

# Hematological and toxicological effects of aqueous leaf extract of *Stevia rebaudiana* Bertoni in normal rat modals

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**Abstract:** *Stevia rebaudiana* Bertoni a non-caloric, safe and natural sweetener has been shown pharmaceutically important in the management of blood disorders. This study was designed to investigate hematology and safety of stevia aqueous extract through animal modeling. For this purpose, fifty albino rats were categorized into 5 groups and all the groups were received aqueous stevia extract at different dosage levels (200, 300, 400 and 500 ppm/kg b. wt) for 8 weeks except control group. Hematological and toxicological analyses were conducted using standard recommended procedures. The results indicated that biochemical parameters (RBC, HB, HCT, MCV, MCH, MCHC, WBC, eosinophils, lymphocytes and neutrophils) of albino rats significantly ( $P < 0.05$ ) increased and PLT, MPV and monocytes levels non-significantly decreased by using aqueous extract of stevia at different levels after eight weeks of study. Furthermore, Stevia aqueous extracts had non-toxic effect on liver functioning tests. However, stevia aqueous extracts were insignificant in their impression regarding organ to body weight ratios. The stevia aqueous extract has positive effect on hematological parameters of albino rats and is toxicologically safe. Therefore it could be used as a natural remedy for the management of hematological disorders without any health hazards.

**Keywords:** *Stevia rebaudiana* Bertoni, stevioside, hematology, toxicology, liver functioning enzymes.

## INTRODUCTION

In the recent years, the problems related hematology that includes abnormalities in the production, functioning and morphology of erythrocytes, leukocytes and platelets are common due to nutrient deficiencies and poor environmental condition (Igwe *et al.*, 2020). Many conventional expensive ways are used for the management of blood disorders that are painful and have many adverse side effects. Therefore use of natural plant products due to presence of minerals, nutrients and antioxidants for the treatment of hematological complications is cheap and have pronounced healing properties (Osonuga *et al.*, 2020).

*Stevia rebaudiana* Bertoni (family Asteraceae) popularly known as stevia, sweet herb of Paraguay, honey leaf and sweet weed (Anbazhaganis *et al.*, 2010; Kurek & Krejpcio (2019). natural, non-toxic, non-caloric and pharmacologically important medicinal herb due to presence of glycosides such as stevioside, rebaudiosides (A, B, C, D, E), dulcoside A and steviolbioside but the major sweet constituents are stevioside and rebaudioside A (Brahmachari *et al.*, 2011; Lemus-Mondaca *et al.*, 2012). Stevioside a natural calorie free sweetener is 100

to 300 times sweeter than sucrose and have been widely used in numerous countries like Paraguay, Japan and Brazil as a safe and calorie free sugar alternate in many functional foods, medicines, cosmetics and wine making (Stoyanova *et al.*, 2011). Stevia extract possess anti-hyperglycaemic, anti-hypertensive, anti-hypolipidemic, anti-tumor, anti-diarrheal, anti-oxidant, anti-viral, anti-diuretic, immunomodulatory properties and has positive effect on hematological problems (Ferrazzano *et al.*, 2016).

According to Joint FAO/WHO Expert Committee on Food Additives (JECFA) and previous studies, the recommended acceptable daily dose level of stevia is 4 mg/kg b. wt (Gardana *et al.*, 2010). Hematological studies in both animals and humans proved that consumption of glycosides present in stevia improved the levels of hematological parameters. Furthermore stevia may not result in any teratogenic, mutagenic or carcinogenic effects and allergic reactions (Brusick *et al.*, 2008; Ruiz-Ruiz *et al.*, 2015).

As the *Stevia rebaudiana* Bertoni a natural sugar substitute has ethno pharmacological relevance. Hence, the present research work was carried out to assess the effect of aqueous extracts of stevia on hematology and toxicology of albino rats.

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## MATERIALS AND METHODS

### *Collection and preparation of plant material*

Fresh leaves of *Stevia rebaudiana* Bertoni were collected from Ayub Agricultural Research Institute (AARI), Faisalabad. For identification of plant, the plant physiology section directorate of Agronomy, AARI, Faisalabad issued the certificate of identification reference No.606/8. Furthermore, the dust, dirt and unwanted material from stevia leaves were washed properly with tap water. After that, stevia leaves were dried at room temperature by spreading them under the shade and then fine powder was made by using grinder (MJ-176-NR-3899) (Kujur *et al.*, 2010).

