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Effect of Methanol Extract of *Swertia chirata* on Various Cellular Components of Blood in Rats

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ABSTRACT

Background: *Swertia chirata has been used for its medicinal purpose worldwide specifically in developing countries. People believe that medicinal plants are safe and economical; However, unaware of their toxic effects. Hematological parameters are the essential tool to assess the degree of toxicity on cellular components of blood.* ***Objective:*** *The aim of this study is to evaluate the effect of Swertia chirata on hematological parameters in rats using an automated analyser.* ***Methodology:*** *Albino Wistar rats were randomly assigned into three groups of six rats each. Group 1 was control, while group 2 (LD) and group 3 (HD) received 250 mg/kg and 500 mg/kg body weight of extract respectively, orally once daily.* ***Results:*** *No significant difference was observed in the WBC count of extract treated groups($P>0.05$). The HD group did not show any significant difference ($P>0.05$) in the RBC count, HCT, Hb, MCV, MCH, MCHC, RDW, platelet count, PDW, MPV, PLCR as compared to control and LD groups. Whereas, MCHC was decreased in the LD group ($P<0.0001$; $P<0.05$) as compared to the control and high dose groups respectively. The RDW in the LD group was significantly increased ($P<0.005$) as compared to the control group. The P-LCR and PDW of the LD group were significantly decreased ($P<0.05$) as compared to the control and HD groups.* ***Conclusion:*** *The oral administration of Swertia chirata at low dose increases RDW and decreases MCHC, P-LCR and PDW, while no significant difference was observed at high dose on different haematological parameters.*

Keywords: Haematological parameters, Haemoglobin, *Swertia chirata*, Platelet count, Red blood cell, WBC count

INTRODUCTION

Since centuries, medicinal herbs have been used all over the world as they have a major role in preventing and treating of various diseases [1]. Due to various adverse effects and contraindications of conventional drugs, medicinal herbs have been popular in developing countries. Cultural beliefs, is one of the reasons that people prefer traditional remedies which are safe, cost effective and easily available [2].

Swertia chirata has been widely used worldwide to treat several diseases particularly in the rural region. It is bitter in taste and belongs to the family Gentianaceae [3], commonly known as chirata, Kirataka, Chiratika [4], an inhabitant Himalayan plant [5] and distributed in the tropical region of Asian countries, Nepal, Europe, America, Africa and as well as in other parts of the world [3,6]. The major biological active compounds in crude extract of different parts of this plant are, mangiferin, amarogentin and swertimarin, secondary metabolites: including glycosides, xanthones, secoirioid, phenolics, alkaloids, flavonoids, triterpenes, tannins, carbohydrates and sterols, these phytochemicals are responsible for its pharmacological effect [7,8]. Studies on Experimental animal studies on *Swertia chirata*, documented the efficacy in diabetes [9], hypertension [10], as an analgesic, anti-inflammatory [11], other studies included anticarcinogenic [12] anticonvulsant, sedative, anxiolytic [13] antioxidant, anti-AChE properties [14], antipyretic [15], anti-bacterial [3], antiviral [16], antiulcer [17], anti-helminthic [18], anti-hyperlipidaemic [19] and hepatoprotective effect [20].

People perceive that herbal therapies are harmless and unaware or ignorant of their toxic effects on the different biological system [21]. Therefore, the data on toxicity of medicinal plants is needed to be explored. Keeping in view the significance of this plant in the treatment of several ailments, the aim of the present study was, to assess the effects of *Swertia chirata* extract on different haematological parameters in albino Wistar rats.

MATERIALS AND METHODS

Collection and identification of plant

The dried aerial part of *Swertia chirata* was purchased from the local market of Karachi, Pakistan. The sample of the plant specimen was identified by Prof. Dr Mansoor Ahmad, Department of Pharmacognosy, University of Karachi and issued a voucher specimen no 20170303-1.

