To evaluate the effect of *Swertia Chirata* on different blood parameters

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Abstract:

Background: *Swertia chirata* has been used for its medicinal purpose worldwide specifically in developing countries. People believe medicinal plants as safe and cost effective however, unaware of their toxic effects. Hematological parameters are essential tool to assess the degree of toxicity on cellular components of blood. Hence, the aim of this study is to evaluate the effect of *Swertia chirata* on hematological parameters in rats using an automated analyzer. **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/kgand500 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 RBC count, HCT, Hb, MCV, MCH, MCHC, RDW, platelet count, PDW, MPV, PLCRas compared to control and LD groups. Whereas,MCHC was decreased in the LD group ($P < 0.0001; P < 0.05$)as compared tothe 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:** 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 hematological parameters.

Key words: Hematological parameters, Hemoglobin,*Swertia chirata*, platelet count, red blood cell, WBC count

1. 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, the medicinal herbs have been popular in developing countries traditionally. Cultural beliefs, is one of the reason that people prefer traditional remedies which are safe, cost effective and easily available[2].

The plant *Swertia chirata* has been widely used worldwide to treat several diseases particularly in 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 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, ssphenolics, 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-hyperlipidemic[19]and hepatoprotective effect[20].

People perceive that herbal therapies are harmless and unaware or ignorant of their toxic effects on different biological system[21]. Therefore, the data on toxicity of medicinal plantsis 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 hematological parameters in albino wistar rats.

2. Materials and Methods:

2.1 Collection and Identification of Plant:

The dried aerial part of swertia chirata was purchased from 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.

2.2 Extract Preparation:

The dried plant material (1000 gram) 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 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.

2.3 Animals handling and dietary protocol:

Adult male wistar albino rats (initially weighing between 180-210 gram) were selected for the present study. Animals were purchased from animal house of Agha Khan University Karachi, Pakistan. All animals were acclimatized one week before commencement of experiment and kept under 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 National Institute of Health (NIH) guidelines for the care and use of Laboratory Animals (National Research Council, 1996), after the approval from Ethical Review Committee (ERC) of Ziauddin University Karachi, Pakistan.

2.4 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 experiment. The methanol crude extract was administered according to their body weight once daily per oral by gastric intubation for 28 days.

2.5 Collection of blood sample:

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

2.6 Analysis of blood parameters:

Blood samples were analyzed by using automated analyzer sysmax (KX-21), accurately programmed for the analysis of red blood cell (RBC) count, hemoglobin (Hb), hematocrit (HCT),mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin 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.

2.7 Statistical analysis:

Statistical analysis was carried out by using SPSS (Statistical Package for Social Sciences) version 20. Data ispresented as mean and standard deviation / standard error of mean.The difference amongst three groups; control, low dose (250mg) and high dose (500mg) of *Swertia chirata*extract 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 pairwise comparison post hoc Tukey-test. A p-value of <0.05 was taken to indicate the statistical significance.

3. Results:

Effect of *Swertia chirata* extract on RBC indices:

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

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.

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

Parameters	Control	Low Dose (LD)	High Dose (HD)
RBC[1](x10^6/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<0.0001vs 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)*

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 Table 2. The Total WBC count was not significantly changed($P > 0.05$) in the LD and HD groups as compared to 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 control group.

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

Parameters	Control	Low Dose (LD)	High Dose (HD)
TWBC ($x10^3/\mu l$) Mean ± SE	7.0 ± 1.1	10.6 ± 1.6^{NS}	14.4 ± 3.1^{NS}
PLT ($x10^3/\mu l$) 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	$6.0 \pm 0.4^{*}$	8.2 ± 1.9^{NS}
PDW (fl) Mean ± SE	9.5 ± 0.5	$8.0 \pm 0.3^{*}$	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 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 crude extract of *Swertia chirata*

	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 have a vital role in our body for instance, WBCs enhance immune system and are capable to fight against infections, RBCs carry hemoglobin that are mainly responsible for transportation of oxygen and carbon dioxide as well as valuable in the diagnosis of anemia; while platelets have a major role in the formation of clot. Hematological 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 hematological parameters is meager. Hence, this study was conducted to investigate the effect of oral administration of *Swertia chirata* extract on hematological 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 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 cells indices including, 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 low dose group; however these parameters remain unaltered in high dose group (Table 1).The MCHC values of low dose group were significantly decreased (p<0.0001, P < 0.05)in comparison with control and high dose group respectively. A decreased MCHC specify 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. 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 / variable MCV can be helpful to differentiate the different types of anemia[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 control as well as phenylhydrazine induced anemic 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 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 low dose in comparison with 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 andother platelet indices i-e MPV, PDW and P-LCR[25, 31, 32]. However, this is not in agreement with our results; since in

current study platelet count was not significantly changed in extract treated groups, besides no direct or inverse relationship was found between platelet count and platelet indices.

The changes in body weight after the administration of *Swertia chirata* extract for 28 days is shown in Table 3.There was a significant increase in the body weight of rats at low dose as compared to control and high dose groups.Whereas, no significant difference was observed at high dose as compared to control and low dose groups.

Conclusion:

No significant difference was observed at high dose of *Swertia chirata* extract on different hematological parameters as well as on body weight of rats.Whereas 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 hematological parameters.

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