### *Preparation of stevia aqueous extracts*

Solvent (water) extraction method was used to extract stevioside from dried ground leaves of stevia plant. First of all, hot water (65°C) at the ratio of 1:45 (w/v) was mixed with dried ground stevia leaves, then shaken properly and kept at room temperature for 24 hours (Abou-Arab *et al.*, 2010). The mixture was stirred 2 to 3 times a day. After one day, mixture was filtered through Whatman filter paper and the filtrate was evaporated using rotary vacuum evaporator (EYELA N-1110S 115V) at 40-45 °C (Kujur *et al.*, 2010).

### *Experimental animals and design*

The research study was approved from the Institutional Review Board, Faculty of Science & Technology, Government College University, Faisalabad, Pakistan (IRB No.0093106, 2/10/2017). After that fifty healthy adult male Albino mice (*Mus musculus*) were procured from National Institute of Health, Islamabad, Pakistan and kept in the animal housing facility of the Department of Physiology, Government College University, Faisalabad. The mice chosen for this study were of the weight around 25-30g and age 8-10 weeks. The rats were kept under standard conditions (temperature 25±2°C and 60±5 % relative humidity with 12 h light-dark cycle) in stainless steel wire bottom cages placed in animal house of College of Pharmacology, Faculty of Science and Technology, Government College University, Faisalabad Pakistan. The rats were received freshly prepared standard diet containing 65% starch, 10% casein, 10% corn oil, 4% salt mixture, 1% vitamins mixture and 10% cellulose (AOAC, 2000) and distilled water for two weeks that meets their requirements for growing ad libitum.

Male albino rats were randomly assigned into five groups with ten animals in each group. The groups were named as 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> groups and were designed as follows: 1<sup>st</sup> group (control group) included normal rats and received only distilled water and standard diet throughout the whole trial. The other experimental groups (2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup>) consumed *Stevia rebaudiana* aqueous extract dissolved at the levels of 200, 300, 400 and 500

ppm/kg B.Wt of albino rats in distilled water and administered orally as a daily dose for eight weeks respectively as shown in table. The dose was selected by carrying out a 21 days safety trial (Data not included) table 1.

### *Collection of blood samples*

Rats of each group were euthanized at the end of treatment period using urethane anesthesia. Blood samples were collected from the killed animals by the cardiac puncture into the dry clean tubes with heparin (anti-coagulant).

### *Determination of hematological parameters*

The non-coagulated blood was used to determine hematological parameters such as red blood cell (RBC), hemoglobin (HB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet counts (PLT), mean platelet volume (MPV), white Blood Cells (WBC) and white blood cell differentials (eosinophils, lymphocytes, neutrophils and monocytes) by using automated full blood count analyzer (Sysmex America Inc., Lincolnshire, IL60069, USA) according to the methods described by Lewis *et al.* (2006).

### *Collection of serum of rats*

For the serum, overnight fasted albino rats were killed using urethane anesthesia. Then blood was collected by cardiac puncture. After that serum was separated by centrifugation in the centrifuge machine (LABCENT 5000) at 3000 rpm for 15 min after allowing the blood to stand for at least 30 min at room temperature as per standard protocols (Uchida *et al.*, 2001).

### *Liver functioning tests*

Levels of aspartate transaminase (AST) and alanine transaminase (ALT) were measured from serum samples by the dinitrophenylhydrazene (DNPH) method using Sigma Kits 59-50 and 58-50, respectively and alkaline phosphatase (ALP) by Alkaline Phosphates-DGKC method (Thomas, 1998; Moss *et al.*, 1999).

### *Organ to body weight ratio*

Animals were killed using urethane anesthesia. The liver, kidneys, lungs, heart, pancreas and spleen were removed and weighed to calculate organ weight ratio. The relative weight of organs (%) was calculated as g/100 g body weight.

### *Ethical approval*

Before doing the research, written informed consent was obtained from all fellows that participate in this experiment and ethical approval was obtained from Institutional Review Board Faculty of Science & Technology, Government College University, Faisalabad, Pakistan. The procedure followed the instructions of Good Laboratory Practice (GLP).