Extract preparation

The dried plant material (1000 g) was grounded into coarse powder using an electrical grinder. The coarse powder was macerated with methanol for 15 days at room temperature. The methanol extract was then filtered, evaporated under reduced pressure in rotary evaporator and stored in the refrigerator until needed. The thick gummy extract was reconstituted with 10% Dimethyl sulfoxide (DMSO) to enhance the solubility of extract in water [13]. All the glass wares used in this study was made of pyrex.

Study design

Animals handling and dietary protocol: Adult male Wistar albino rats (initially weighing between 180 g - 210 g) were selected for the present study. Animals were purchased from the animal house of Agha Khan University Karachi, Pakistan. All animals were acclimatized one week before the commencement of the experiment and kept under the controlled room temperature at 23 ± 2°C in 12 h light/dark cycles with free access to food and water. The experiment was performed in accordance with the National Institute of Health (NIH) guidelines for the care and use of Laboratory Animals (National Research Council, 1996), after the approval from the Ethical Review Committee (ERC) of Ziauddin University, Karachi, Pakistan.

Grouping of animals and extract administration: The animals were divided into three groups of six rats in each group, as follows.

Group 1: Received DMSO 10% as placebo (10 ml/kg body weight).

Group 2: Received 250 mg/kg body weight of extract.

Group 3: Received 500 mg/kg body weight of extract.

All the animals were weighed before starting the experiment. The extract was administered according to their body weight once daily, per oral by gastric intubation for 28 days.

Collection of blood sample

On the 29th day, all the animals were reweighed, starved for 24 hours and sacrificed under chloroform anaesthesia. Blood samples approximately 2.5 ml were collected by cardiac puncturing into vacutainers containing EDTA, inverted about 6 times to mix the sample with EDTA [22]. The vacutainers stored in the refrigerator until analysis was done, the haematological parameters were analysed within 24 hours after collecting blood samples.

Analysis of blood parameters

Blood samples were analysed by using automated analyser by Sysmex (KX-21), accurately programmed for analysis of the red blood cell (RBC) count, haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), RBC distribution width (RDW), platelet count, mean platelet volume (MPV), platelet distribution width (PDW), platelet large cell ratio (P-LCR) and total white blood cell (TWBC) count.

Statistical analysis

Statistical analysis was carried out by using SPSS (Statistical Package for Social Sciences) version 20. Data is presented as mean and standard deviation/standard error of mean. The difference amongst three groups; control, low dose (250 mg) and high dose (500 mg) of *Swertia chirata* was measured by using one-way analysis of variance (ANOVA) and where data violated the assumptions of normality, Kruskal Wallis H test (non-parametric test) was used, followed by pair-wise comparison post hoc Tukey-test. $P<0.05$ was taken to indicate the statistical significance.

RESULTS

Effect of *Swertia chirata* extract on RBC indices

The effects of the oral administration of *Swertia chirata* on RBC indices in the control, low dose (LD) and high dose (HD) groups are presented in the Table 1.

Table 1 Effect of the crude extract of *Swertia chirata* on RBC indices after 28 days of treatment

Parameters	Control	Low Dose (LD)	High Dose (HD)
RBC[1] (× 10^6/µl) Mean ± SD	6.5 ± 0.8	6.6 ± 0.3[NS]	6.9 ± 0.4 [NS]
Hb (g/dl) Mean ± SE	12.7 ± 0.3	12.6 ± 0.4 [NS]	13.1 ± 0.2 [NS]
HCT (%) Mean ± SE	39.3 ± 1.0	42.0 ± 1.3 [NS]	42.2 ± 1.1 [NS]
MCV (fl) Mean ± SE	61.1 ± 0.8	63.3 ± 0.9 [NS]	61.0 ± 0.4 [NS]
MCH[2] (pg) Mean ± SD	19.6 ± 1.0	18.8 ± 0.6 [NS]	19.1 ± 0.5 [NS]
MCHC (g/dl) Mean ± SE	32.5 ± 0.5	30.0 ± 0.2***	31.2 ± 0.3*
RDW-SD (fl) Mean ± SE	33.0 ± 1.1	37.8 ± 1.1**	34.5 ± 0.6 [NS]

