9	0	0	10
Effect on breathing	10	0	0	9	0	1	6	0	4	7	0	3	1	0	9	0	0	10
Autonomic nervous system																		
Abnormal piloerection, salivation, lacrimation, urination, diarrhoea	10	0	0	4	5	1			4			3			9	0	0	10

(+): present; (-): not present; N/A : cannot be observed (died)

ence in the haemoglobin levels of male rats and there was a significant difference in female rats between the control group, the test, and the satellite groups. The hematocrit levels of male rats were not significantly different between the treatment groups but the female rats showed significant differences between the control group and other treatment groups.

Blood biochemical analysis

The levels of SGOT, SGPT, and urea in all groups were above normal, while in the satellite group, the values were lower than in the test group (Table 7), indicating that recovery occurred after the extract was discontinued.

Organ index

The test group animals experienced a decrease in the liver organ index and an increase in the kidney which indicated the occurrence of kidney lesions, where the size of the glomerulus was enlarged due to toxicants that accumulated in the glomerulus. Based on the statistical analysis (Table 8), there was only a significant difference in the liver organ index in female rats from the satellite group to other groups.

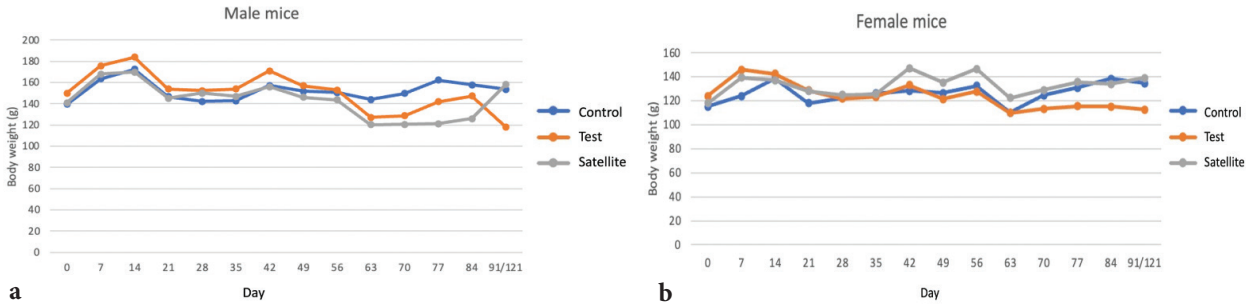

Figure 2. Average body weight of male (**a**) and female mice (**b**) during sub-chronic toxicity examination.

Table 5. The statistical analysis result of effect of HS extract on body weight.

	Sum of squares	df	Mean square	F	Sig	Duncan test (.05) Sig.
Male						
Between groups	3663.128	2	831.564	3.686	0.027	0.792
Within groups	76029.558	153	496.925			
Total	79692.686	155				
Female						
Between groups	1859.885	2	929.924	4.158	0.17	1.000
Within groups	34217.346	153	223.643			
Total	36077.231	155				

Table 6. Haematology examination.

	Hb (g/dL)	HCT (%)	Leukocyte (10^3/mm^3)	Erythrocyte (10^6/mm^3)
Male				
Normal Min.	11.52	37.24	6.63	6.76
Control	14.65	50.25	3.55	8.59
Test	16.22	54.25	3.14	8.31
Satellite	16.12	53.25	3.82	8.80
Normal Max.	16.13	50.63	12.63	9.75
Female				
Normal Min.	11.53	37.24	6.63	6.76
Control	13.43	41.22	3.85	6.70
Test	16.22	53.75	2.52	8.66
Satellite	16.32	51.75	3.81	8.88
Normal Max.	16.1	50.6	12.6	9.75

The gastric mucosa index

There was no evidence of gastric ulcers in the test and satellite groups (Table 9), which means that the long-term consumption of the HS extract does not cause gastric disorders.

Histopathological organ examination

Histopathological examination of the liver revealed mild damage namely steatosis, damaged sinusoids, narrowing of the central vein (only in the female rat group), irregular hepatocytes, pyknosis cell nuclei undergoing necrosis or apoptosis, and increased Kupffer cells (Fig. 3).

