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Research article

Effects of Aqueous and Methanolic Extracts of *Hibiscus rosa-sinensis* Leave on the Blood Cells of Mice *In vivo*

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ABSTRACT

Present study focused on *in vivo* effect of aqueous and methanolic leave extracts of *Hibiscus rosa-sinensis* plant on different blood cells count of mice that was carried out by using 40 mg / kg of plant extracts orally. Effects of plant extracts were examined after 3 and 7 days of feeding orally. The results showed significant increases (P≤ 0.05) for all parameters (cell types) that included in current study, total white blood cells (WBCs), lymphocytes, eosinophil, red blood cells (RBCs) and platelets. However, there is no significant difference in Hb values as compared with control.

Keywords: : *Hibiscus rosa-sinensis*, Hb, RBCs, WBCs.

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INTRODUCTION

Plants are important sources for medicines that were used in the treatment of various categories of human diseases. Historically, all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex forms of crude extracts, mixtures, etc. Today a substantial number of drugs were developed from plants which active against a number of diseases [1]. One of these important medicinal plants is *Hibiscus rosa-sinensis*, Linn. Malvaceae was known as China rose or beauty rose. Medicinally, all parts of this plant such as Leaves, flowers and roots were used in treatment of diseases like aphrodisiac, menorrhagia, oral contraceptive, laxative, etc [2]. *Hibiscus rosa-sinensis* is a bushy, evergreen shrub or small tree growing 2.5–5 m (8–16 ft) tall and 1.5–3 m (5–10 ft) wide, with glossy leaves and solitary, brilliant red flowers in summer and autumn [3]. Many varieties exist differing in size and color, in single or double forms. The important colors include red, white, yellow, and light red [4]. Many plants have serious adverse effects, which include positive and negative effects (toxic or harmful side effects). The plant contains phytochemicals materials that are chemical compounds formed during the normal metabolic processes. These chemical materials are often referred to as "secondary metabolites" of plants [5].
Different extracts of *H. rosa-sinensis* plant contains many secondary metabolites such as alkaloids, glycosides, fatty materials, reducing sugars, resin, sterols and the lack of tannins and saponins. Leaves of *H. rosa-sinensis* were investigated for their fatty alcohol, fatty acids and hydrocarbon content. Two cyclic acids viz., malvalic and sterculic are also identified [6]. Therefore, many of the commonly used plants

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need to be evaluated for their potential hemolytic activity such as *H. rosa-sinensis*. Natural products from plants are potent sources of potent anticancer agents. Previous studies had showed that *H. rosa sinensis* possesses anti-complementary, anti diarrhetic and anti-phologistic activity [7]. Leaves are used as laxative while root is used in cough. Leaves and flowers of *H. rosa-sinensis* leaves are used as an antiseptic for boils and ulcers. In traditional medicine, the leaves of the plant are used in fatigue and skin disease [8]. Thus, this work is aimed to study the effects of *H. rosa-sinensis* plant on the blood cells of mice *(in vivo)*.

MATERIAL and METHODS

Collection and drying of plants

Leaves of *H. rosa-sinensis* plant were collected locally from different places in Baghdad, the plants were authenticated by department of biology in college of science, University of Baghdad, Baghdad, Iraq; during November 2011 to March 2012. The collected plant was washed with clean tap water for removing dust from the plant leaves. The collected clean plant was left at room temperature at (22-25) °C for (3-4) weeks for drying (aseptically) and then was grinded by electric blander to convert the dried plants into coarse powder.

Preparation of crude extracts of plant leaves

Crude plant extract was prepared by using methanol 80%. Dried leaves powder (100 gm) were kept in a thimble and extracted by soxhlet apparatus which contained 500 ml of the solvent at 50°C for 36 h with an extraction ratio of (1:5) [9]. Extracted samples were filtered through a filter paper (Whatman No.1) and the filtrate was concentrated using rotary evaporator.

The concentrated extract was kept in a glass container at 4°C. While, aqueous extract was carried out by adding (100 mg) of dried leaves powder to flask contained 450 ml of distilled water and extracted on a hot plate stirrer apparatus at 80°C for 24 h with an extraction ratio of (1:5) [10]. Then the extract was filtered through a filter paper (What man No. 1), and the sample was concentrated by using rotary evaporator. The extract was kept in a glass watch container at 4°C in a refrigerator.

