Research Article

The Effect of Ethanolic Extract of *Hibiscus sabdariffa* on some Physiological and Antioxidant Parameters in Female Rabbits

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Abstract | The aim of this study was to investigate the effects of ethanolic extract of 70% *Hibiscus sabdariffa* (HS) on some physiological parameters and the alteration in enzymatic and non-enzymatic antioxidants in female rabbits. Ten female adult rabbits were divided into two groups (5 rabbits/group), group1: Control group received orally normal saline for four weeks, group2: the treated group received (200 mg/Kg) B.W of the ethanolic extract of HS. Blood samples were collected from animals by cardiac puncture technique. At the end of experiment the results showed that there were significant increase in red blood cell count (RBCC), hemoglobin concentration (Hb) with significant decrease in platelet count (Plt) and non-significant increase in the white blood cell count (WBCC), lymphocyte, monocyte and significant increase in granulocyte count in animals treated by HS extract as compared with control group. The results of liver enzymes showed that there were non- significant increase in activity of serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), and non-significant decrease in serum alanine aminotransferase (ALT) in HS extract treated group as compared with the control group. The results of kidney function parameters explained that there were non-significant increase in level of urea but there were significant ($p < 0.05$) decrease in level of uric acid in animals treated with HS as compared with control. Moreover, the results of this study showed that there were significant ($p < 0.05$) decrease in serum triacylglycerol (TAG), cholesterol, superoxide dismutase (SOD), glutathione peroxidase (GPx), but there was non-significant ($p > 0.05$) changes in catalase activity (CAT), malondialdehyde (MDA), glutathione (GSH), vitamin E (Vit E), and vitamin C (Vit C). The results obtained from this study explained that 70% ethanolic extract of HS increase (RBCC) count, enhance immunity by increasing granulocyte count, and had hypolipidemic and antioxidant effect in female rabbit.

Keywords | *Hibiscus sabdariffa*, TAG, RBCC, Female Rabbits

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INTRODUCTION

Hibiscus sabdariffa L., commonly known as Bissap (Senegal), Roselle (English), Oseille de Guinée (French) and Karkadeh (Arabic) is an erect annual herb cultivated for its seeds, petals and leaf. *Hibiscus sabdariffa* L. (Roselle) is belong to the family Malvaceae, it is known for delicacy and also for medicinal properties. This plant is used by people in Africa via direct or indirect ways in the treatment of several diseases. Roselle juice is also known as hibiscus tea, bissap, agua de Jamaica, Lo-Shen, red sorrel, sudan tea, sour tea or karkadè, The plant is widely grown in Africa, South East Asia, and some tropical countries of America. Roselle produces red edible calyces with unique brilliant red colour, that widely been extracted (Abou-Arab et al., 2001; Sagayo-Ayerdi et al., 2007; Tsai et al., 2002). Anthocyanins present in roselle are dephinidin 3-sambubioside, cyanidin 3-sambubioside, delphinidin 3-glucoside and cyanidin 3-glucoside. These contribute benefit for health as a good source of antioxidants as well as a natural food colorant (Tsai and Huang, 2004; Duangmal et al., 2008).

The approach of *H. sabdariffa* (HS) is equally significant and is quite convensing in alternative medicine. *H. sabdar-*

iffa is an aromatic, astringent, cooling herb that is currently used in tropical areas. It is known to have diuretic effects, to help in lowering fevers and is an antiscorbutic. The plant is also reported to be antiseptic, astringent, cholagogue, demulcent, digestive, purgative and resolvent. It is used as a folk remedy in the treatment of abscesses, bilious conditions, cancer, cough, debility, dyspepsia, fever, hangover, heart ailments; and hypertension (Vilasinee et al., 2005; Lin et al., 2007). The calyces of HS are prolific in many modern commercial blends of cold and hot drinks due to its pleasing taste, as well as having decorative, culinary and medicinal uses (Ngamjarus et al., 2010). In Egypt and Sudan, it is used as a beverage that helps to lower the body temperature, to treat cardiac conditions, and as a diuretic. In African folk medicine it has been used for its spasmolytic, antibacterial, cholagogic, diuretic and anthelmintic properties. Other uses in North Africa include cough and sore throat, while the leaf pulp is used into a topical application for external wounds and abscesses. In Europe, the dried calyces (the cup-like structures that are formed by the sepals) are used mostly as a tea. Historically, folk medicine has used HS for the treatment of high blood pressure, liver diseases and fevers. Hibiscus tea acts as a mild laxative (Gurrola-Diaz et al., 2010). In present study, *H. sabdariffa* extract was used to investigate some physiological and antioxidant effects in female rabbits.

