Effect of *Hibiscus sabdariffa* extract on the body weight, complete blood count, liver function, and lipid profile in rabbits

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Abstract

The objective of the study conducted here was to investigate effect of *Hibiscus sabdariffa* extract on the body weight, complete blood count (CBC), liver function, and lipid profile in rabbits. A total of 20 local male rabbits were randomly divided into four experimental groups, including five rabbits in each group: (i) control; (ii) starvation; (iii) *H. sabdariffa* extract; and (iv) *H. sabdariffa* extract and starvation. Blood samples were collected directly from the heart at the end of the experiment (day 28) and used for complete blood count and estimation of cholesterol, triglycerides, ALT, and AST. Aorta and liver sections were histopathologically examined. Results indicated that the Body weight at the end of the study was significantly decreased in starvation, extract, and starvation and extract groups, compared to the beginning of the study for each group. There was no difference in the body weight between groups at the end of the study. Rabbits in the extract group showed significant increase in lymphocytes, RBC, hemoglobin, and platelets, compared to those in control and in starvation groups. ALT activity significantly increased in starvation, extract, and starvation and extract groups, compared to those in control group. AST activity significantly increased in both extract and starvation and extract group, compared to those in control and starvation groups. Cholesterol and triglycerides decreased in starvation, extract, and starvation and extract groups, compared to those in control group. Histopathological sections indicated simple changes in aorta layers in starvation group rabbits including few number foam cells in tunica media. Liver sections from starvation group rabbits showed coagulative necrosis of hepatocytes, hyperplasia of bile duct endothelia, dilation and congestion of central veins. In extract group there was dilation of central veins, vacuolar degeneration of the hepatocytes, infiltration of inflammatory cells and hepatic artery congestion. Similar changes were revealed in liver sections from starvation and extract group. In conclusion, aqueous *H. sabdariffa* can decrease the body weight as alternative to the starvation, decrease blood cholesterol and triglycerides, and increase the immunity; however, daily consumption can have impact on the liver.

Keyword: *Hibiscus sabdariffa*, starvation, rabbits, body weight, lipid, liver

INTRODUCTION

Hibiscus is a large genus plant from the family “Malvaceae” includes 200 to 220 species primarily cultivated for the use of the flowers, leaves, and seeds, and known as rosella or rosemallow (Hussein et al., 2010). Although hibiscus is a native to tropical Africa, it can also be found in Indian and Southeast Asia including Thailand and Malaysia (Mahadevan et al., 2009). Fresh or dried calyces are used for preparation of herbal drinks and various hot or cold beverages (Bako et al., 2010; Ismail and Nazri, 2008). In Egypt, Sudan and Nigeria, fresh calyces are used in preparing a drink known as Caredy tea, Karkade, or Zoborodo, respectively (Gibbor and Pain, 1985; Kochhar, 1986).

The nutritional composition of fresh calyces *Hibiscus sabdariffa* can vary depending on genetic, environmental, ecological, and harvest conditions of the plant (Da-Costa-Rocha et al., 2014). The leaf contains protein, fat, carbohydrate, fiber, ash, calcium, phosphor, iron, thiamine, β-carotene, riboflavin, niacin and ascorbic acid (Duke and Atchley, 1984). The calyces contain the flavonoids, alkaloids, β-sitosterol, anthocyanin, citric acid wax, pectin, crude protein and different minerals (Hirunpanich et al., 2006; Singh et al., 2017).

Hibiscus is considered safe medical plant (Abbas et al., 2011). Aqueous extract of *H. sabdariffa* petals exhibited antihypertensive and cardio-protective effects in rats (Odigie et al., 2003). Consumption of three servings of *H. sabdariffa* tea daily lowered the blood pressure in pre-and mid hypertensive adults (McKay et al., 2010). In addition, *Hibiscus sabdariffa* can have the antihypertensive effect via three major mechanisms: diuretic (Jiménez-Ferrer et al., 2012), vasodilator (Sarr et al., 2009), and angiotensin converting enzyme inhibitor (Ojeda et al., 2010). However, *H. sabdariffa* extract can reduce high blood pressure on long term as antioxidant (Chen et al., 2004), which considered a cardio-protective effect. In addition, hibiscus buds can remove burning sensations and relieve pain (Alka et al., 2009). The analgesic effect of the flower or leaves extracts is thought to be via inhibiting prostaglandin synthesis; however, the methanolic extracts endowed with analgesic effect mediated through central inhibitory mechanism (Zahid et al., 2014). Furthermore, *H. sabdariffa* can manage different medical conditions including cancer, inflammatory disease, and different cardiovascular problems (De-Casta-Rochal et al., 2014), as well as antibiotics resistance (Voon et al., 2012).

