

## Anti-obesity and haematological effects of Malaysia *Hibiscus sabdariffa* L. aqueous extract on obese Sprague Dawley rats

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### ABSTRACT

**Background:** *Hibiscus sabdariffa* L. (Hs; Malvaceae) is commonly known as roselle or red sorrel in English or karkadè in Arabic. It is a tropical plant native to India and Malaysia. Roselle extracts have been widely reported to have beneficial health effects. The aim of this study was to determine the effective dose of Hs aqueous extract, which is a possible reducing agent in diet-induced obese rats, and monitoring any toxicological effect.

**Methods:** Male Sprague Dawley rats (n= 24) aged 6-7 weeks and weighing 210 ± 3.5 g (mean ± S.E.M) were used. The rats were fed a high fat diet (HFD) for 8 consecutive weeks to induce obesity. The animals were then randomly assigned to one of five groups based on the concentration of Hs aqueous extract. The normal group (NG) received a normal diet (commercial chow) throughout the study. The obese group (ObG) included obese rats administered with tap water ad libitum. Groups 1 (150 mg/kg), 2 (200 mg/kg), 3 (250 mg/kg), and 4 (300 mg/kg) were obese rats continuously fed with HFD in combination with the Hs extract for 10 weeks. The effect of Hs on some haematological and blood biochemical parameters were also evaluated.

**Results:** Hs aqueous extract is a rich source of anthocyanins. The main compounds detected are delphinidin-3-*O*-sambubioside and delphinidin-3-*O*-sambubioside. The extract also possesses

high antioxidant properties which may be caused by these anthocyanins. The anti-obesity effect of Hs aqueous extract was demonstrated by the significant reduction in the weight gain and abdominal weight ( $p < 0.05$ ) between treated and non-treated groups, which was dose-dependent. Overall, our study demonstrated that oral administration of Hs at doses of 150, 200, 250, and 300 mg/kg for ten weeks did not cause any toxicity effect within the obese rats.

**Conclusion:** The results of the study implied Hs aqueous extract at 300 mg/kg is the dose which can the most weight reduction effect with no severe haematological and biochemical changes in all experimental animals.

**Keywords:** *Hibiscus sabdariffa*, obese rats, roselle, aqueous extract, and body weight

## INTRODUCTION

Overweight and obesity are chronic disorders which are defined as abnormal or excessive fat accumulation caused by imbalance in energy intake and expenditure. Being obese or overweight can lead to various metabolic disorders such as cardiovascular disease, dyslipidaemia, hypertension, and diabetes [1, 2].

Furthermore, diets which contain high-fats foods generally contribute to obesity. Accordingly, the inhibition of the digestion and absorption of dietary fats is a promising remedy for obesity. The potential effect of natural products to counteract obesity have been widely documented [3], along with reports on the multiple natural product combinations that can lead to synergistic effects which increase their bioavailability and mechanism of action. These natural products are preferable over current obesity drugs such as Orlistat, Lorcaserin, and Sibutramine which have various side effects such as the development of cardiovascular problems, restlessness, insomnia, and stomach pain [4].

*Hibiscus sabdariffa* L. (Hs; Malvaceae) is commonly known as roselle or red sorrel in English and as karkadè in Arabic [5]. This tropical plant is native to India and Malaysia and is also widely grown in South East Asia and other tropical countries such as America and Africa [6]. *Hibiscus sabdariffa* L. is an erect annual plant that consists of red calyx. Red calyx is thick, fleshy, and has a brilliant red colour. The plant is consumed worldwide as a cold beverage and hot drink (sour tea) [7]. Roselle is also used for making jellies, jams, preserves, and sauces [8]. Due to the high pigment content in their calyces they are also a good source of natural food colorant [9]. In addition to being used in beverages and food based products, roselle has been used as animal feed and within nutraceutical, cosmetics, and pharmaceutical industries [10]. Pharmacologically, this plant has been reported to have anti-obesity [11], cardio protective, antihypertensive, and antioxidant effects [12, 13].

Hs calyx extract contains phenolic compounds, which recently attracted a great deal of attention due to their beneficial effects in promoting human health and well-being [14]. Several organic and phenolic acids such as citric acid, hibiscus acid, or (+)-hydroxycitric acid (HCA) are reported to be present. The latter is known as (+)-HCA in which its isomer (-)-HCA is the active ingredient also reported to be present in *Garcinia indica* and *Garcinia cambogia* fruits, the inhibitor of citrate lyase [15]. Flavanoids such as anthocyanins are the main compounds present in calyces, which are widely known to be effective in controlling body weight and can help in the prevention or treatment of obesity [16, 17]. Accordingly, the present study was designed to

investigate the effects of Hs aqueous extract at different doses as a possible reducing agent in diet-induced obese rats and its toxicological effects.