MATERIALS AND METHODS

Plant Extraction

Hibiscus Sabdariffa extract preparation: After grinding the dried flowers (Hibiscus calyx) the plant material was extracted with 70% ethanol. The extract was filtered and evaporated in vacuum rotatory evaporator to yield extract according to procedure of Harborn (1984).

Experimental Design

The experiment was conducted at the animal house of biology department in college of science for Women/university of Baghdad and all experimental protocols were approved by the institutional committee. Ten adult female rabbits weighting 1000-1250 g were used in this study. The animals were housed for two weeks for adaptation. They were housed in cages in a room with controlled temperature and humidity and under good hygienic conditions. Animals were maintained on a natural 12h light and 12h dark cycle, received a balanced diet, pallets, water *ad libitum* throughout the experimental period. Rabbits were divided into two group (n=5) as follow:

Control Group: received standard diet and normal saline orally daily for four weeks.
Treated Group: received orally ethanolic extract of HS at a dose of 200 mg/kg B.W for four weeks (Buko and Mabrouk, 2009).

At the end of the experimental period, after overnight fasting blood samples were collected from animals by cardiac puncture technique and divided into two parts, one part kept in tubes containing EDTA anticoagulant for hematological study, Hemoglobin concentration according to Van kampen and zulstra (1961), White blood cell (WBC) count according to Harris-young (1995), Red blood cell count (RBCC) using the technique of Rodak (1995), total platelets (Plt) count according to Voigt (2000). Another part of blood sample kept in gel clot activator tubes and then serum was separated from coagulant blood by centrifugation at 5000 rpm for 10 minutes and stored at -20C for study the following: liver enzymes (AST, ALT, ALP) by using enzymatic kit respectively (Reitman and Frankel, 1975; Belfield and Goldberg, 1971), kidney function parameter (Urea, Uric acid) according to diamond enzyme kit (Palton and Croush, 1977; Henry, 1974). triacylglycerol (TAG) by using enzymatic assay kit (Rojkin et al., 1974), total cholesterol concentration by using enzyme assay kit (Ellefson and Garaway, 1976), Antioxidant parameters including SOD according to Misra and Fridovich, (1972), GPX according to Paglia and Valentine (1967), CAT according to Beers and Sizer (1952), MDA according to Ohkawa et al. (1979), GSH according to Beutler et al. (1963), Vit E according to Bieri et al. (1979) and Vit C according to Lin (1982).

Statistical Analysis

The Statistical Analysis System was used to compare the effects of (HS) treated group parameters with control. Least significant difference –LSD test was used to compare significance between means in this study (SAS, 2012).

RESULTS

The results of current study explained that there were significant increase ($p<0.05$) in the red blood cell (RBC) count, hemoglobin concentration (Hb), with significant decrease ($p<0.05$) in platelet count (Plt) in animals treated with 200 mg/kg B.W of the ethanolic extract of HS as shown in Table 1.