People are excessively use herbal plants, including hibiscus tea, particularly in some Arabic regions. However, effect of hibiscus on some body function is not well studied. The objective of this study was to investigate effect of *H. sabdariffa* extract with starvation on the body weight, complete blood count (CBC), liver function, and lipid profile in rabbits.

MATERIALS AND METHODS

Hibiscus sabdariffa extract preparation

Dry calyxes of *H. sabdariffa* were obtained from local market in Mosul, Iraq, and identified at the Herbarium of Botany Department, University of Mosul. A 10% aqueous extract was prepared as the following: 10 gram of dry *H. sabdariffa* calyx was added to 100 ml of boiled distilled water. The mixture was kept for 15 minutes at room temperature, and then filtered using Whatman filter paper, and stored at 4 °C.

Study Animals

A total of 20 local male rabbits 9 to 10 months old were used in this study. The rabbits were kept in an appropriate environment (temperature 28 °C, and 12 hrs dark/light). The rabbits were fed with a standard diet prepared locally and included 47% wheat bran, 38% crushed local barely, 10% soybeans, 2% protein concentrate, 1% limestone powder, 1.5% NaCl, and 0.5% minerals and vitamins mixture.

Experimental groups

The rabbits were randomly divided into four experimental groups, including five rabbits in each group: (i) control group (CG), where rabbits fed 3 times per day (8 am, noon, 6 pm) and received distilled water once daily at 6 pm (1ml water / 1kg BW); (ii) starvation group (SG) for 16 hrs; where rabbits were fed and received distilled water once daily at 6 pm (1ml water / 1kg BW); (iii) *H. sabdariffa* extract group (HG), where rabbits fed 3 times per day (at 8 am, noon, 6 pm) and received 10% aqueous *H. sabdariffa* extract once daily at 6 pm(1ml extract/ 1kg BW); and (iv) *H. sabdariffa* extract and starvation group (HSG), where rabbits were fed 1 time per day (6 pm) and received 10% aqueous *H. sabdariffa* extract (1ml extract/ 1kg BW). Water was open to all rabbits all the time. Rabbits in all groups were initially weighted and allowed one week of acclimatization before starting the experiment. In each groups, the rabbits received the treatment (distilled water or the extract) once daily at 6 pm for four weeks. At the end of the experiment, five ml of blood were collected from the heart directly, and then all rabbits were slaughtered by cervical dislocation. Liver and aorta (from the arch to the iliac bifurcation) were harvested.

Blood analysis

Blood EDTA samples were tested for complete blood count (CBC) including total red blood cells count, deferential leukocytes count, hemoglobin, and platelets count using hematology analyzer (Siemens Healthcare Diagnostics, Eschborn, Germany). Blood serum samples were analyzed via cobas c111 analyzer (Roche DiaLog, New Zealand) to estimate cholesterol, triglycerides using commercial kits (Biocon, Bengaluru, India), and ALT and AST using commercial kits (Biolabo, Maizy, France)

Histopathological sections

Liver and aorta sections were prepared according to Drury et al (1985) including the following steps: washing, .dehydration, clearing, infiltration and embedding, and trimming and sectioning. Finally, the sections were stained using hematoxylin and eosin (Luna, 1968).

Statistical Analysis

Differences between study groups were examined using one-way analysis of variance (ANOVA), and the difference between body weight at day 0 and 28 in each group was examined using t-test (Moore et al., 2009). The data were presented as Mean ± Standard Deviation. The difference with value of $P \leq 0.05$ was considered statistically significant. Statistical analysis performed using STATA 13.0 (StataCorp, College Station, TX).