## **MATERIALS AND METHODS**

### ***Sample collection and preparation***

Matured fresh dark-red calyces of Hs were purchased from HerBagus, Penang, Malaysia. The sample was authenticated by the Forest Research Institute Malaysia (FRIM). A voucher specimen No PID 050515-05 was deposited at the Herbal Medicine Research Centre, Institute for Medical Research, Malaysia.

Fresh Hs calyces (1 kg) with seeds removed were washed with water and air dried. The dried samples were grounded to a fine powder using a universal cutting mill (Fritsch, Germany). The powdered form of Hs was extracted in hot water (80°C) with 1% trifluoroacetic acid (TFA) and filtered by gauze. The filtrates were then evaporated using Virtis freeze mobile (Massachusetts, USA) to yield the aqueous extract. The extract was stored at -4°C until further analysis.

### ***Standards, chemicals and reagents***

All solvents were of High Performance Liquid Chromatography (HPLC) grade. All chemicals were of analytical grade. Formic acid, methanol, and acetonitrile were purchased from Fisher Scientific (Malaysia). Ultrapure water was obtained from the Milli-Q gradient water purification system (Millipore MA, USA). Folin ciocalteau reagent was purchased from Sigma-Aldrich (USA) and 2, 2-diphenyl-1-picrylhydrazyl was from Extrasynthase (Genay, France). Anthocyanin standards (cyanidin-3-*O*-sambubiside and delphinidin-3-*O*-sambioside) were purchased from Extrasynthase (Genay, France). Individual stock solution was made using appropriate dilution in methanol: water (w:v ; 1:1) and stored in amber glass vials at -20°C.

### ***Determination of anthocyanins by HPLC***

HPLC analyses were carried out in a UHPLC system from Thermo Scientific (USA) and consisted of an autosampler, quaternary pump, thermostated column compartment, and a diode array detector (DAD). UV/Vis spectra were recorded from 200-600 nm, with a selected wavelength at 520 nm for anthocyanins.

Chromatographic separation was performed on a reversed phase column Kinetex C18 (150 x 4.6 mm, 5 µm) from Phenomenex (USA) at a flow rate of 1.2 mL/min. The column compartment was maintained at 40°C. The sample was dissolved in the methanol (10 mg/mL) and passed through a 0.22 µm PTFE membrane filter prior to injection. The mobile phases used were mobile phase A (water with 0.1% formic acid) and mobile phase B (acetonitrile with 0.1% formic acid). Separation (10 µL of injection volume) was carried out using the following multi-step gradient as follows: 0-2 min, 10% B-isocratic; 1-15 min, 10-40% B-gradient; 10.1-13 min, 90% B-isocratic; and a conditioning cycle of 2 min utilizing these initial conditions for subsequent analysis.

### ***Total polyphenols***

The total polyphenols assay was performed using a Folin ciocalteau assay based on a method of Singleton and Rossi [18]. The method allows determination of oxidized phenols by producing a blue colour from heteropoly phosphomolibdate-tungsten anions. The darkness of the blue colour indicates the presence of phenols. The darker the shade the more phenols are present.

### ***Ferric Reducing Antioxidant Power (FRAP)***

The antioxidant activity of Hs extract was measured using FRAP assay after a method of Benzie and Strain [19] with some slight modifications. 10  $\mu\text{L}$  of Hs extract was mixed with 200  $\mu\text{L}$  of FRAP reagent and incubated for 10 minutes at room temperature. The absorption of the samples was measured on a spectrophotometer (Biotek) at 515 nm and results were expressed as mM  $\text{Fe}^{2+}/\text{mg}$ .

### ***Animals and feeding protocols***

The experimental protocol was approved by the Animal Care and Use Committee (ACUC), Ministry of Health Malaysia (ACUC/KKM/02(1/2015). Male Sprague Dawley rats ( $n=24$ ) aged 6-7 weeks and weighing  $210 \pm 3.5$  g (mean  $\pm$  SEM) were supplied by Laboratory Animal Resource Unit, Institute for Medical Research, Malaysia.

