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Some immunological effects of Roselle *Hibiscus sabdariffa* L. calyces in albino mice

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Abstract

It was aimed to evaluate the immunological effects of cold and hot aqueous extracts of *H. sabdariffa* calyces in albino mice. Three oral doses (9.78, 19.56 and 29.34 mg/mouse for cold extract, and 13.72, 27.44 and 41.16 mg/mouse for hot extract) of each extract were evaluated, in addition to the immune suppressive drug etoposide (0.05 mg/mouse/day) and interferon (0.25 mg/mouse/day). Metaphase index, T-rosette formation (bone marrow, thymus, spleen and lymph nodes) and ADA specific activity (serum and thymus) in the investigated organs were significantly decreased in animals treated with etopside as compared with normal controls, while in mice treated with calyx extracts of *H. sabdariffa*, the investigated parameters were significantly enhanced, especially at the third dose of both extract, and such dose almost shared the effect of interferon in mice treated with it. It was almost universal to observe that hot extract was better than the cold extract. These findings suggests the immune enhancement effects of the investigated extract, and they were in a dose-dependent manner.

Key words: Medicinal plants, *Hibiscus sabdariffa*, Immunology, Albino mice.

Introduction

The consumption of plants for medicinal purposes has been an age-long tradition by mankind in many regions of the world. One of the plants, which is considered medicinal, is *Hibiscus sabdariffa* Linn (Family Malvaceae). It is known as Rosella or Red Sorrel in English and "Karkadeh" in Arabic, and is mainly cultivated in tropical and subtropical regions of Africa and Asia (Bako *et. al*., 2009). Typically, the calyces of the plant are used in the manufacture of beverages, jam and vegetable gelatin (Amusa *et.al*., 2005). However, *H. sabdariffa* has many other applications. Among the nourishing applications, the leaves are used like vegetables in the preparation of soups and sauces (Fasoyiro *et.al*., 2005). Moreover, many medicinal applicat-ions of this plant have been developed around the world. It is used to treat hypertension, pyrexia, and liver damage. Recently an aqueous extract of dried flowers of *H. sabdariffa* has been used as an effective treatment against and gastric carcinoma, due to its high content of polyphenol (Tseng *et.al*., 2000; Lin *et.al*., 2007; Hussein *et.al*., 2010).

Further studies have demonstrated that the calyx extracts of *H. sabdariffa* possess hypoglycaemic (Mantruad *et.al*., 2010), hypolipidaemic (Hirunpanich *et.al*., 2006), antioxidant (Hirunpanich *et.al*., 2005). The calyces of *H. sabdariffa* are also rich sources of vitamins and antioxidants, which are essential as health foods in the building up of body immune system and in preventing diseases (Jamaludin *et.al*., 2012). Accordingly, the present investigation was planned to evaluate the immunological effects of cold and hot extracts of *H. sabdariffa* calyces in albino mice.

Materials and Methods

Plant collection and identification:

The fruits of *H. sabdariffa* were collected from the botanical garden of College of Education Ibn Al-Haitham, and classified as *H. sabdariffa* at the herbarium of Biology Department at the college.

Plant extraction and doses:

After collection of fruits, the calyx leaves were separated and air-dried. The dried calyces were powdered by a coffee grinder, and then two aqueous extracts were prepared.

In the first (cold extract), 50 g of dried calyces were In the first (cold extract), 50 g of dried calyces were soaked in 250 ml of distilled water for 24 hours at 4°C (Dafalla and Mustafa, 1996). while for hot extract, the dried calyces were boiled in distilled water for 3 hours. In both cases, the extract solution was filtered sterilized (0.22µm Millipore filter), and doses corresponding to 9.78, 19.56 and 29.34 mg/mouse for cold extract, and 13.72, 27.44 and 41.16 mg/mouse for hot extract, were prepared.