RESULTS AND DISCUSSION

Body Weight

Means of the body weight between the groups were not different at the beginning of the study ($P = 0.19$). Body weight at the end of the study (day 28) was significantly decreased ($P \leq 0.05$) in SG, HG, and HSG, compared to the beginning of the study (day 0) for each group. However, there was no difference in the body weight between groups at the end of the study ($P = 0.27$) (Fig 1).

In starvation, loss of body weight occurs due to loss of adipose tissue subcutaneous and around different internal organs in addition to muscle atrophy (Madea, 2005). In our study, treatment with aqueous *H. sabdariffa* extract decreased the body weight as same as for that for starvation. However, effect of *H. sabdariffa* extract on decreasing adipose tissue has not been examined, yet. A consumption of 500 mg per day of LC-HS for two months in significantly reduced body weight, abdominal circumference, and percentage of body fat overweight women and in obese volunteers consuming the polyphenolic extract (Herranz-Lopez et al., 2019; Al-Snafi, 2018). One explanation for *H. sabdariffa* extract decreased body weight is through reduce of dietary carbohydrate absorption; as the plant contains enzyme inhibitor blocks production of amylase, an important enzyme for carbohydrate break down (Singh et al., 2017). An additional possible explanation for the anti-obesity effect for *H. sabdariffa* is through the effect of the extract on leptin and ghrelin, as the extract increased glucagon-like peptide-1 and decreased ghrelin hormones (Herranz-Lopez et al., 2019).

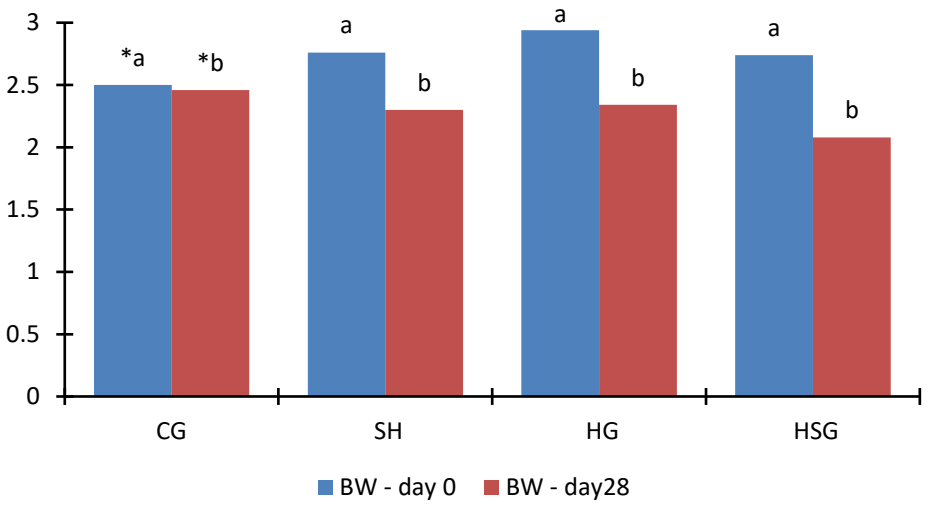


Fig 1. Differences in body weight (kg) between study groups; control (CG); starvation (SG); *H. sabdariffa* extract (HG); and *H. sabdariffa* extract and starvation (HSG) groups, compared within groups and between day 0 (beginning of the study) and day 28 (end of the study). Different letters (i.e., a, b) refer to statistical difference ($P \leq 0.01$), star (*) means no statistical difference between the groups.

Hematology

Differences in hematological parameters between groups of the study were summarized in Table 1. Rabbits in HG group showed significant increase in RBC, hemoglobin, lymphocytes, and platelets, compared to CG and SG groups. Rabbits in HSG group did not differ from HG group, except for platelets. Hematological parameters in CG and SG groups were not different, except for platelets. Monocytes were not different between all groups.

One explanation for increase of RBC in HG is that *H. sabdariffa* extract might stimulate release of erythropoietin in the kidney, the humeral regulator of RBC production (Elsner et al., 2004). In addition, Kaur and Kapoor, (2005) showed that anthocyanins, flavonoids exist in *H. sabdariffa*, can induce renal secretion of erythropoietin. Another possible explanation is that bone marrow and lymphoid organs are stimulated by different compounds in *H. sabdariffa* calyx such as alkaloids, flavonoids, polyphenolics, ascorbic acid and other vitamins, and thus different blood cells are increased (Mungole and Chaturvedi, 2011).