High fat diet (HFD) was prepared as follows: a standard animal chow (Gold Coin, Malaysia) was the source of dietary fat, with cholesterol (32%) and Vitamin D were added respectively based on the method by Nik Norliza et al [20]. Twenty four rats were randomly and equally divided into six groups. The first group was a normal group with a normal commercial diet, while the remaining five groups were fed the HFD diet. For obese groups, the animals were fed with HFD for eight weeks in order to yield the obese rats of body mass index (BMI)  $\geq 0.68$ . Afterwards, they were divided into five groups of four rats each. Obese Control Group (ObG) was maintained with HFD as a control. The remaining four groups (Group 1, 2, 3, and 4) received HFD followed by treatment with 150, 200, 250, and 300 mg/kg of aqueous extract of Hs respectively. Two rats were maintained in each cage, under a controlled temperature, humidity, and proper illumination conditions with distilled water and HFD for ten weeks. Food ingestion and body weight were recorded weekly. In the normal group (NG), animals were fed only with the standard diet (commercial chow) throughout the experiment. At the end of the experimental period, the diets were withdrawn for at least 4 hours before necropsy.

### ***Blood collection for haematology and biochemistry analyses and fat measurement***

All animals were euthanized at the end of week 10 with sodium pentobarbiturate (60 mg/kg body weight intraperitoneal). Blood (5 mL) was collected via cardiac puncture and was transferred into two tubes. The first tube with EDTA was analysed immediately for determination of haematology parameters by SYSMEX KX-21N Haematology Analyser (Sysmex Cooperation, Kobe, Japan) which include haemataocrit (HCT), haemoglobin concentration (HB), erythrocyte counts (RBC), total and differential leucocyte count (WBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), lymphocyte percentage, lymphocyte number, and platelet were measured.

In the second tube without EDTA, blood was immediately centrifuged at 1,500 g for 4°C for 10 min. The serum was carefully aspirated with Pasteur pipette into sample tube and was stored at -20°C. Biochemical examinations were performed using Vitalab Selecta, E series (Netherlands). Clinical biochemistry values such as albumin (ALB), total bilirubin, blood urine nitrogen (BUN), phosphatase (ALP), Alanine Aminotransferase (ALT), and cholesterol were also determined.

### ***Statistical analysis***

All values were expressed as mean  $\pm$  standard error of mean (S.E.M) in each experiment. When appropriate, one-way ANOVA with post-hoc or unpaired t-test was used to determine significant differences between the groups using GraphPad Prism version 6.00 for Windows (GraphPad

Software, La Jolla California USA). All tests were two-tailed and the significance level was set at  $p < 0.05$ .