Histopathological examination of the kidneys showed no changes or minor damage in the form of enlargement of the glomerulus and narrowing of Bowman's space (Fig. 4).

Table 7. Blood biochemical analysis.

	Control	Test	Satellite	Normal score
Male				
SGOT (IU/L)	192.2	233.2	138.0	63.3
SGPT (IU/L)	87.2	112.7	116.5	23.9
Urea (mg/dL)	40.2	63.4	55.2	14.7
Creatinine (mg/dL)	0.2	0.2	0.3	0.5
Female				
SGOT (IU/L)	113.7	189.2	145.2	63.3
SGPT (IU/L)	69	105.7	79.3	23.9
Urea (mg/dL)	35	63.2	56.2	14.7
Creatinine (mg/dL)	0.2	0.3	0.3	0.5

Table 8. The statistical analysis result of the HS effects on the liver organ index.

	Sum of squares	df	Mean square	F	Sig	Duncan test (.05) Sig.
Male						
Between groups	0.011	2	0.005	1.185	0.349	–
Within groups	0.041	9	0.005			
Total	0.052	11				
Female						
Between groups	0.013	2	0.007	9.476	0.006	0.155
Within groups	0.006	9	0.001			
Total	0.019	11				

Table 9. The gastric mucous index.

	U_N	U_S	U_P	UI
Male				
Control	1.00	1.00	0	2.00
Test	1.00	1.00	0	2.00
Satellite	1.00	1.00	0	2.00
Female				
Control	1.00	1.00	0	2.00
Test	1.00	1.00	0	2.00
Satellite	1.00	1.00	0	2.00

Discussion

Hibiscus sabdariffa L. (Malvaceae) tea is widely consumed as a beverage and as a treatment for hypertension and hyperlipidemia (Hopkins et al. 2013). Roselle drink has been reported could elevate plasma high-density lipoproteins, and significantly reduced both systolic and

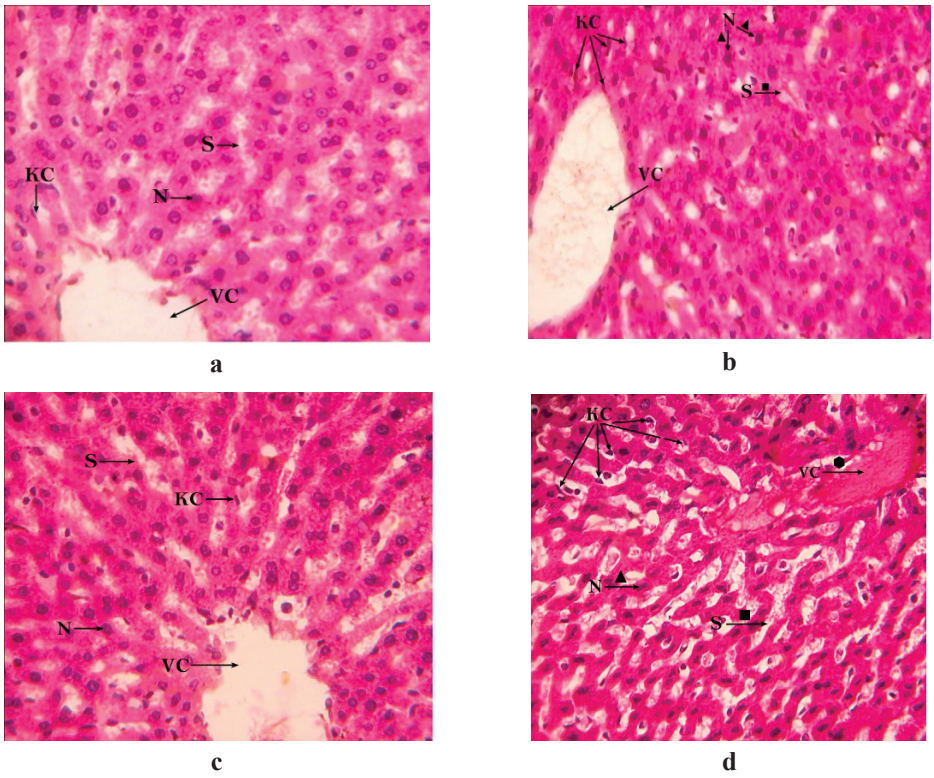

Figure 3. Histopathological examination of rat liver organ with (H-E), (400X): **(a)** male control group, **(b)** male test group; **(c)** female control group, **(d)** female test group. Kuppfer cel **(KC)**, Nucleus **(N),** vena centralis **(VC)**, sinusoid **(S)**. Histopathological changes on the organ were marked with: **black rectangle:** minor damage; **black circle:** cell are constricted; **black triangle:** pyknosis.

