Physiological effects of Roselle calyces extract in some blood parameters in laying hens

R. J. T. Al-Baghdadi
Coll. of Vet. Med./ Univ of Al-Qadisiya

Abstract

This study was designed to investigate the physiological effects of alcoholic extract of Calyces of Roselle (*Hibiscus sabdariffa)* in some blood parameters in laying hens. 30 layers, 49 week old belonging to the Isa Brown breed were randomly divided into two groups of 15 layers each, both are received basal diet. Group 1 served as control which was orally administered 2ml/kg body weight of distilled water. Group 2 served as treated group which was orally administered alcohol extract of *Hibiscus sabdariffa* calyces at a dose of 200mg/kg BW dissolved in 2 ml of distilled water. The groups received the water and extract daily to individual layers at 6:00 o'clock in morning, after that drinking water and feed were provided *ad libitum*, the experiment lasted 8 weeks from 25/١٢/2009 until 25/٢/2010. The effect of the extract was assessed by estimating red blood cells count, Hb concentration, white blood cell count, and serum: total protein, cholesterol, LDL and HDL, The results revealed that Roselle calyces extract produced significant increase in the levels of Hb conc., white blood cells and HDL, and significant decrease in the level of LDL, while the numbers of red blood cells and levels of total serum protein & cholesterol had non significant elevation in Group 2 compared to Group 1.

Introduction

Roselle (*Hibiscus sabdariffa L.*) is an annual, erect, bushy, herbaceous subshrub to 2.4 m tall, with smooth or nearly smooth, cylindrical, typically red stems, flower leaves are deeply 3 - 5 or even 7-lobed; the margins are toothed the typically red calyx, consisting of 5 large sepals with a collar of 8 to 12 slim, pointed bracts around the base, begins to enlarge, becomes fleshy, crisp but juicy, (3.2-5.7 cm) long and fully encloses the velvety capsule, (1.25-2 cm) long (1). Flowers are given many names at different parts of the world this indicates it's widespread over variety of lands under variable growing conditions (2). The plant chemicals, pharmacological, and toxicological properties have been investigated in many studies consequently, many of its constituents are well known, some with multiple health-promoting properties in humans, they include the following biologically active compounds: antioxidants, including anthocyanins, ascorbic acid, beta carotene, beta-sitosterol, campestrol, citric acid, delphinidium, delphinidium-3-glucoside,myristic acid, procatechuic acid, selenium, and tartaric acid; hypotensive or blood pressure lowering agents including ascorbic acid, calcium, chromium, fiber, magnesium, manganese, potassium, zinc; antioxidant synergist (enhances the work of antioxidants) including citric acid and tartaric acid; vasodilators including anthocyanins, potassium; heart protective compounds, including chromium, niacin, and procatechuic acid (3). Animal studies have suggested that hibiscus might have a blood pressure–lowering effect (4). It was concluded that *H. sabdariffa* calyces contain potential antioxidant and antibacterial agents that need further investigation (5). The extract of calyces is reported to contain 17 amino acids and possess antibacterial (6), antinociceptoic (7) and antipyretic activities (8). It has also been shown to protect cells against oxidative stress in rats (7). In spite of the numerous researches in laboratory animals and humans, there are still limited studies in poultry, some of which revealed that Roselle calyces could be used in ameliorating the negative effect of heat stress on laying hens reared under hot climatic conditions (9), other study

suggested that aqueous extract of H.sabdariffa may possess hypotriglyceridemic effect in Shika Brown laying hens but caused increase in serum and egg yolk cholesterol (10).