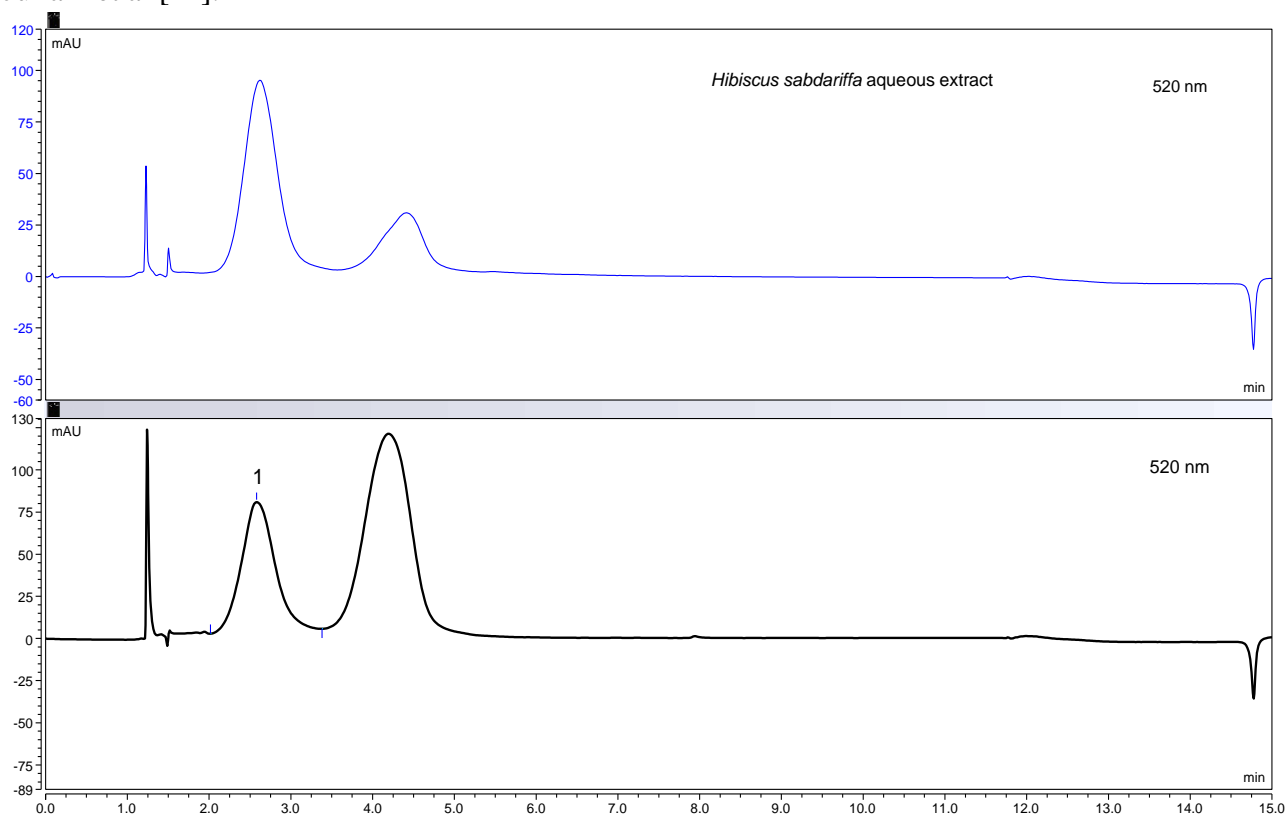
## RESULTS

### *Content of total phenol and antioxidant activity in Hs extract*

Hs aqueous extract yielded  $510.7 \pm 0.0$  mg gallic acid equivalent (GAE)/100 g of total phenolic content and FRAP assay resulted  $0.751 \pm 0.0$  mM  $\text{Fe}^{2+}$ .

### *HPLC quantification of anthocyanins*

HPLC absorbance profile at 520 nm is presented in Fig 1. Standard of delphinidin-3-*O*-sambubioside (1) displayed retention time at 2.7 min and cyanidin-3-*O*-sambubioside 4.6 min respectively. Standard curves were linear with  $R^2 = 0.99$  for compound 1 and  $R^2 = 0.98$  for compound 2. Injection of six replicates of Hs extract displayed the data corresponding to the compound 1 ( $104 \pm 0.0$   $\mu\text{M}$ ) and 2 ( $402.6 \pm 0.0$   $\mu\text{M}$ ) respectively. These results represent concentration of the main anthocyanins in aqueous extract of Hs (10 mg/mL) [21, 22]. Delphinidin and cyanidin are the main anthocyanins presence in roselle as reported by Cisse et al. [21] and Juliani et al [22].



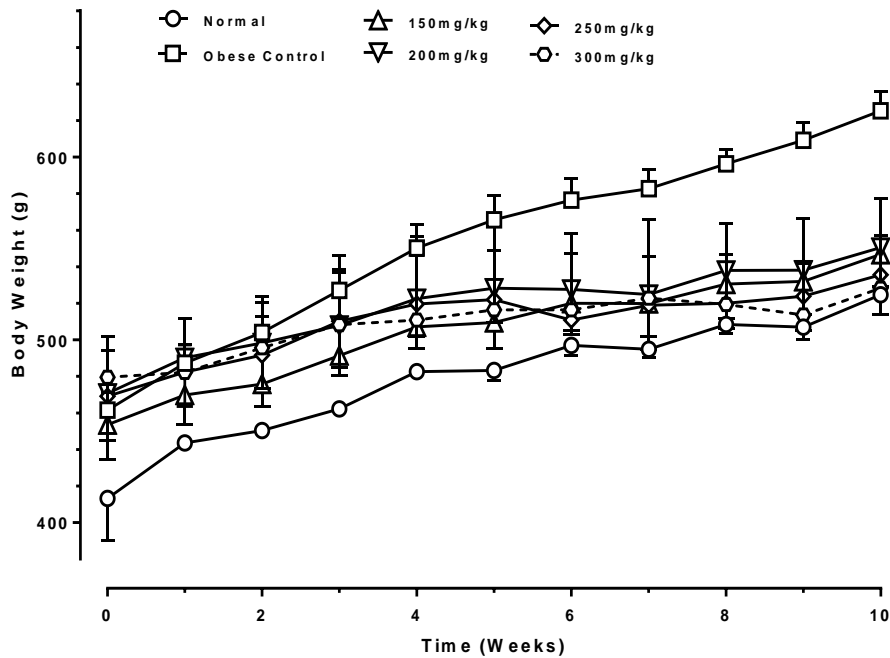
**Figure 1.** HPLC profile of *H. sabdariffa* aqueous extract and standard of anthocyanins at 520 nm. Compound 1- Delphinidin-3-*O*-sambubioside and compound 2-Cyanidin-3-*O*-sambubioside