Experimental design:

Male and female albino mice were the mammalian system of this study and their age ranged between 8-10 weeks. The animals were administrated orally (0.25ml) of each dose extract for 7 days as single dose per day. Three controls were included. The first were mice dosed (0.25 ml/day) with distilled water (normal controls); the second included mice dosed (0.05 mg/mouse/day) with the immune suppressive drug etoposide (negative controls); and the third were mice dosed (0.25 mg/mouse/day) with interferon (positive controls). The mice were distributed into males (9 groups) and females (9 groups), and each group included 4 mice; therefore the total number of mice was 72.

Immunological parameters:

At the end of each treatment period, the mice were sacrificed and subjected to laboratory evaluations, which were metaphase index of cells obtained from bone marrow, thymus, spleen and lymph nodes (Brown *et.al*., 1978; Allen *et.al*., 1977), T-rosette formation of cells obtained from the same organs (Brown *et.al*., 1978; Mackenzie, 1998), and adenosine deaminase (ADA) specific activity of serum and cells obtained from thymus (Hess, 1965).

Statistical analysis:

Data are presented as mean ± standard error (SE), and differences between means were assessed by ANOVA table followed by Duncan test, in which $P \leq 0.05$ was considered significant. The analyses were carried out using the statistical package SPSS version 13.

Results and Discussion

Metaphase index, T-rosette formation an ADA specific activity in the investigated organs were significantly decreased in animals treated with etopside as compared with normal controls, while in mice treated with calyx extracts of *H. sabdariffa*, the investigated parameters were significantly enhanced, especially at the third dose of both extract, and such dose almost shared the effect of interferon in mice treated with it. It was almost universal to observe that cold extract was better than the hot extract. These findings suggest the potential of immune enhancement effects mediated by the investigated extracts, and such effects were in a dose-dependent manner, as shown in tables 1, 2, 3, 4 and 5.

The present results demonstrated that cold and hot extracts of *H. sabdariffa* enhanced the values of the investigated parameters (metaphase index, rosette forming index and ADA specific activity), especially when male and female mice treated with the third dose of cold and hot extracts (29.34 and 41.16 mg/mouse, respectively). These findings suggest the immune-enhancement of the tested extract. In agreement with such findings, the literature depicted that *H. sabdariffa* calyces are rich source of phytoconstituents, which are reported to incite para-immunity and also affects the non-specific immunomodulation especially of granulocytes macrophages, natural killer cells and complement system (Sainis *et.al*.,1997; Sharififar *et.al*.,2009; Agrawal *et.al*.,2010; Lee *et.al*.,2010). The most important phytoconstituents are flavonoids, anthocyanin, protocatechuic acid, vitamin C, glycosides and polysaccharides (Ologundudu *et.al*., 2009; Mozaffari-Khosravi *et.al*., 2009), and accordingly, these components made the plant to be useful for pharmacological investigations with different scopes; such as anti-oxidant and anti-mutagenic potentials. In agreement with this, anthocyanins and protocatechuic acid extracted from the calyces of plant have shown to have strong antioxidant (Lee *et.al*., 2002) and anti-tumor effects (Lin *et.al*., 2005). Furthermore, a high antioxidant activity of infusions obtained from Roselle's calyx has been observed; an observation that suggests that its daily consumption may be beneficial to human health (Serrano-Cruz *et.al*., 2013). From animal models (mainly rodents) to *in vitro* studies some authors have found that Roselle's infusion can be used to inhibit the oxidation of low density lipoproteins and to prevent some types of hyperlipidemia (Alarcon-Aguilar *et.al*., 2007; Chen *et.al*., 2004). Together, these findings established the immunoenhancing properties of the investigated extracts of this plant confirming that the

Table (1): Metaphase index of bone marrow, thymus, spleen and lymph node cells in albino male mice treated with cold and hot extract of *Hibiscus sabdariffa* calyces.