Materials and Methods

Dry calyces of *Hibiscus sabdariffa* purchased from the local market. The extraction of the calyx was as described by (8). Each fifty grams of the dry calyces were pulverized using miller; the resulting powder was dissolved into 200 ml of 70% ethanol and put the baker that containing the mixture on hot plate magnetic stirrer to the next day, then the production was filtered using gauze and then filter papers. The filtrate was evaporated in aeration oven at 40°C. The dried residue was scrapped and kept in a capped bottle. From the dried extract, a fresh solution was prepared on each day of the experiment. A total of 30, 49 week old layers belonging to the Isa Brown breed were divided into two groups of 15 layers each, both were received basal diet according to (11). Group 1 served as control which was orally administered 2ml/kg body weight of distilled water, Group 2 served as treated group which were orally administered alcohol extract of *Hibiscus sabdariffa* calyces at a dose of 200mg/kg BW dissolved in 2 ml of distilled water (12). The groups received the water and extract daily to individual layers at 6:00 a.m, after that drinking water and diet were provided *ad libitum*, the experiment lasted 8 weeks. Five birds from each group are randomly selected for blood sample collection via venepuncture, using wing veins. The specimens are collected into heparinized tubes for evaluating red blood cell count, Hb & white blood cell count, and the other specimens put into serum tubes centrifuged at 2000 rpm for 20 min and the serum separated using Pasteur pipette. Serum was used to assay total protein, cholesterol, LDL and HDL. The sampling was done at the last day of treatment for determination the following parameters:

1. Red blood cells count: by using Natt and Herrick solution (13).
2. Hemoglobin: by using Drabkin`s reagent according to (14).
3. White blood cells count: by using Natt and Herrick solution (13).
4. Total protein: by using Biuret method (15).
5. Cholesterol: according to (16).
6. Low density lipoprotein LDL: according to (17).
7. High density lipoprotein HDL: according to (17).

Statistical analysis

Data were expressed as mean ± standard error, significance between groups was determined by T test, ($P<0.05$) was used as a criterion for significance (18).

Results

The results revealed that group 2 had a significant elevation ($P<0.05$) in the levels of hemoglobin, white blood cells and high density lipoprotein (HDL), and significant reduction ($P<0.05$) in the level of serum low density lipoprotein (LDL) compared to the group 1, while there was no significant elevation ($P>0.05$) in the levels of red blood cells, total protein and cholesterol between the groups, as in table- 1.

Table (1): The RBCs, Hb, WBCs, serum protein, cholesterol, LDL and HDL levels (mean ± standard error) of the studied groups

Groups / Parameters	Group 1	Group 2
Red blood cells (× ١٠^١٢/ml)	3.46 ±١.01 a	3.76 ± ١.6٦ a
Hemoglobin (%)	8.14 ± 2.21 a	9.68 ± 1.42 b
White blood cells (×١٠^٩/ml)	19.39 ±1.57 a	21.72 ± 2.13 b
Total protein (g/dl)	5.11 ± 0.33a	5.18 ± 0.59a
Cholesterol (mg/ dl)	199.2 ± 5.02 a	200.11± 7.31 a
LDL(mg/ dl)	79.32 ± 3.05a	43.51± 1.89 b
HDL (mg/dl)	61.9 ± 2.41 a	77.97 ± 3.01 b

The different letters refer to significant differences between the groups (P< 0.05).
Group 1: control Group 2: treatment

Discussion

Results revealed that group 2 had a non significant increase (P>0.05) in red blood cells level and protein, significant elevation (P<0.05) in the levels of hemoglobin and white blood cells, this may return to the treatment with *H. Sabdariffa* calyces extract which contain high percent of protein in its composition (19) so the level of protein elevated in the treated group, but this elevation not reach the significant degree, and the other reasons for these results may be the presence of anthocyanin, flavonoids, glycosides, vitamin C and other substances, which are powerful antioxidants prevent cells and tissues from oxidative damage, lipid perioxidation and also improve immunity and productivity against stress including challenges of diseases (20), these have fundamental contribution in maintenance of red blood cells and white blood cells life and increase hemoglobin level in the treated group, the ameliorating mechanism is through the ability of the hydroxyl groups and other features of the antioxidants found in this extract in scavenging the harmful free radicals and reactive oxygen species (21). Also the study revealed that there was slight elevation in the levels of cholesterol in Group 2, this may be due to increased intestinal absorption and/or increased endogenous synthesis of cholesterol since certain biological activities in plants may vary with the animal species (22), the extract may contain a substance that potentiates the activity of 3-hydroxymethylglutaryl-CoA (HMG-CoA) reductase, an enzyme that catalyzes the committed step of the cholesterol biosynthetic pathway (10). The study revealed a significant reduction (p<0.05) in the level of serum low density lipoprotein (LDL), & significant elevation (P<0.05) in the level of high density lipoprotein (HDL) this attributed by (23) which have reported that HDL has a role in preventing LDL oxidation *in vitro*. The HDL transports cholesterol from the peripheral tissues to the liver for conversion into bile (24). In conclusion the administration of Roselle calyces extract for laying hens has positive effect on some of blood parameters & negative effect on others.