***P<0.0001 vs control; **P<0.005 vs control; *P<0.05 vs LD; (n=6); [NS] Not Significant vs Control; [1-2]ANOVA; SD=Standard Deviation; SE: Standard Error of Mean; Red Blood Cell (RBC) count; Hematocrit (HCT); Hemoglobin (Hb); Mean Corpuscular Volume (MCV); Mean Corpuscular Hemoglobin (MCH); Mean Corpuscular Hemoglobin Concentration (MCHC); RBC Distribution Width-Standard Deviation (RDW-SD)

The result of the study showed that the MCHC values in the LD group were significantly decreased (P<0.0001; P<0.05) as compared to the control and HD groups respectively. However, no significant difference was found (P>0.05) in the HD group as compared to the control group.

The RDW values in the LD group were significantly increased (P<0.005) as compared to the control group. However, the RDW values in the HD group were not significantly different (P>0.05) as compared to the LD and control groups. Other parameters including the RBC, Hb, HCT, MCH and MCV of the LD and HD groups were not significantly different (P>0.05) as compared to control.

Effect of *Swertia chirata* on Total WBC Count and Platelet indices

The effects of *Swertia chirata* extract on Total WBC count and platelet indices are shown in the Table 2. The Total WBC count was not significantly changed (P>0.05) in the LD and HD groups as compared to the control group. The P-LCR and PDW values of the LD group were significantly decreased (P<0.05) as compared to the control and HD groups. Other Parameters including, platelet count and MPV in the LD and HD groups were not significantly (P>0.05) different as compared to the control group.

Table 2 Effect of the crude extract of *Swertia chirata* on TWBC and Platelet indices after 28 days of the treatment

Parameters	Control	Low Dose (LD)	High Dose (HD)
TWBC (× 10^3/µl) Mean ± SE	7.0 ± 1.1	10.6 ± 1.6 [NS]	14.4 ± 3.1[NS]
PLT (× 10^3/µl) Mean ± SE	781 ± 93	740 ± 53 [NS]	851 ± 111[NS]
MPV (fl) Mean ± SE	7.6 ± 0.3	6.8 ± 0.1 [NS]	6.9 ± 0.2[NS]
P-LCR (%) Mean ± SE	10.5 ± 1.6	6.0 ± 0.4*	8.2 ± 1.9[NS]
PDW (fl) Mean ± SE	9.5 ± 0.5	8.0 ± 0.3*	8.4 ± 0.6[NS]

*P<0.05 vs control; [NS] Not significant vs Control, (n=6); SE: Standard error of mean, platelet count (PLT), Mean platelet volume (MPV), platelet distribution width (PDW), Platelet large cell ratio (P-LCR) and white blood cell (WBC) count

Effect of *Swertia chirata* extract on body weight of rats

The effect of *Swertia chirata* extract on body weight of Wistar rats after 28 days treatment was observed (Table 3). The mean difference of weight (initial weight-final weight) of the control, LD and HD groups were compared with one another as shown in the Table 3. There was a significant weight gain in the LD group (P<0.01; P<0.05) as compared to the control and HD groups respectively. However, no significant alteration in weight was observed in the HD group (P>0.05) as compared to the control group.

Table 3 Alteration in weight among different experimental groups after 28 days treatment with the crude extract of *Swertia chirata*

Variables	Control	Low dose (LD)	High dose (HD)
Initial weight (Mean ± SD)	190.8 ± 3.1	190.3 ± 2.9	196.8 ± 4.2
Final weight (Mean ± SD)	217.2 ± 3.3	223.0 ± 2.4	224.3 ± 6.5
Mean Difference (Final – Initial weight) (Mean ± SD)	26.3 ± 2.7	32.7 ± 3.4**	27.5 ± 2.4*

**P<0.01 vs control group; *P<0.05 vs LD group, (n=6); SD: Standard deviation

DISCUSSION

Blood is a fundamental circulatory tissue composed of red blood cells (RBCs), white blood cells (WBCs), and platelets which are suspended in a fluid called plasma, with the important function of maintaining homeostasis. Every blood cell has a vital role in our body; for instance, WBCs enhance the immune system and are capable to fight against infections, RBCs carry haemoglobin that are mainly responsible for transportation of oxygen and carbon dioxide as well as valuable in the diagnosis of anaemia; while platelets have a major role in the formation of clot. Haematological studies are essential in the diagnosis of several diseases as well as it could be a helpful tool in early sign of toxicity to cellular components of blood in response to certain natural or chemical agents [23].