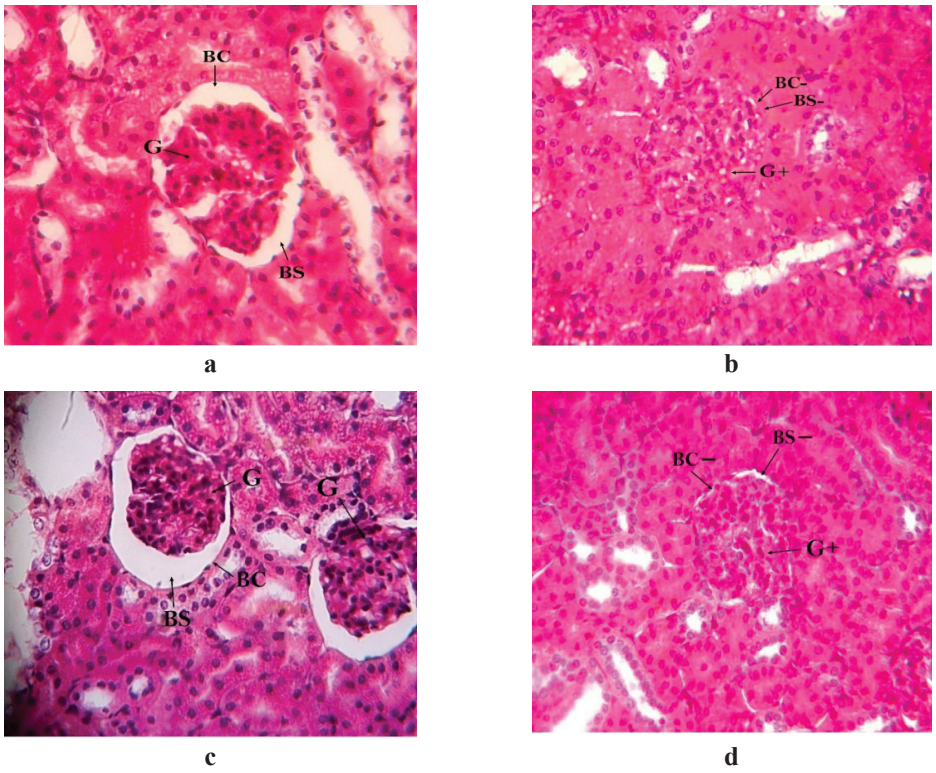

Figure 4. Histopathological examination on rat kidney organ with (H-E),(400X): (**a**) male control group, (**b**) male test group, (**c**) female control group, (**d**) female test group. Glomerulus (G), Capsule Bowman (CB), Bowman's space (BS). Histopathological changes in the kidney were marked with: (+) enlargement and (-) narrowing on Bowman cell.

diastolic blood pressure in healthy subjects (Diantini et al. 2021). Moreover, a recent clinical trial reported that 6 months of administration of roselle drink, could improve the levels of glutathione, superoxide dismutase (SOD), glucose-6-phosphate dehydrogenase (G-6-PDH), glutathione peroxidase (GSH-Px), and glutathione reductase (GSH-Rd) in healthy subjects (Chiu et al. 2022). In traditional medicine, either maceration with cold water or hot decoction is the most widely applied extraction method for the preparation of roselle herbal teas (Salem et al. 2022). In their study, Hopkins (2013) concluded that there are consistent results on the beneficial effects of HS extracts on lowering blood pressure. Although the same effects on blood pressure occur in normotensive animal models, the positive effects are greater in hypertensive animals. There is evidence that the positive effects are dose-dependent at lower doses.