### *Weight gain*

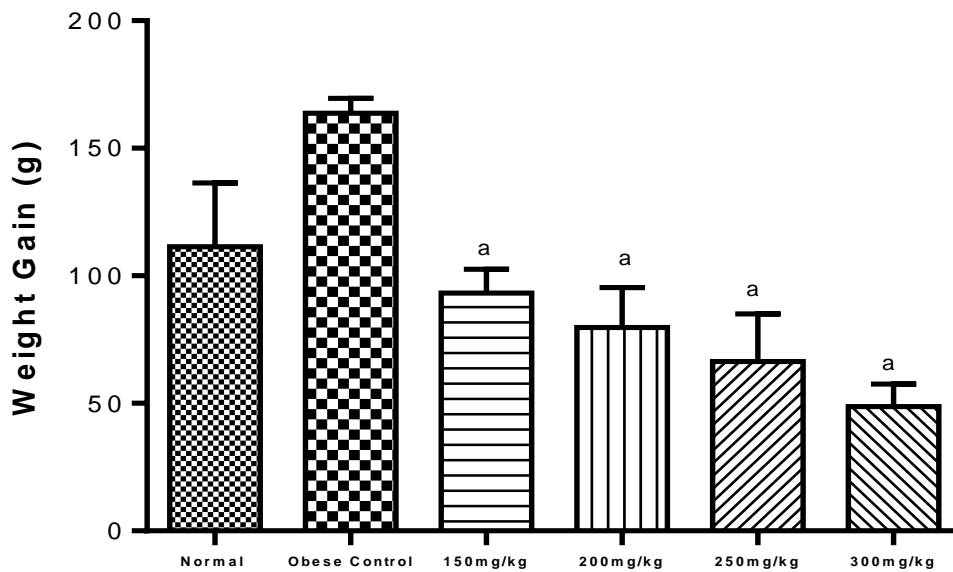
Generally, Hs extract suppresses the weight gain of the obese rats (Fig 2) with the lowest body weight gain was demonstrated in group receiving 300 mg/kg of Hs extract (Fig 3). For all control and treated groups; changes in body and organ weight are summarized in Table 1. Following 10

weeks of treatment with Hs extract, all obese rats had significantly ( $p < 0.05$ ) lower body weight compared to ObG, except for Group 3 (150 mg/kg).

In terms of weight gain, a positive trend was seen where the higher the dose the lower the weight gain observed among these obese rats. Abdominal fat (%) was also varied between all obese groups with the lowest observed in Group 4. With liver weight significant data was not obtained as all the experimental animals were shown to have 3-4% of weight gain of the liver.



**Figure 2.** The effects of oral administration of Hs extract (150, 200, 250, and 300 mg/kg) on body weight (mean  $\pm$  S.E.M) in obese rats.



**Figure 3.** The effects of oral administration of Hs extract (150, 200, 250, and 300 mg/kg) on weight

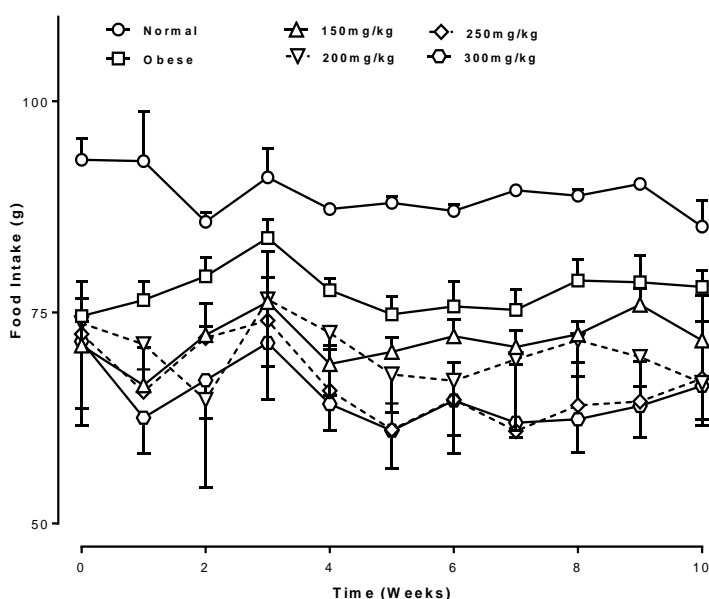
gain (mean  $\pm$  S.E.M) in obese rats. Values are mean  $\pm$  SEM of the mean. <sup>a</sup> $p < 0.05$  when compared with the Obese Group.