References

1. Mortn, J. (2010). Roselle. Fruits of warm climates. P. 281-286. Miami, FL.
2. Suliman,G. M.; Babiker, S.A. and Eichinger, H.M. (2009). Growth performance of Sudan Baggara bulls fed diets containing Hibiscus (Karkade) seeds as a non-conventional protein source.

Livestock Research for Rural Devilopment. 21 (6).

3. Mozaffari-Khosravi, H.; Jalili-Khanabadi, B-A.; Afkhami-Ardekani, M.; Fatehi, F. and Noori-Shadkam, M. (2009). The effects of sour tea (Hibiscus sabdariffa) on hypertention in patients with type II diapetes. Journal of Human Hypertension. 23, 48-54.
4. Adegunloye, B. J.; Omoniyi, J. O.; Owolabi, O.A.; et al. (1999). Mechanisms of the blood pressure lowering effect of the calyx extract of *Hibiscus sabdariffa* in rats. Afr. J .Med .Sci.;25: 235–8.
5. Onibi, G. E. & Osho, I. B. (2007). Oxidative stability and bacteriological assessment of meat from broiler chickens fed diets containing Hibiscus sabdariffa calyces. African Journal of Biotechnology, Vol. 6, No. 23, pp. 2721-2726.
6. Oboh,G. (2004). Nutrient composition and antimicrobial activity of Sorrel Drinks (Soborodo). J. Med. Food, 7:340-342.
7. Wang, C. T.; Wang, J. M. and Lin, W.L. (2000). Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxidase indused hepatic toxicity in rats. Food Chem. Toxicol. 51: 411-416.
8. Harborne, J. B.; Mabray, T. J. & Mabray. H. (1975). Physiology and function of flavonoids. P: 970- 1042. The flavoids. Acad. Press, New York, San Francisco.
9. Minka, N.S.; Fayomi, A. and Ayo, J. O. (2007). Protective Influence of Calyces of Hibiscus sabdariffa Against Heat Stress in Laying Hens During the Hot Season. Research Journal of Poultry Sciences 1 (1): 7-11.
10. Habibullah, S. A.; Bilbis, L. S.; Ladan, M. J. Ajagbonna, O. P. and Saidu, Y. (2010).but Aqueous Extract of *Hibiscus sabdariffa* Calyces Reduces Serum Laying Hens Increases Serum and Egg Yolk Cholesterol of Shika Brown
11. NRC (National Research Council). (2002). Nutrient Requirements for poultry .7th ed. National Academic Press. Whashington, USA.
12. Campbell, T. W. (1988). Avian Hematology and Cytology. First Edition, Iowa state University Press. Amess, IOWA.
13. Coles, E.H. (1980). Veterinary Clinical Pathology . 4th edition. W . B . Sanders co.
14. Tietz, N. (1995). Clinical Guide to laboratory test. Philadelphia: WB Saunders
15. Elias, A. and Franey, R. J. (1968). Serum cholesterol mesurment based on ethanol extraction and ferric chloride sulfuric acid . Clinical Chemistry Acta, 2: 225-263.
16. Assmann, G.; Jabs, H. U.; Kohnert, U. and Nolte, W. (1984). LDL cholesterol determination in blood serum following precipitation of LDL with polyvinyl sulfate. Clin. Chem. Acta. 140:77-83.
17. Warnick, G. R.; Benderson, J. and Albers, J. J. (1982). Dextran sulfate Mg^{+} precipitation procedure for quantitation of high density lipoprotein cholesterol. Clin. Chem. 28: 1379-1388.
18. Niazi, A. D. (2004). Statistical analysis in medical research. 2nd ed. Dep. of Community Medicine, Iraq.
19. Ali, B. H.; Wabel, N. and Blunden, G. (2005). Phytochemical and toxicological aspects of H. sabdariffa L. :A review. Phytother Res., 19: 369-375.
20. Essa, M. M.; Subrammanian, P.; Suthakar, G.; Subash, S.; Manivasagam, T.; Dakshayani,