Groups	Dose (mg/mouse)	Metaphase Index (%)*			
		Bone marrow	Thymus	Spleen	Lymph Node
Control	D.W	17.00±1.15^{BC}	7.75±0.50^{BC}	11.50±1.29^{B}	5.00±0.81^{A}
Etopside	0.05	10.50±1.00^{D}	3.50±0.57^{D}	7.50±0.57^{C}	3.25±0.95^{B}
Interferon	0.25	24.25±0.95^{A}	10.75±1.25^{B}	14.00±0.81^{A}	7.75±0.50^{A}
Cold Extract	9.78	16.75±1.50^{C}	6.75± 0.95^{C}	11.00±0.81^{B}	4.75±0.95^{AB}
	19.56	18.25±1.25^{BC}	8.25±1.25^{B}	13.00±1.63^{AB}	5.75±0.50^{A}
	29.34	24.25±1.70^{A}	11.50±1.29^{B}	16.25±1.25^{A}	7.75±1.25^{A}
Hot Extract	13.72	16.00±1.41^{C}	6.00±0.81^{C}	10.75±0.95^{B}	5.00±0.81^{A}
	27.44	18.75±0.95^{BC}	8.00±1.41^{B}	12.50±1.29^{B}	6.25±0.95^{A}
	41.16	21.50±2.38^{AB}	15.00±1.29^{A}	15.00±0.81^{A}	7.25±0.95^{A}

^{A,B} different superscripts in a column differ significantly (P<0.05).

Table (2): Metaphase index of bone marrow, thymus, spleen and lymph node cells in albino female mice treated with cold and hot extract of *Hibiscus sabdariffa* calyces.

Groups	Dose (mg/mouse)	Metaphase Index (%)*			
		Bone marrow	Thymus	Spleen	Lymph Node
Control	D.W	18.25±0.95^{BC}	7.50± 1.00^{B}	11.75±1.25^{B}	4.50±0.57^{BC}
Etopside	0.05	11.50±0.57^{D}	3.00±0.81^{C}	8.00±0.81^{C}	3.00±0.81^{C}
Interferon	0.25	25.00±0.81^{A}	10.25±0.95^{AB}	14.00±0.81^{A}	7.00±0.81^{A}
Cold Extract	9.78	17.25±0.9^{BC}	7.00± 1.15^{B}	10.75±0.95^{BC}	4.25±0.95^{BC}
	19.56	19.00±1.15^{B}	8.00± 1.63^{B}	13.00±0.81^{AB}	5.50±0.57^{AB}
	29.34	25.25±0.95^{A}	12.75±1.70^{A}	16.00±1.82^{A}	8.50±0.57^{A}
Hot Extract	13.72	16.50±1.29^{C}	7.00±0.81^{B}	10.50±0.57^{BC}	4.25±0.50^{BC}
	27.44	19.00±0.81^{BC}	8.00±0. 81^{B}	13.75±1.25^{AB}	5.25±0.50^{AB}
	41.16	22.25±2.21^{AB}	11.25±0.95A	15.25±1.70^{A}	7. 00±1.15^{A}

^{A,B} different superscripts in a column differ significantly (P<0.05).

Table (3): Rosette forming of bone marrow, thymus, spleen and lymph node cells in albino male mice treated with cold and hot extract of *Hibiscus sabdariffa* calyces.

Groups	Dose (mg/mouse)	Rosette forming (%)			
		Bone marrow	Thymus	Spleen	Lymph Node
Control	D.W	2.50±0.57^{AB}	13.00± 0.81^{B}	6.00±0.81^{C}	11.50±1.29^{B}
Etopside	0.05	1.75±0.95^{B}	6.25± 0.95^{C}	5.25±1.25^{C}	4.50±1.29^{C}
Interferon	0.25	4.00±0.81^{A}	16.25±0.95^{A}	13.00±2.94^{A}	15.00±0.81^{A}
Cold Extract	9.78	2.25±0.50^{AB}	13.00± 0.81^{B}	6.50±1.00^{C}	12.00±0.81^{B}
	19.56	3.00±0.81^{AB}	14.25± 0.95^{AB}	8.50±0.57^{BC}	12.50±1.73^{B}
	29.34	3.25±0.95^{AB}	15.50±1.29^{AB}	11.50±0.57^{AB}	13.75±0.50^{AB}
Hot Extract	13.72	2.00±0.81^{AB}	12.50±0.57^{B}	6.00±0.81^{C}	11.50±0.57^{B}
	27.44	2.50±0.57^{AB}	14.00±0.81^{AB}	8.00±0.81^{BC}	12.25±1.70^{B}
	41.16	3.25±0.95^{AB}	14.75±1.25^{AB}	11.50±0.57^{AB}	13.00±0.81^{AB}