### Food intake

The effect of the Hs on the food intake is shown in Fig 4. At the initial stage (week 0), the NG was shown to have a significantly higher food intake compared to obese groups. The high food intake demonstrated in NG was consistent throughout the study. In obese rats, the amount of food intake was similar at week 0 in all groups.

However, by the end of the experiment (week 10), the tendency in food consumption varied between ObG and treated groups. The food intake was significantly lower in all treated groups when compared to both ObG and NG.

The results demonstrated how concentration of Hs was inversely associated with the food intake (150 > 200 > 250 > 300 mg/kg).



**Figure 4.** The effects of oral administration of Hs extract (150, 200, 250, and 300 mg/kg) on the food intake on obese rats.

### Biochemical and haematology analyses

The corresponding mean values ( $\pm$  S.E.M) of the biochemical and parametric values for the normal, obese, Group 1, 2, 3, and 4 rats with Hs are shown in Table 2 and 3.

The platelet levels (PLT) were also significantly higher among obese rats when compared with normal group. White blood cells (WBC) also significantly increased in all obese groups except for Group 2 when compared to the normal group.

The effect of the Hs on the biochemical parameters is shown in Table 3. Rats treated with 250 and 300 mg/kg of Hs significantly decreased the ALT levels when compared with the control groups ( $p < 0.05$ ). The cholesterol levels (CHOL) in the animal group decreased when treated at the higher dose (200, 250, and 300 mg/kg). However, these differences are not significant.

**Table 1.** The effect Hs extract on body weight, weight gain, liver weight and abdominal fat in obese rats following 10 weeks treatment of the extract.

Parameter	Dietary Group					
	Normal Group	Obese control	150 mg/kg	200 mg/kg	250 mg/kg	300 mg/kg
Initial body weight (g)	413 ± 22	461 ± 10	453 ± 18	470 ± 12	469 ± 25	479 ± 22
Final body weight (g)	525 ± 3.2*	625 ± 10	547 ± 17*	550 ± 18*	535 ± 42	528 ± 29*
Initial food intake (g)	93.1 ± 2.5	74.6 ± 4.1*	71.1 ± 2.8*	73.7 ± 4.6*	72.4 ± 1.6*	71.6 ± 3.0*
Final food intake (g)	85.2 ± 3.1	78.0 ± 1.9	71.7 ± 2.3*	66.7 ± 1.7**	67.2 ± 3.7**	66.3 ± 1.8**
Body weight gain (g)	111 ± 50	164 ± 6	93 ± 9**	80 ± 16**	66 ± 19**	49 ± 9**
Liver weight (g)	18 ± 0.6	17 ± 0.9	18 ± 0.7	19 ± 1.0	16 ± 1.4	16 ± 0.7
Liver weight <sup>a</sup> (%)	3 ± 0	3 ± 0	3 ± 0	4 ± 0**	3 ± 0	3 ± 0
Abdominal fat (g)	0	40.0 ± 1.7	26.0 ± 2.2**	22.0 ± 2.3**	26.0 ± 7.6	18.0 ± 2.2**
Abdominal fat <sup>b</sup> (%)	0	6 ± 0	5 ± 0**	4 ± 1**	5 ± 1**	3 ± 0**

\*  $p < 0.05$  when compared to Normal Group. \*\*  $p < 0.05$  when compared to Obese Group.

<sup>a</sup> Liver weight (g) / body fat (g) x 100; <sup>b</sup> (abdominal fat (g)/ body fat (g) x 100)

**Table 2.** The haematological values (mean ± S.E.M) in obese rats following 10 weeks treatment with Hs extract.

	Dietary Group					
	Normal Group	Obese control	150 mg/kg	200 mg/kg	250 mg/kg	300 mg/kg
RBC (x 10 <sup>6</sup> mm)	6.8 ± 0.4	6.2 ± 1.5	8.0 ± 0.0*	6.6 ± 0.7	7.9 ± 0.1*	8.4 ± 0.4*
MCV (µm)	47.7 ± 0.7	48.3 ± 1.0	47.7 ± 0.8	48.8 ± 0.8	47.3 ± 0.7	47.1 ± 0.6
HCT (%)	32.8 ± 1.8	30 ± 7.4	38.3 ± 0.5	32.5 ± 3.6	37.5 ± 0.3	39.8 ± 0.2
PLT (x10 <sup>3</sup> /mm)	101.3 ± 7.8	645.5 ± 1.7*	849.7 ± 117*	509.5 ± 192*	882.5 ± 8.5*	1016.3 ± 33*
MPV (µm)	7.5 ± 0.1	4.9 ± 1.7	6.7 ± 0.1	7.0 ± 0.4	6.8 ± 0.13	6.9 ± 0.2
WBC (x10 <sup>3</sup> /mm)	2.2 ± 0.5	4.6 ± 1.3	4.5 ± 0.4*	3.87 ± 1.2	5.2 ± 1.1*	6.0 ± 1.0*
HGB (g/dl)	12.5 ± 0.7	11.4 ± 2.8	14.4 ± 0.1*	18.5 ± 0.1*	14.2 ± 0.2	15.0 ± 0.6*
MCH (pg)	18.2 ± 0.3	18.4 ± 0.4	17.9 ± 0.2	17.9 ± 0.2	17.9 ± 0.4	17.8 ± 0.3
MCHC (g/dl)	38.2 ± 0.2	38.2 ± 0.2	37.6 ± 0.2	38.0 ± 0.5	37.6 ± 0.3	37.8 ± 0.1