Table 1. Differences in hematological parameters between study groups, control (CG); starvation (SG); *H. sabdariffa* extract (HG); and *H. sabdariffa* extract and starvation (HSG) groups, at the end of the study. Different letters (i.e., a, b, c, d) for each parameter refer to statistical difference ($P \leq 0.05$).

Hematological parameter	Mean ± Standard deviation			
	CG	SG	EG	ESG
Red blood cells (cell×10^6µL)	4.29 ± 0.57 ac	3.75 ± 0.46 a	5.37 ± 0.39 b	4.96 ± 0.46 cb
Hemoglobin (g/dL)	7.2 ± 0.22 a	7.08 ± 0.73 a	8.5 ± 0.58 b	8.36 ± 0.38 b
Lymphocytes %	4.72 ± 0.75 ac	4.22 ± 0.54 a	6.06 ± 1.44 b	5.52 ± 0.62 bc
Monocytes %	1.84 ± 0.52 a	1.46 ± 0.34 a	1.8 ± 0.32 a	1.56 ± 0.30 a
Granulocytes %	1.17 ± 0.14 a	1.38 ± 0.36 ac	1.6 ± 0.33 ac	1.7 ± 0.23 bc
Platelets (cell×10^9µL)	139.6 ± 8.32 a	126.2 ± 12.54 b	219.8 ± 27.62 c	152 ± 12.63 a

In this study, increase of hemoglobin in HG and HSG can be attributable to the high concentration of minerals, particularly iron, and ascorbic acid in *H. sabdariffa* (Da-Costa et al., 2014). Previous studies showed that *H. sabdariffa* improve iron status and increase hemoglobin in women (Kubuga et al., 2019).

Lymphocytes and granulocytes increased in *H. sabdariffa* groups in our study. The presence of phytochemicals like flavonoids and tannins in the *H. sabdariffa* extract could have hemopoietic stimulating effect (Al-Jarah et al., 2017). Studies identified effect of *H. sabdariffa* in improve of hematological parameters such as RBC, WBC, Hb, and PCV in Swiss albino mice (Bhakta and Das, 2019). This evidence could boost immune system and defensive mechanism in the body.

In this study, platelets increased in *H. sabdariffa* group, which could be attributable to presence of components that produce blood cells such as vitamin C, thiamin and riboflavin, as well as β-carotene that transform to vitamin A, a helpful vitamin in treatment of platelate disorder (Carvajal-Zarrabal et al., 2012). In our study, on the other hand, platelets decreased in rabbits treated with starvation. Feng et al (2019) showed that autophagosome formation induced in platelets by starvation because of unwanted activation of platelets during starvation.

Liver Function

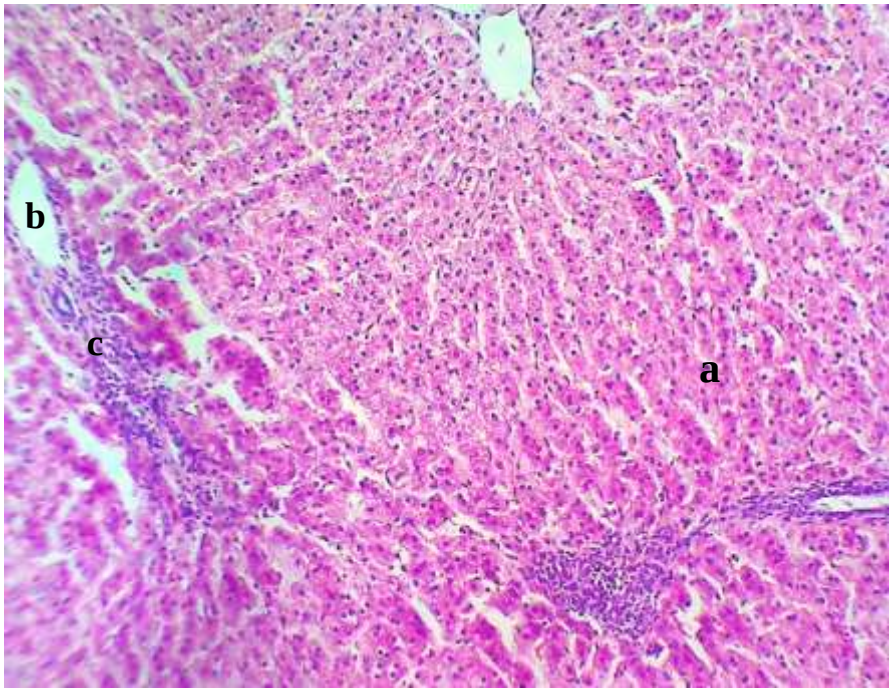
Liver function test in this study revealed that ALT activity significantly increased in SG, HG, and HSG groups, compared to CG group, although it was the same in both HG and HSG groups (Table 2). Moreover, AST activity significantly increased in both HG and HSG groups, compared to CG and SG groups (Table 2). In addition, histopathological sections of the liver revealed changes in the liver tissue in SG, HG, and HSG (Fig. 1, 2, 3). The Histopathological changes included coagulative necrosis of hepatocytes, hyperplasia of bile duct endothelia, dilation and congestion of central veins, vacuolar degeneration of the