Administration HS extract and fractions markedly reduced the elevated blood pressure that could be related to the anthocyanins' hypotensive constituent of *Hibiscus sabdariffa*, including delphinidin-3-*O*-sambubioside (hibiscin) and cyanidin-3-*O*-sambubioside (gossypicyanin), which were reported to possess the ACE inhibition activity (Salem et al. 2022).

In our study, the ethanol extract and ethyl acetate fraction showed significant antihypertensive effects and were known to contain secondary metabolites of the flavonoid and polyphenol groups. This was also reported by other studies which stated that the main bioactive compounds in roselle flower petals were flavonoids, polyphenols and anthocyanins, as well as organic acids (Da-Costa-Rocha et al. 2014).

Medicinal plants are presumed to be safe without any compromising health effects. Appropriate use of medicinal plants in dietary supplementation is very important in the maintenance of health (Manaharan et al. 2014). Many studies have reported the harmful effects of the improper use of medicinal plants. Therefore, evaluating the toxicological effects of any medicinal plant extract intended to be used in animals or humans is a crucial part of its assessment for potential toxic effects. This study finds that *H.sabdariffa* belongs to the slightly toxic category because the dose is in the 5–15 g/kg BW.

Different from several previous similar studies, Sari et al. (2016) reported that the administration of ethanol extract of rosella calyces to SD rats found the LD_{50} at 850.90 mg/kg BW. Adeyemi et al. (2014) reported that methanol extract of roselle calyces also showed LD_{50} at a dose of 3200 mg/kg BW within 72 hours. Sireetawong et al. (2013) reported that water extract with a single dose 5,000 mg/kg BW did not cause death in test animals for 14 days (Sireeratawong et al. 2013; Adeyemi et al. 2014; Sari et al. 2016).

While sub-chronic toxicity deals with the adverse effects of single doses, many chemical substances which are given in repeated doses do not produce immediate toxic effects. Delayed effects may occur due to the accumulation of the chemical in tissues or to other mechanisms. Thus, it is important to identify any toxic potential of the substance by a sub-chronic toxicity test.

The use of a satellite group of test animals, given the highest dose and then observed after the ending of dosing, is to give additional information on the persistence or reversibility of effects (Sireeratawong et al. 2013).

According to Manaharan et al. (2014), after some exposure to potentially toxic substances, there will be a slight reduction in body weight gain. Our study finds that the average body weight of male and female rats from each group was significantly different, which in the test group was lower than in the control group. This suggests that administration of the HS extract affects the body weight of the rats. We also observed, there was a significant difference in the liver organ index in female rats from the satellite group to other groups, while the kidney organ index had no significant difference.

The gross and microscopic observations conducted in all the above-mentioned organs further suggested there is minor damage caused by the administration of the extract at the concentrations studied for 90 days consecutively.

The previous reports on the toxicity profile of HS showed no observed toxicity at 15 g/kg calyces (high dose) of aqueous and ethanol extracts in mice within 7 days after oral administration. Sireeratawong et al. (2013) also reported the safety HS (red species) aqueous calyces extract when administered a single oral dose of 5,000 mg/kg BW after fourteen days. They also reported safety at doses between 50 and 200 mg/kg BW of extract after oral administration every day for two hundred and seventy days.

This study revealed *H.sabdariffa* extract has some toxic effects in rats on sub-chronic administration. In addition, the extracts produced a significant diuretic activity. Hence, prolonged oral consumption of the extract may not be recommended (Njinga et al. 2020).

Conclusions

Based on the comprehensive analysis, it is evident that the ethyl acetate fraction of *Hibiscus sabdariffa* L. petals exhibits remarkable antihypertensive activity comparable to that of the positive control. In the context of acute toxicity, the ethanolic extract from roselle calyces falls within the classification of slight toxicity. Neuropharmacological assessment indicates that the test preparation has a profound impact on both central and autonomic nervous system activities. Sub-chronic toxicological evaluations reveal significant adverse effects in specific physiological parameters for both male and female Wistar rats, in addition to minor hepatic and renal damage. Therefore, caution is advised when administering the extract, particularly at doses approaching or exceeding the identified threshold, underscoring the necessity for further mechanistic studies and clinical evaluations to substantiate the safety and efficacy of this plant-based intervention.

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