Table 1: The effect of 70% ethanolic extract of *Hibiscus sabdariffa* on blood picture in female rabbits

Group	Mean ± SD		
	RBC x 10^6	Hb (g/dl)	Plt x 10^3
Control	5.3 ± 0.17	10.80 ± 2.64	677.0 ± 86.16
Treatment	6.08 ± 0.09	11.724 ± 2.48	493.3 ± 68.24
LSD value	0.428 *	3.71 *	92.18 *

* ($P<0.05$); Values expressed as mean ± SE; **n**=5 each group; control group; **treatment group**: animals received 200 mg/kg B.W of the ethanolic extract of *Hibiscus sabdariffa* (HS); **RBC**: Red blood cells; **Hb**: Hemoglobin; **Plt**: Platelets

The group that received ethanolic extract of HS showed

a non-significant increase in the WBCC, Lymphocyte count and monocyte count and a significant increase in the Granulocyte count as compared with control group (Table 2). Table 3 showed that there were non-significant increase in the activity of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), non-significant decrease in Alkaline phosphatase (ALP) and Urea, while there was significant decrease in the uric acid in animals treated with 200 mg/kg B.W of the ethanolic extract of HS as compared with control.

Furthermore, the present results showed that there was a significant decrease (P<0.05) in serum triacylglycerol (TAG) in treated group as compared with control. Also there was significant decrease (P<0.05) in cholesterol (Chol) in treated group as compared with control and the same for glutathione peroxidase (GPx), and superoxide dismutase (SOD). But there was non-significant changes in the level of catalase activity (Cat), malondialdehyde (MDA), glutathione (GSH), vitamin E (Vit E) and vitamin C (Vit C) as shown in Table 4.

Table 2: The effect of 70% ethanolic extract of *Hibiscus sabdariffa* on white blood cell (WBC) count and differential white blood cell count in female rabbits

Group	Mean ± SD			
	WBC x 10^9	Lympho-cyte x 10^9	Monocyte x 10^9	Granulocytes x 10^9
Control	6.7 ± 0.38	2.10 ± 0.04	0.46 ± 0.007	3.96 ± 0.29
Treatment	7.4 ± 0.24	2.24 ± 0.19	0.46 ± 0.005	4.70 ± 0.36
LSD value	0.893 NS	0.426 NS	0.073 NS	0.519 *

* (P<0.05); **NS**: Non-significant; Values expressed as mean ± SE n=5 each group; **control group and treatment group**: animals received 200 mg/kg B.W of the ethanolic extract of *Hibiscus sabdariffa* (HS)

Table 3: The effect of 70% ethanolic extract of *Hibiscus sabdariffa* on liver enzymes (AST, ALT, ALP) and kidney function parameters (Urea, Uric Acid) in female rabbits

Group	Mean ± SD				
	AST	ALT	ALP	Urea	Uric acid
Control	32.5 ± 1.59	44.3 ± 0.94	7.75 ± 1.33	33.75 ± 1.64	1.50 ± 0.72
Treatment	33.5 ± 1.33	42.5 ± 0.87	8.00 ± 0.71	34.00 ± 1.27	0.30 ± 0.006
LSD value	5.38 NS	4.92 NS	0.96 NS	4.09 NS	0.392 *

* (P<0.05); **NS**: Non-significant. Values expressed as mean ± SE n=5 each group; control group; **treatment group**: animals received 200 mg/kg B.W of the ethanolic extract of *Hibiscus sabdariffa* (HS); **AST**: aspartate aminotransferase; **ALP**: alkaline phosphatase; **ALT**: alanine aminotransferase

DISCUSSION

The results of the present study showed that there were significant elevation in the level of RBC that is in agreement with Ashafa et al. (2011) that indicated that the extract has beneficial properties that result in increased red blood cell count. This clearly indicated that there was an increase in the rate of production of RBCs within the study period. The extract may stimulate erythropoietin release in the kidney, which is the humoral regulator of RBC production (Polenakovic and Sikole, 1996; Sanchez-Elsner et al., 2004). The increment in the blood cells could be due to the stimulation of the bone marrow and lymphoid organs by the compounds such as alkaloids, flavonoids, polyphenolics, ascorbic acid and other vitamins that of the different cell lines and hence the observedincrement in the various blood cell types (Kuriyan et al., 2010; Mungole and Chaturvedi, 2011). As shown in this study there was significant increase in Hb concentration in aniare found in the herb, these compounds may stimulate the hemopoietic tissue leading to the increased activity mals treated with HS as compared with control. This finding is in agreement with previous worker (Adigun et al., 2004; Fakeye et al., 2008), that indicated the fact that oxygen