hepatocytes around the central veins, and presence of inflammatory cells, particularly macrophages, around the central veins.

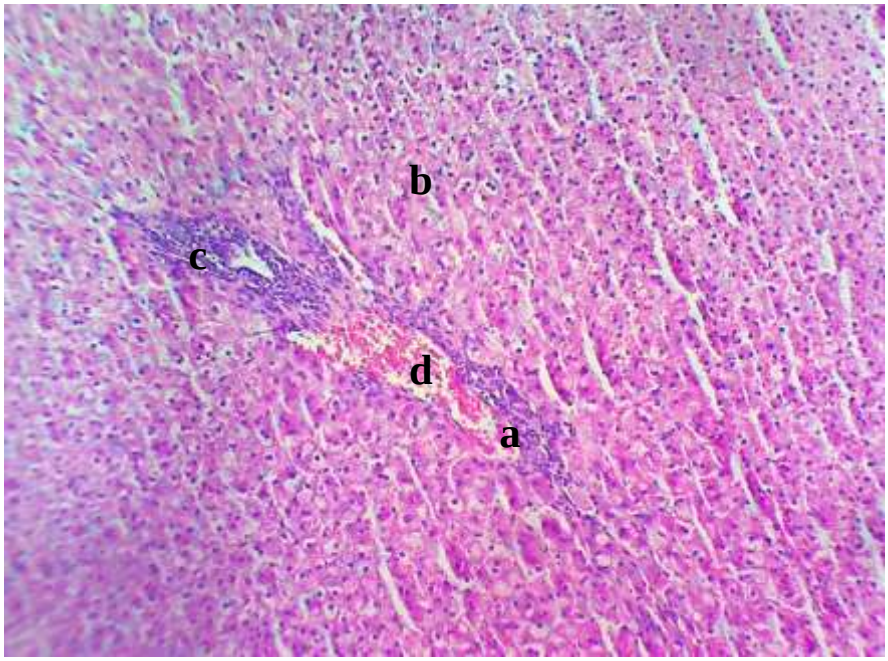
Increase the activity of ALT and AST in the circulation indicates hepatocellular damage, toxicity, inflammation, hypoxia, or tissue trauma (Igwilo et al., 2019; Huang et al., 2015; Chapman and Hostutler, 2013). It is known that inflamed or injured liver cells leak high amount of certain chemicals including ALT and AST into blood stream (Ejere et al., 2013; Esposti et al., 2012). A previous study showed that prolonged use of aqueous extract of calyx *H. sabdariffa* caused liver injury even at dose level lower than 150 mg/Kg B.W. (Ojokoh, 2010). In addition, ALT increased in starvation group, suggesting that alanine is an important amino acid involved in neoglucogenesis during starvation (Sharma et al., 1985). Changes in ALT and AST can suggest a rapid mobilization between intra- and extra hepatic fat stores that can cause histopathological changes (Gasteyger et al., 2017). Fasting can cause increase of hepatic content of triacylglycerol resulting in accumulation of lipid in the liver parenchyma that appear as vacuoles (Namazi et al., 2016; Lonardo et al, 2015; Marks et al., 2015).