^{A,B} different superscripts in a column differ significantly (P<0.05).

Table (4): Rosette forming of bone marrow, thymus, spleen and lymph node cells in albino female mice treated with cold and hot extract of *Hibiscus sabdariffa* calyces.

Groups	Dose (mg/mouse)	Rosette forming (%)*			
		Bone marrow	Thymus	Spleen	Lymph Node
Control	D.W	2.25±0.50^{A}	12.25± 0.50^{B}	6.25±1.25^{C}	11.25±1.50^{A}
Etopside	0.05	2.00±0.81^{A}	5.50± 0.57^{C}	5.00±1.82^{C}	4.75±0.95^{B}
Interferon	0.25	3.75±0.95^{A}	15.25±0.95^{A}	12.25±0.50^{A}	14.00±0.81^{A}
Cold Extract	9.78	2.50±0.57^{A}	12.00±1.41^{AB}	6.75±0.95^{C}	11.25±0.95^{A}
	19.56	2.75±0.50^{A}	13.75±0.95^{AB}	8.25±0.50^{BC}	12.75±0.95^{A}
	29.34	3.50±0.57^{A}	15.25±1.70^{A}	11.75±1.25^{A}	13.50±1.29^{A}
Hot Extract	13.72	2.25±0.95^{A}	11.75±0.95^{B}	6.25±1.25^{C}	11.00±0.81^{A}
	27.44	2.75±0.95^{A}	13.75±1.25^{AB}	8.00±0.81^{BC}	12.50±0.57^{A}
	41.16	3.50±0.57^{A}	14.50±0.57^{AB}	11.00±1.15^{A}	13.25±0.95^{A}

^{A,B} different superscripts in a column differ significantly (P<0.05).

Table (5): Adenosine deaminase of thymus and serum in albino male and female mice treated with cold and hot extract of *Hibiscus sabdariffa* calyces.

Groups	Dose (mg/mouse)	Specific activity of ADA U/mg protein			
		Thymus		Serum	
		Male	Female	Male	Female
Control	D.W	1571.3±6.3^{C}	1550.3±4.7^{C}	1.54±0.01^{D}	1.53±0.02^{D}
Etopside	0.05	558.6±8.6^{D}	546.4±3.6^{D}	0.47±0.02^{F}	0.46±0.02^{F}
Interferon	0.25	2873.1±126.9^{A}	2868.9±168.9^{A}	1.88±0.01^{A}	1.87±0.05^{A}
Cold Extract	9.78	1453.0±3.0^{C}	1427.6±127.4^{C}	1.51±0.02^{D}	1.50±0.03^{D}
	19.56	1653.0±3.0^{C}	1647.6±2.4 ^{C}	1.72±0.01^{B}	1.70±0.01^{B}
	29.34	2637.2±2.2^{A}	2633.1±1.9^{A}	1.86±0.01^{A}	1.85±0.02^{A}
Hot Extract	13.72	1347.0±94.2^{C}	1366.6±93.4^{C}	1.48±0.01^{E}	1.48±0.02^{E}
	27.44	1565.5±24.5^{C}	1544.6±37.3^{C}	1.64±0.01^{C}	1.63±0.01^{C}
	41.16	1960.4±5.4^{B}	1954.1±304.1^{C}	1.84±0.02^{A}	1.83±0.01^{A}

^{A,B} different superscripts in a column differ significantly (P<0.05).

immunomodulatory activity is cell mediated as suggested by the increased metaphase index especially in spleen cells and increased percentage of T-rosette formation, especially in thymic cells. Of course, a significant increased specific activity of ADA, especially in thymus of extract-treated mice, can not be ignored. However, further investigations are certainly required to shed more light on the immunological potential of *H. sabdariffa*.