K.B.; Sivaperu Mul, R. and Vintothini, G. (2006). Influence of Hibiscus sabdariffa (Gangura) On the levels of circulatory lipid perioxidation products and liver marker enzymes in experimental hyperammonemia. J. Appl.Biomed., 4: 53-58.
21. Sofowora, A. (1992). Medicinal Plants and Traditional Medicine in Africa. 24th Edn., John Wiley and Sons, New York, pp: 8.
22. Heinecke, J.W. and A.J. Lusis. (1998). Paraoxonase-gene polymorphism associated with coronary heart disease: Support for the oxidative damage hypothesis. Am. J. Hum. Genet., 62: 20-24.
23. Lenfant, C.J., A. Shepherd, P. Gotto, A. Kwiterovich, R. Scanu and O. Superk.(1997). Lowering LDLs plays only a part in preventing heart disease. Modern Med., 65: 14-17.
24. Chen, C.C., J.D. Hsu, S.F. Wang, H.C. Ching and M.Y. Yang *et al.*,(2003). *Hibiscus sabdariffa* extracts inhibits the development of atherosclerosis in cholesterol-fed rabbits. J. Agric. Food Chem., 51: 5472-5477.

التأثيرات الفسلجية لمستخلص كؤوس أزهار نبات الكجرات في بعض المعايير الدمية في دجاج بيض المائدة

رنا جابر طارش البغدادي
كلية الطب البيطري/ جامعة القادسية

الخلاصة

صممت هذه الدراسة لتقييم التأثيرات الفسلجية التي يحدثها المستخلص الكحولي لكؤوس أزهار نبات الكجرات في بعض المعايير الدمية في دجاج بيض المائدة. قسمت ٣٠ دجاجة بياضة بعمر ٤٩ أسبوع من سلالة الايسا براون عشوائياً الى مجموعتين ضمت كل مجموعة ١٥ دجاجة تناولت العليقة الاساسية.المجموعة الاولى مثلت السيطرة التي تم اعطاؤها الماء المقطر بمقدار ٢ مل /كغم من وزن الجسم عن طريق الفم. اما المجموعة الثانية فقد مثلت المجموعة المعاملة التي التي تم اعطاؤها المستخلص الكحولي لكؤوس أزهار نبات الكجرات بجرعة ٢٠٠ ملغم /كغم من وزن الجسم المذابة بـــ ٢ مل من الماء المقطر.تم تجريع المجموعتين بالمواد اعلاه يومياً عند الساعة السادسة صباحاً، بعد ذلك تكون التغذية وماء الشرب بصورة حرة طيلة اليوم، استمرت التجربة ٨ أسابيع من٢٠٠٩/١٢/25 ولغاية 2010/٢/25 وفي نهايتها تم دراسة تأثير المستخلص وذلك بقياس بعض المعايير الدمية كعدد كريات الدم الحمر، تركيز خضاب الدم، عدد خلايا الدم البيض وتركيز البروتين، الكولسترول، تركيز البروتين الدهني واطئ الكثافة وتركيز البروتين الدهني عالي الكثافة في مصل الدم. أظهرت النتائج أن مستخلص كؤوس أزهار نبات الكجرات أدى الى حدوث إرتفاع معنوي في مستويات هيموغلوبين الدم، خلايا الدم البيض والبروتين الدهني عالي الكثافة وانخفاض معنوي في تركيز البروتين الدهني واطئ الكثافة اما عدد كريات الدم الحمر وتركيز البروتين الكلي والكولسترول فقد اظهرت ارتفاعاً غير معنوي في المجموعة الثانية مقارنة بالمجموعة الأولى .