\*  $p < 0.05$  when compared to Normal Group.



**Table 3.** Blood biochemical values (mean  $\pm$  S.E.M) in obese rats following 10 weeks treatment with Hs extract.

Parameter	Dietary Group					
	Normal Group	Obese control	150 mg/kg	200 mg/kg	250 mg/kg	300 mg/kg
Alb (g/L)	15.3 $\pm$ 8.9	14.5 $\pm$ 1.6	16 $\pm$ 1.1	11.8 $\pm$ 0.6	13.0 $\pm$ 0.4	12.8 $\pm$ 0.5
ALP I (U/L)	150 $\pm$ 86	138 $\pm$ 26	169 $\pm$ 24	121 $\pm$ 25	113 $\pm$ 12	112 $\pm$ 9.8
ALT I (U/L)	491 $\pm$ 180	113 $\pm$ 16	139 $\pm$ 27	206 $\pm$ 93	69 $\pm$ 3.0*	60 $\pm$ 4.7*
BUN (mmol/L)	5.2 $\pm$ 3.2	4.9 $\pm$ 0.2	3.5 $\pm$ 0.2	3.8 $\pm$ 0.6	4.1 $\pm$ 0.4	4.4 $\pm$ 0.3
CHOL (mmol/L)	1.8 $\pm$ 1.0	2.1 $\pm$ 0.3	2.1 $\pm$ 0.1	1.8 $\pm$ 0.0	1.9 $\pm$ 0.2	1.9 $\pm$ 0.1
TBI (Umol/L)	3.3 $\pm$ 1.9	3.0 $\pm$ 0.5	4.0 $\pm$ 0.4	3.3 $\pm$ 0.3	2.5 $\pm$ 0.5	3.5 $\pm$ 0.6

\* $p < 0.05$  when compared to Normal Group.

## DISCUSSION

A previous report conveyed how an effective drug of anti-obesity has the ability to decrease energy/food intake, increase energy expenditure, decrease preadipocyte differentiation and proliferation, and decrease lipogenesis and fat oxidation [10]. Regardless, besides drug treatments, incorporating functional foods which enhance satiety and inhibit appetite can also be an alternative for treating obesity [23].

The anti-obesity efficacy of Hs aqueous extract was determined by looking into its effects on the body weight, weight gain, and abdominal mass of HFD induced obese rats. Results demonstrated that 10 weeks of Hs oral administration (150, 200, 250, and 300 mg/kg) caused a dose-dependent decrease in (a) final body weight, b) body weight gain, c) abdominal fat, and d) blood cholesterol. These results are in line with previous findings on the effect of Hs as an anti-obesity agent [11, 24, 25].

The actual mechanisms by which the Hs extract causes reduction in body weight gain are not entirely clear. There are many possibilities that may be contributing to the anti-obesity effect of Hs, which includes the inhibition of adipocyte differentiation, carbohydrate digesting enzyme, or inhibition of pancreatic lipase [26]. Secondary metabolites like flavan-3-ols are known to act as pancreatic lipase inhibitors [27] which decrease fat absorption and energy intake that leads to weight reduction. These groups of polyphenol compounds include anthocyanins and non-phenolics such as organic acid. Hydroxycitric acid has also been discovered in the Hs extract.

These reported compounds not only result in beneficial antioxidant properties in the Hs extract but may also contribute to effectiveness as an anti-obesity agent. Another possible explanation for the weight reduction seen in rats treated with Hs extract may be due to how Hs suppresses appetite.