Table 4: The effect of 70% ethanolic extract of *Hibiscus sabdariffa* on Triacylglycerol (TAG), Cholesterol (Chols) and oxidative stress parameters in female rabbits

Group	Mean ± SD								
	TAG (mg. dl)	Chols. (mg/ dl)	SOD (µ/L)	GPx (u/ ml)	Cat (µ/ ml)	MAD (µM)	GSH (µM)	Vit E (nmo-l/L)	Vit. C (nmo-l/L)
Control	97.8 ± 8.93	135.8 ± 14.72	18.0 ± 1.63	10.5 ± 0.83	9.2 ± 0.71	2.2 ± 0.37	2.3 ± 0.09	0.28 ± 0.07	2.26 ± 0.05
Treatment	75.2 ± 5.68	86.4 ± 9.52	13.56 ± 1.06	8.8 ± 0.74	9.04 ± 0.64	1.7 ± 0.04	2.3 ± 0.06	0.28 ± 0.007	2.28 ± 0.13
LSD value	11.48 *	15.72 *	3.57 *	1.74 *	1.66 NS	0.83 NS	0.072 NS	0.052 NS	0.63 NS

* (P<0.05); NS: Non-significant; Values expressed as mean ± SE n=5 each group; **control group and treatment group**: animals received 200 mg/kg B.W of the ethanolic extract of *Hibiscus sabdariffa* (HS); **SOD**: superoxide dismutase; **GPx**: glutathione peroxidase; **Cat**: catalase; **MAD**: malondialdehyde; **GSH**: glutathione; **Vit E**: vitamin E; **Vit C**: vitamin C

uptake and transfer was very adequate in the treated rabbits (Ots et al., 1998). Significant elevation in the levels of hemoglobin may due to the treatment with *H. Sabdariffa* calyces extract which contain high percent of protein in its composition (Ali et al., 2005). There was significant decrease in level of platelets in agreement with Olatunji et al., (2005). According to the result of this study there was significant increase in granulocyte count as compared with control in agreement with Ejere et al. (2013). The significant elevation observed on the granulocyte count following the administration of the extract could be suggestive of its capacity to boost the defensive mechanism of the body against invaders. This is indicative of immune enhancer effect (Ejere et al., 2013). According to the result of the present study there was significant decrease in the level of uric acid in animals treated with HS as compared with control group, this agreement with Chih et al. (2012) which shows HS extract contains polyphenol, flavonoids, and anthocyanins. Antioxidant anthocyanins can potentially lower serum uric acid, polyphenols can affect the prevention of cancer and atherosclerosis, lower blood pressure, reduce inflammation and aging, and inhibit free radical activity (Kamboh et al., 2015), this suggests that HS extract may be able to lower serum uric acid (Lin et al., 2005). Also there was significant decrease in level of TAG and cholesterol in animals treated with HS as compared with control in agreement with Chen et al. (2003). This effect may be caused by the ability of the extract to control the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues (Hirunpanich et al., 2006). The results of the present study explained that there were significant decrease in the level of SOD and GPX. Treatment of rabbits with *Hibiscus sabdariffa* calyx extract greatly restored the levels of these antioxidant enzymes to near normal which is an indication of good antioxidant activity and protection from free radicals which cause tissue damage (Rice-Evan and Burdon, 1993).

CONCLUSION

The findings from current study indicated that *Hibiscus sabdariffa* extract may increase Hb and RBC thus may be useful in treating anaemia and also has the potential to increase blood volume, enhance immunity by increasing granulocyte count. Furthermore it has protective effect to kidney by decreasing uric acid, and also possesses strong hypolipidemic as well as antioxidant properties.

AUTHORS CONTRIBUTION

The authors Ali AH, Abdul-Azeez LA, Humood JK, Ali ZA, Helal ZH and Wahab FL were the investigators who contributed equally in the research work. Dr. Ali AH was the supervisor of the team.

CONFLICT OF INTEREST

Author declares no conflict of interest.

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