Several species of *Swertia* have been used worldwide for medicinal purpose, among all of them *Swertia chirata* is believed to be the most beneficial herb due to its pharmacological properties [4]. Despite of its multiple uses in different ailments, the data regarding its effect on haematological parameters is meagre. Hence, this study was conducted to investigate the effect of the oral administration of *Swertia chirata* extract on haematological parameters in rats.

Our study showed that the WBC count was not significantly altered ($P>0.05$) in the low dose and high dose groups as compared to the control group although an increase count of WBC was observed in the high dose group (Table 2). The normal WBC count in extract treated groups disclosed that *Swertia chirata* does not have any significant effect on white blood cells or leukocytes.

The results of our study indicate that red blood cell indices including, the RBC count, Hb, HCT, MCV and MCH were not significantly altered ($P>0.05$) in high dose and low dose groups (Table 1). Significant alteration was observed in MCHC and RDW values in the low dose group; however, these parameters were remained unaltered in the high dose group (Table 1). The MCHC values of the low dose group were significantly decreased ($P<0.0001$, $P<0.05$) in comparison with control and high dose group respectively (Table 1). A low MCHC signifies hypochromia in early sign of iron deficit and could be observed with any of the disease that can lead to microcytosis [24].

The RDW values at low dose were significantly increased ($P<0.005$) as compared to the control and high dose groups (Table 1). As documented, RDW is a numerical determinant of the variation in size or extent of anisocytosis of circulating RBCs [25]. It has been suggested that the raised RDW together with decreased MCHC and normal MCV can be helpful to differentiate the different types of anaemia [26].

In our study, HCT and Hb were not significantly increased at high and low dose as previously discussed (Table 1); Our results seem to be contradictory with the results of earlier study by Turasker, et al. who reported an increase in HCT and Hb following four weeks administration of the *Swertia chirata* leaves extract to the control as well as phenyl hydrazine induced anaemic rats. They concluded that the mineral and vitamin constituents of the leaf is responsible for its hematinic effect [27].

The results of the current study showed that the high dose of *Swertia chirata* extract does not have any significant effect ($P>0.05$) on platelet indices as compared to the control and low dose groups (Table 2). At low dose, no significant difference was observed ($P>0.05$) on platelet count and MPV values. Whereas, P-LCR and PDW values were significantly decreased ($p<0.05$) at the low dose in comparison with the control and high dose groups (Table 2). High PDW is an indicator of platelet activation [28], whereas P-LCR is an indicator of circulating larger platelets [29]. Platelet indices altogether could provide better understanding of platelet disorders [30]. In several studies, the inverse relationship was found between platelet count and other platelet indices i.e., MPV, PDW and P-LCR [25,31,32]. However, this is not in agreement with our results; Since, in the current study platelet count was not significantly changed in extract treated groups as well as no direct or inverse relationship was found between platelet count and platelet indices.

At low dose, a significant increase ($P<0.01$; $P<0.05$) in body weight of rats was observed as compared to the control and high dose groups respectively (Table 3). Whereas, no significant difference was observed at high dose as compared to control and low dose groups. It showed that high dose does not lead to weight gain as compared to the control group.

CONCLUSION

No significant difference was observed at the high dose of *Swertia chirata* on different haematological parameters as well as on body weight of rats. Whereas, the low dose of extract significantly affected the values of MCHC, RDW, P-LCR, PDW and body weight. Based on these facts, we can conclude that the high dose of *Swertia chirata* may not

have toxic effects on cellular components of blood. However, further studies should be carried out to evaluate the effect of *Swertia chirata* on haematological parameters.

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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