This explanation is supported by our result on how the amount of food intake was inversely associated with the dose given. Hs at a higher dose (200, 250, and 300 mg/kg) significantly lowered the food intake compared to those of normal and untreated obese rats. *Hoodia gordonii* is one example of appetite suppressant that regulates appetite, significantly reduce calorie intake and boost weight loss [28]. Compounds such as (-)-hydroxycitric acid (HCA) from *G. cambogia* is among one of the reported potent natural appetite suppressants. Besides anthocyanins, Hs also contains HCA, which is the principal organic acid found in the calyces. However, the (2S, 3R)-HCA from Hs calyces is different from (2S, 3S) from *G. cambogia*, which thereby raises the question of whether both diastereomers possess similar pharmacological properties [29].

Admittedly, the blood cholesterol lowering effect of Hs extract observed at higher dosage (200, 250, and 300 mg/kg) was not that significant. This result may be due to the active ingredient of delphinin-3-*O*-sambubioside and cyanidin--3-*O*-sambubioside which are significantly present in the Hs aqueous extract and act not only as an antioxidant but also as an anti-hypertensive and hypocholesterolemic [11, 30-32]. The results from HPLC analyses confirmed that these two compounds were the main anthocyanin detected similar to the finding by Khafaga et al [33]. In order to generate better results as a hypocholesterolemic agent, one possible solution is to recommend a higher dose of Hs. However, the correct dose must be chosen carefully in order to avoid saturation and any side effects. As previously reported, a dose of 2000mg/kg of the Hs extract caused severe weight loss, diarrhoea, and death in rats [34, 35].

The toxicity of the extract at different dosage was investigated by measuring the haematological and biochemical parameters of the blood sample taken from the experimental animals. No deaths, abnormalities in behaviours, or physical signs of toxicity were observed in rats after 10 weeks oral administration of the Hs. All haematological parameters after administration of the extract revealed no significant abnormality, except an increase in platelet (PLT) level as shown in Table 2. This parameter is useful as the PLT indices conditions which can cause problems with clot formation while also being used as a tool to monitor people with underlying conditions or who are undergoing treatment with drugs known to affect platelets. Therefore, further study is warranted to confirm whether this extract may have the adverse side effects on platelets.

Blood biochemical values were determined to assess any changes in the parameters. In general, if the biochemical values differ more or less than one fold than the normal values they were to be noted accordingly [36]. The ALT test is normally used to detect liver injury for people who are at an increased risk for liver disease. Many people with mild liver damage will have no signs or symptoms which include people who are overweight and/or have diabetes. In this study, the level of ALT significantly decreased with Hs at a higher dose (250 and 300 mg/kg), results which are similar to previous studies [32]. In contrast, a study by Fayeke et al. [35] reported that doses of 300 mg/kg of Hs over 3 months has an adverse effect on liver enzyme. ALT is a leakage enzyme and its elevation in circulation indicates hepatocellular damage. Low levels of ALT after Hs intake may indicate a decrease in fat deposition and necrosis in liver cells. However, further study is necessary to study the effect of the Hs extract on liver cells. Although there were slight changes in the haematological and biochemical parameters observed, it can concluded overall that Hs extract at various doses following 10 weeks of treatment do not possess any toxic effect to the experimental animals.

## CONCLUSION

The effects of different doses (150, 200, 250, and 300 mg/kg) of Hs aqueous extract on obese rats and the biological safety from toxicology point of view was investigated. The results obtained demonstrated how most of the biochemical parameters remained in the normal range, indicating there was no hepatic or renal toxicity with the oral administration of the extracts. In conclusion, this study supported previous findings of the effect of the aqueous extract of Hs on weight management. Therefore, we can recommend that the collateral effect of Hs extract at a greater concentration used in this experiment should be considered as a possible weight management agent in addition to its ability to reduce blood cholesterol.

**List of Abbreviations:** ALB, albumin; ALT, Alanine Aminotransferase; ALP, phosphatase; BUN, blood urine nitrogen; CHOL, cholesterol; GAE, gallic acid equivalent; HB, haemoglobin; HCA, (-)-hydroxycitric acid (HCA); HFD, High fed diet; HTC, haemataocrit; Hs, *Hibiscus sabdariffa*; MCHC, mean cell haemoglobin; MCV, mean cell volume; NG, normal group; ObG, obese control group; PLT, platelet; RBC, erythrocyte counts; WBC, white blood cell.

**Competing Interests:** The authors report no declaration of interest.

**Authors' Contribution:** Maizatul Hasyima Omar and Norsyafawati Shamsahal designed and performed the experiments. Hussin Muhammad performed the toxicology studies. Wan Amir Nizam Wan Ahmad helped carry out the preparation of high fed diet for obese rats. Maizatul Hasyima Omar wrote the manuscript in consultation with Hussin Muhammad, Wan Amir Nizam Wan Ahmad and Mohd Isa Wasiman.

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