Phytochemical investigation of H. rosa-sinenss leaves

For the phytochemical investigation, hydro methanolic solvent (methanol 80%) was used for extraction of leaves by soxhalet apparatus [11] and analyzed the extract for determination the presence of different plant constituents (secondary plant products) that were detected by usual prescribed methods. Preliminary phytochemical analysis of the leaves extract that mentioned above was subjected to various qualitative phytochemical tests for detection of plant constituents such as flavonoids (alkali test), alkaloids (Dragendroff reagents) and tannins (ferric chloride test) [12], saponins (olive oil test), resins (acetic anhydride test) and volatile oils (fluorescence test) [13], and glycosides (Liebermann's test) [14].

Preparation of 40 mg/ml concentration of crude plant leaves extracts

Concentration 40 mg/kg of crude plant extracts were prepared by dissolving a certain weight of concentrated extracts in a suitable volume of distilled water (concentration mg/ml = weight/volume *1000).

Laboratory animals

Fifteen albino mice laboratory animals were used. They were procured from National Center for Drug Control and Research, Ministry of Health, Baghdad, Iraq. All mice have same age (8-10 weeks) and same weight (23-27 gm) for both sex. They were divided into five groups, and each group was kept in a separate plastic cage (details of these groups are given in the paragraph of experimental design). The animals were kept at 23 – 25°C, and they had free excess to food (standard pellets) and water. The study was conducted following approval from the animal ethics committee of University of Baghdad, Baghdad, Iraq.

Experimental design

Mice were divided into five groups and each group contained three mice. Group 1, Not treated animals (control); group II, animals were administrated orally with methanolic extract of *H. rosa sinsis* leaves per day at concentration 40 g/kg of body weight for 3 days; group III and Group IV, animals were administrated orally with aqueous extract of *H. rosa sinsis* leaves per day at concentration 40 g/kg of body weight for 3 days; group V, animals were administrated orally with methanolic extract of *H. rosa sinsis* leaves per day at concentration 40 g/kg of body weight for 7days; group VI, animals were administrated orally with aqueous extract of *H .rosa sinsis* leaves per day at concentration 40 g/kg of body weight for 7days.

Collection of blood samples

Blood samples were collected at 3rdday and 7th day post treatment, heart puncher of mice for measuring of hematological parameters. Five hundred of blood was collected into bottles containing anticoagulant (EDTA) then the containers were shacked genteelly and hematological parameters were measured. At day 7 post treatment, animals were euthanized by using chloroform.

Determination of hematological parameters

Blood was collected into EDTA-containers at day 3th and 7th day from the administration of crude extract plant to experimental animals for hematological analysis for daily. This study established the hematological changes such as hemoglobin concentration (Hb), RBCs and total WBCs, eosinophil, lymphocytes, and platelet, and differential count of WBCs were measured in an automatic hematology analyzer, cell-DYN-3700.

Statistical analysis

The statistical analysis system- SAS (2012) program was used for determination of significant effect of different factors on parameters of study. Least significant difference –LSD test was used for significant compression between the means of data in this study.

RESULTS and DISCUSSION

Effect of crude extract of *H. rosa-sinesis* leaves on blood cells after 3 days

Effect of control, methanolic and aqueous extracts of leaves of *H. rosa-sinesis* induced a changes in erythrocytes and related at the dose levels of 40 mg/kg. The results showed that the numbers of WBC were 4.8, 4.9 and 6.3; lymphocytes, 56, 58 and 39); eosinophil, 0, 0 and 0.6; RBC, 5.8, 5.8 and 7.8;

platelets, 756, 599 and 745; the concentrations of Hb were 10.5, 13 and 14 in control, aqueous and methanol extract, respectively of treated animals. There were showed significant increasing at level ($p \leq 0.05$) for all parameters (**Table 1**).