Table 2. Differences in ALT and AST activity between study groups, control (CG); starvation (SG); Hibiscus *sabdariffa* extract (HG); and Hibiscus *sabdariffa* extract and starvation (HSG) groups, at the end of the study. Different letters (i.e., a, b, c, d) for each parameter refer to statistical difference ($P \leq 0.05$).

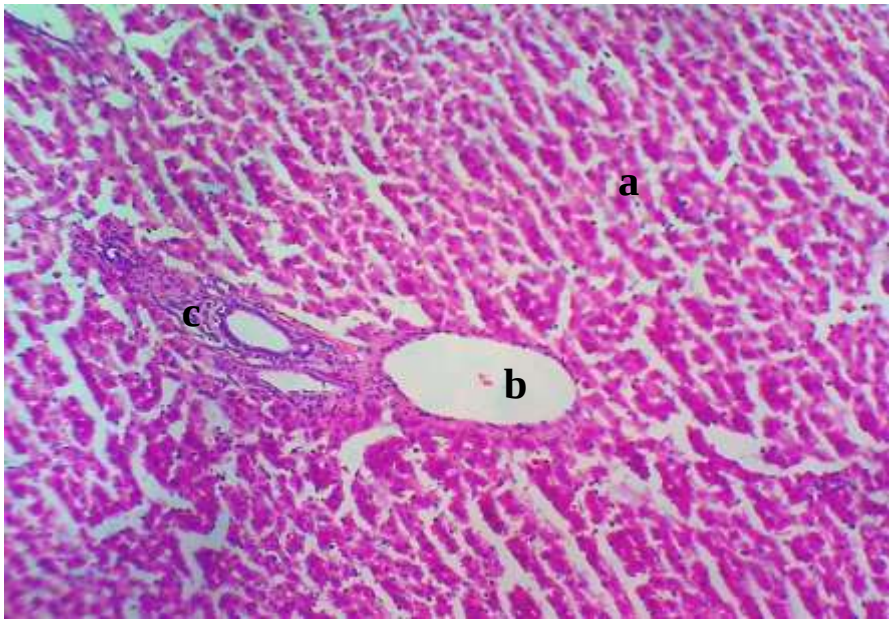
Liver function	**Mean ± Standard deviation**			
Parameter	**CG**	**SG**	**HG**	**HSG**
ALT (U/L)	113.06 ± 4.68 a	121.98 ± 3.24 B	149.26 ± 19.13 c	143.36 ± 15.27 c
AST (U/L)	80.46 ± 6.55 a	83.6 ± 4.59 A	116.88 ± 5.93 b	116.52 ± 3.89 b



(Fig. 1) Histopathological section of liver from starvation group. Coagulative necrosis (a) of hepatocytes including pylenosis, karyorehexis, and karyolysis. There is hyperplasia of bile duct endothelia, dilation and congestion of central veins (b), and presence of inflammatory cells (c), particularly macrophages, around the central veins.



(Fig. 2) Histopathological section of liver from hibiscus extract group. Histopathological changes included dilation of central veins (a), vacuolar degeneration of the hepatocytes around the central veins (b), infiltration of inflammatory cells in the portal area (c), portal vein and hepatic artery congestion (d), and focal infiltration of inflammatory cells in the hepatic tissue and around central veins.