References

Agrawal, S., Khadase S. and Talele G., 2010. Bioactive immunomodulatory fraction from Tridax procumbens. Asian J. Biol. Sci., 3: 120-127.

Ali B.H., Al-Wabel N. and Blunden G., 2005. Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L. a review. Phytother. Res., 5: 369-375.

Allen, J.W., Shuller C.F., Mendes R.W. and Latt S.A., 1977. Simplified technique for in vivo analysis of sister chromatid exchange using 5-bromodeoxy uridine tablets. Cytogenetics, 18: 231-137.

Amusa, N.A., Ashaye O.A., Aiyegbayo A.A., Oladapo M.O., Oni M.O. and Afolabi O.O., 2005. Microbiological and nutritional quality of hawked sorrel drinks (soborodo)(the Nigerian locally brewed soft drinks) widely consumed and notable drinks in Nigeria. J. food Agric. Environ., 3(4):47-50.

Bako, I.G., Mabrouk M.A. and Abubakar A.,2009. Antioxidant effect of ethanolic seed extract of *Hibiscus Sabdarrifa Linn* (Malvaceae) alleviate the toxicity induced by chronic administration of sodium nitrate on some haematological parameters in wistars rate. Advan. J. Food Sci. Technol., 1: 39-42.

Brown, C.D., Brown I.W. and Siljivic V.S., 1978. Enhancement of the antibody response in vitro by BCG In: Griffith, A.H. and regameny, R.H. ed., Development in Biolo-gical Standardization, Academic Press, London. Pp: 138-154.

Dafallah, A.A. and al-Mustafa Z., 1996. Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibiscus sabdariffa*. Am. J. Chem. Med., 24:263-269.

Fasoyiro, S.B., Ashaye O.A., Adeola A. and Samuel F.O., 2005. Chemical and storability of fruit-flavoured (*Hibiscus sabdariffa*) drinks. Word J. Agric. Sci., 2:165-168.

Giusti, G., 1981. Adenosine deaminase. In: Bergmeyer H.U. ed., Methods of enzyme analysis 2nd ed., Academic Press, 2: 1092-1099.

Hirunpanich, V., Utaipat A., Molales N.P., Bunyapraphtsala N., Sato H., Herunsale A., 2006. Hypocholesterolemic and antioxid-ant effect of aqueous extracts from the dried calyx of *Hibiscus sabdariffa L*. in hypercholesterolemic rats. J. Ethnopharm-acology, 103:252-260.

Hirunpanich, V., Utaipat A., Molales N.P., Bunyapraphtsala N., Sato H., Herunsalee A., Suthisisang C., 2005. Antioxidant effect of aqueous extracts from dried calyx of *Hibiscus sabdariffa Linn*.(Roselle) in vitro using rat low-density lipoprotein (LDL). Biol..Pharm. Bull., 28:481-484.

Hou, D.H., Tong X., Terahara N., Luo D. and Fuji M., 2005. Delphinidin 3-sambubioside,a Hibiscus anthocyanin, induces apoptosis in human leukemia cells through reactive oxygen species-mediated mitochondrial pathway. Arch. Biochem. Biophys., 440: 101-109.

Hussein, R.M., Shahein Y.E., El Hakim A.E. and Awad H.M., 2010. Biochemical and molecular characterization of three colored types of roselle (*Hibiscus sabdariffa L*.). J. Am. Sci., 11:726-733.