Table1. Effect of *Hibiscus rosa-sinesis* leaves on blood cells after 3 days. * ($P \leq 0.05$). LSD =Wbc (1.675) Lymphocytes (14.336) Eosinophil (1.149) RBC (2.134)Hb(2.097) Platelets(102.675)

Blood characters	Control (No.the cells)	Conc. 40 mg/ml		MEAN
		Aqueous	Methanol	
WBC	4.8	4.9	6.3*	5.6
Lymphocytes	56	58	39*	48.5
Esionphile	0	0.6	1.3*	0.95
RBC	5.8	7.8	8.6*	8.2
Hb	10.5	13	14*	13.5
Platelets	756	599*	745	672

Significant increasing of erythrocytes and WBC number was observed after oral administration of methanolic and aqueous extracts of *H. rosa-sinesis* because the extracts may contain phytochemicals compounds that stimulate the forming or secretion of erythropoietin in the stem cells of normal mice. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells [15]. Erythropoietin affects the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and hemoglobin are very important in transferring respiratory gases [16]. It may also suggest that the extracts can cause polychethermia. Previous studies have indicated that an increasing of erythrocytes number is suggestive of polycythermia and positive erythropoiesis [17]. The presence of antioxidant phytochemicals like flavonoids and tannins in the methanolic extracts of *H. rosa-sinesis*may is responsible for the haemopoietic stimulating effects [18]. Phytochemical analyses showed presence of alkaloids, glycosides, flavonoids, saponins and tannin in hibiscus leaf extract [19]. From the preliminary screening, it has been identified that the methanolic extract of hibiscus exhibits phytomedical property which may due to the presence of biologically active compounds in hibiscus whose activity are enhanced in the presence on methanol solvent [19].

Effect of crude extract of Hibiscus rosa-sinesis leaves on different blood cells after 7 days

Effect of control, aqueous and methanolic extracts of *H. rosa sinensis* leaves on mice after 7days of oral administration showed that WBC, 4.8, 5.3 and 6.2; lymphocytes, 56, 70 and 67, eosinophil, 0, 0.6 and 1.3; RBC, 5.8, 7.4 and 7.1; Hb, 10.5, 12 and 11.2 and platelets, 756, 1284 and 1081 for control, aqueous and methanolic extracts, respectively (**Table 2**). There were significant increasing ($P \leq 0.05$) for all parameters of hematology analysis including WBC, lymphocytes, eosinophil, RBC and platelets, but the Hb did not show significant increasing ($P \leq 0.05$).

Administration of any chemical compound for long duration may bring about significant changes in the structure, function, metabolic transformation and concentration of biomedical enzymes and even metabolic pathways. These alterations may be rapid or slow and may lead to different biochemical mechanisms, producing a pathological case [20]. Actually, assessments of hematological analysis of blood parameters are used to determine the extent of deleterious effect of the extracts on blood of an animal. On the contrary, an increasing of RBC number is suggestive of polycythemia summarized and positive erythropoiesis [21].

Table 2. Effect of *Hibiscus rosa-sinesis* leaves on blood cells after 7 days. * P<0.05, NS: Non-significant, LSD = Wbc (1.887) Lymphocytes (12.532) Eosinophil(1.149) RBC (1.546) Hb (2.037) Platelets (139.74).

Blood cells	Control (No.the cells)	Concentration (40 mg/ml)		MEAN
		Aqueous	Methanol	
WBC	4.8	5.3	6.2*	5.75
Lymphocytes	56	70*	67	68.5
Eosinophil	0	0.6	1.3*	0.95
RBC	5.8	7.4*	7.1*	7.25
Hb	10.5	12	11.2	11.6
Platelets	756	1284*	1081	11825

Administration of any chemical compound for long duration may bring about significant changes in the structure, function, metabolic transformation and concentration of biomedical enzymes and even metabolic pathways. These alterations may be rapid or slow and may lead to different biochemical mechanisms, producing a pathological case [20]. Actually, assessments of hematological analysis of blood parameters are used to determine the extent of deleterious effect of the extracts on blood of an animal. On the contrary, an increasing of RBC number is suggestive of polycythemia summarized and positive erythropoiesis [21]. Hence, a significant increasing of RBC with no alteration in Hb in *H. rosa sinensisi* treated animals indicates that the extract causes did not show any toxic effect on RBC. Reports about WBC count have pointed out that whereas increased count of WBC is supposed to be helpful in boosting immune system [22]. The present study proved the role of *H. rosa sinensisi* leveaves extract in increase the number of blood cells but not affect on the level of Hb concentration *in vivo*.

Conflict of interest

The authors declare that they have no conflict of interests.

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