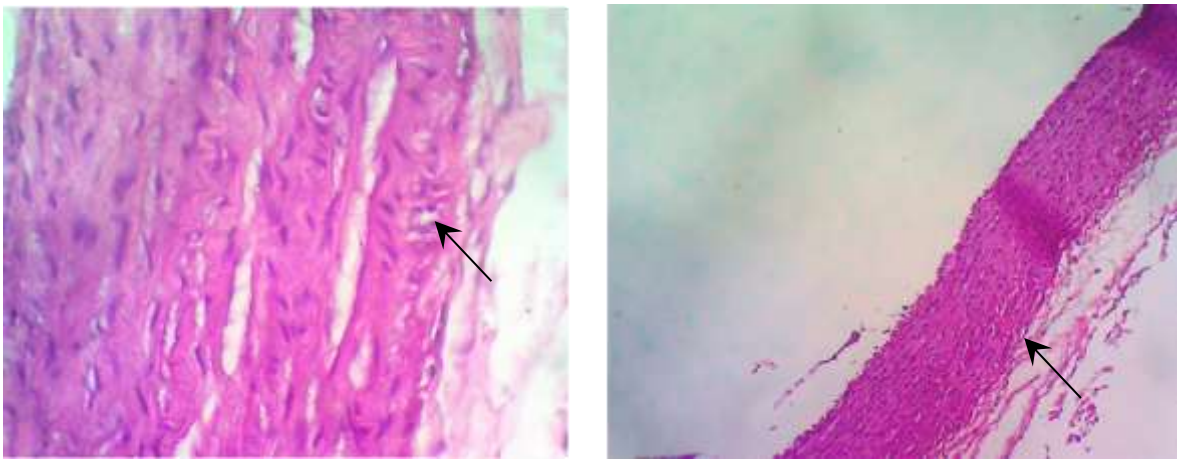
(Fig. 3) Histopathological section of liver from hibiscus extract with starvation group. Histopathological changes included vacuolar degeneration (a), coagulative necrosis, dilation of sinasoide, dilation of central veins (b) and infiltration of inflammatory cells in the portal area (c).

Lipid Profile

In this study, cholesterol and triglycerides decreased in SG, HG, and HSG groups, compared to CG group; however, there was no difference between SG and ESG groups (Table 3). In addition, no histopathological changes revealed in rabbits treated with *H. sabdariffa*. On the other hand, aorta sections from rabbits in starvation group revealed simple changes in aorta layers including few number of foam cells in tunica media, which appeared colorless as vacuoles.

Table 3. Differences in cholesterol and triglycerides concentrations between study groups, control (CG); starvation (SG); Hibiscus *sabdariffa* extract (HG); and Hibiscus *sabdariffa* extract and starvation (HSG) groups, at the end of the study. Different letters (i.e., a, b, c, d) for each parameter refer to statistical difference ($P \leq 0.01$).

Lipid profile parameter	Mean ± Standard deviation			
	CG	SG	HG	HSG
Cholesterol (mg/dL)	134.1 ± 9.34 a	115.04 ± 6.17 B	99.63 ± 12.85 c	119.46 ± 1.99 B
Triglycerides (mg/dL)	153.22 ± 3.54 a	137.77 ± 4.43 B	121.16 ± 4.05 c	121.80 ± 3.76 C



(Fig. 4) Histopathological section of aorta from starvation group. Simple changes in aorta layers including few number foam cells (→) in tunica media, which appeared colorless as vacuoles.

In this study, the decrease in serum levels of cholesterol and triglycerides in rabbits treated with *H. sabdariffa* could be mediated by the antioxidant characteristics in the extract (Aba et al., 2016). Moreover, the *H. sabdariffa* extract is able to synthesis of HDL from the liver helping cleaing excess cholesterol from the body in a process of inverse cholesterol transport (Elijah et al., 2017). Our result is similar to those reported by Aguirre-García et al (2019). In addition, Lin et al (2007) observed that *H. sabdariffa* can decrease serum cholesterol levels in human.

The normal histopathological sections in rabbits treated with *H. sabdariffa* extract could be attributable to the antioxidant effect of the extract that can protect the aorta from the oxidative damage and prevent development of atherosclerotic lesions (Zheoat et al., 2019; Zainalabidin et al., 2018; Yusof et al., 2018). On the other hand, a mild histopathological changes observed in starvation group that can be attributable to intracellular lipid mobilization as a result of utilizing the fat as a source of energy (Kartin et al., 1944). In this situation, a gradual accumulation of lipid into aortic subendothelial space occurs, which is taken by macrophage and transform into foam cells (Maguire et al., 2019). These changes are most likely prevented in rabbits with starvation and extract group as a result of antioxidant effect of the extract, an action that requires more investigation.

CONCLUSIONS

This study concluded that the aqueous *Hibiscus sabdariffa* can decrease the body weight as alternative to the starvation, decrease blood cholesterol and triglycerides, and increase the immunity; however, daily consumption can have impact on the liver.

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