Jamaludin, M., Nor Azlinda Z., Muhd Hanis M.I. and Siti Balkis B., 2012. Effects of aqueous extract of *Hibiscus sabdariffa L*. (Roselle) calyx on bovine sperm membrane. Rev. Global Med. Healthcare Res., 2:67-73

Lee, M.J., Chou F.P., Tseng T.H., Hsieh M.H., Lin M.C. and Wang C.J., 2002. Hibiscus protocatechuic acid or esculetin can inhibit oxidative LDL induced by either copper ion or nitricoxide donor. J. Agric. Food Chem., 50: 2130-2136.

Lee, S.H., Lillehoj H.S., Hong Y.H., Jang S.I., Lillehoj E.P., Ionescu C., Mazuranok L. and Bravo D., 2010. In vitro effects of plant and mushroom extracts on immunological function of chicken lymphocytes and macrophage. Br. Poult. Sci., 51: 213-221.

Lin, H.H., Chen J.H., Kuo W.H., Wang C.J., 2007. Chemopreventive properties of *Hibiscus sabdariffa* L. on human gastric carcinoma cells through apoptosis induction and JNK/P38 MAPK signaling activation. Chem. Biol. Interact., 1:59-75.

Lin, H.H., Huang H.P., Huang C.C., Chen J.H. and Wang C.J., 2005. *Hibiscus* polyphenol-rich extract induces apoptosis in human gastric carcinoma cells via p53 phosphorylation and p38 MAPK/FasL cascade pathway. Mol. Carcinog., 43:86- 99.

Mantruad, A., Pannangpetch P., Kongyingyoes B., Kukongviriyapan U., Chuanta S., Nakmareong S., Itharat A., 2010. Roselle extract and gallic acid improve vascular reactivity of diabetic rats. Srinagarind Med. J., 25 (Suppl.).

Mozaffari-Khosravi, H., Jalali-Khanabadi B.A., Afkhami-Ardekani M.,Fatehi F. and Noori-Shadkam M., 2009. The effects of sour tea (*Hibiscus sabdariffa*) on hypertension in patients with type II diabetes. J. Human Hyperten., 23:48-54.

Ologundudu, A., Ologundudu A.O., Ololade I.A. and Obi F.O., 2009. Effect of *Hibiscus sabdariffa* anthocyanins on 2,4-dinitrophenylhydrazine-induced hematotoxi-city in rabbits. African J. Biochem. Res., 4: 140-144.

Sainis, K.B., Sumariwalla P.F., Goel A., Chintalwar G.J., Sipahimalani A.T. and Banerji A., 1997. Immunomodulatory Properties of Stem Extracts of *Tinospora Cordifolia*: Cell Targets and Active Principles. In: Immunomodula-tion, Upadhyay, S.N. ed., Narosa Publishing House, New Delhi, India, Pp: 95.

Serrano-Cruz, M.R., Villanueva-Carvajal A., Morales Rosales E.J.; Ramírez Dávila J.F., Dominguez-

Lopez A., 2013. Controlled release and antioxidant activity of Roselle (*Hibiscus sabdariffa L.*) extract encapsulated in mixtures of carboxymethyl cellulose, whey protein, and pectin. Food Sci. Technol., 50: 554-561.

Sharififar, F., Pournourmohammadi S., Arabnejad M., 2009. Immunomodulatory activity of aqueous extract of *Heracleum persicum* Desf. In mice. Iran. J. Pharm. Res., 8(4): 287-292.

Tseng, T., Kao T., Chu C., Chou F., Lin W., Wang C., 2000. Induction of apoptosis by Hibiscus Protocatechuic Acid in Human Leukemia Cells via Reduction of Retinoblastoma (RB) Phosphorylation and Bcl-2 Expression. Biochem. Pharmacol., 60: 307-315.

Tseng, T.H., Wang C.J., Kao E.S., Chu C.Y., 1996. Hibiscus protocatechuic acid protects against oxidative damage induced by tert-butylhydroperoxide in rate primary hepatocytes. Chem. Biol. Interact., 101:137-148.