**Table 1:** Addition of aqueous Stevia extract in the distilled water of rats at different substitution levels

G <sub>0</sub>	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>
Control (Basal diet+ distilled water)	Basal diet + 200 ppm SAE	Basal diet+ 300 ppm SAE	Basal diet+ 400 ppm SAE	Basal diet+ 500 ppm SAE

G<sub>0</sub>= Basal diet and distilled water

G<sub>1</sub>= Basal diet and distilled water with 200 ppm Stevia leaf Aqueous extract

G<sub>2</sub>= Basal diet and distilled water with 300 ppm Stevia leaf Aqueous extract

G<sub>3</sub>= Basal diet and distilled water with 400 ppm Stevia leaf Aqueous extract

G<sub>4</sub>= Basal diet and distilled water with 500 ppm Stevia leaf Aqueous extract

**Table 2:** Effect of Stevia aqueous extract on hematology and coagulation parameters of rats after 8 weeks

Parameters	Treatments				
	G <sub>0</sub>	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>
RBC (10 <sup>6</sup> /mL)	5.25±0.22d	5.77±0.33c	5.80±0.44bc	5.85±0.55b	5.90±0.57a
HB (g/dL)	11.7±0.11e	12.20±0.14d	12.33±0.16c	12.42±0.18b	12.88±0.20a
HCT (%)	33.47±0.11e	34.62±0.13d	34.77±0.14c	35.22±0.16b	35.87±0.18a
MCV (fL)	55.32±0.25e	55.82±0.28d	57.33±0.30c	57.72±0.34b	58.09±0.36a
MCH (pg)	18.45±0.03d	18.55±0.06c	18.57±0.06bc	18.62±0.09b	18.67±0.10a
MCHC (pg)	34.82±0.33c	34.86±0.35bc	34.88±0.34b	34.88±0.23b	34.90±0.22a
PLT(10 <sup>3</sup> /μL)	680±0.66	679±0.65	679±0.69	677±0.70	677±0.76
MPV(fL)	655±0.67	654±0.63	653±0.60	653±0.65	652±0.62

RBC = red blood cells; HB = hemoglobin; HCT = hematocrit; MCV = mean cell volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MPV = mean platelet volume and PLT = platelets count.

Values represent mean ± standard deviation (SD) (n = 10).

Values with lower case letters (a, b, c) along the column differ significantly (P < 0.05).

G<sub>0</sub>= Basal diet and distilled water

G<sub>1</sub>= Basal diet and distilled water with 200 ppm stevia leaf aqueous extract

G<sub>2</sub>= Basal diet and distilled water with 300 ppm stevia leaf aqueous extract

G<sub>3</sub>= Basal diet and distilled water with 400 ppm stevia leaf aqueous extract

G<sub>4</sub>= Basal diet and distilled water with 500 ppm stevia leaf aqueous extract

## STATISTICAL ANALYSIS

Results are represented as mean ± standard deviation (SD). One way Analysis of variance (ANOVA) and least significance difference (LSD) were applied on the result data at 95 % confidence level using SPSS statistical software package, version 17 (SPSS Inc., Chicago).

## RESULTS

Table 2 showed the effect of aqueous stevia extract on the levels of hematological parameters such as red blood cell (RBC), hemoglobin (HB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet counts (PLT) and mean platelet volume (MPV) in G<sub>0</sub>, G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub> and G<sub>4</sub>. According to results the level of RBC recorded in control group was 5.25±0.22 10<sup>6</sup>/mL. While RBC level increased from 5.77±0.33 to 5.90±0.57 10<sup>6</sup>/mL (G<sub>1</sub> to G<sub>4</sub>) in the experimental groups received 200, 300, 400 and 500 ppm/kg b. wt of stevia extract. Control group (G<sub>0</sub>) had least Hb level (11.7±0.11 g/dL). While highest Hb level (12.88±0.20 g/dL) was observed in G<sub>4</sub> that received

500 ppm/kg b.wt of stevia extract. Regarding the results of HCT, highest level of HCT (35.87±0.18%) was demonstrated in the albino rats that drank 500 ppm/kg b. wt of stevia extract (G<sub>4</sub>) and lowest level of HCT (33.47±0.11%) was observed in control group (G<sub>0</sub>). Furthermore, the results presented in table 2 depicted that levels of MCV, MCH and MCHC increased from 55.82±0.28 to 58.09±0.36%, 18.55±0.06 to 18.67±0.10% and 34.86±0.35 to 34.90±0.22% with the addition of stevia aqueous extract respectively. According to results (table 2) the levels of PLT and MPV in albino rats given stevia aqueous extract decreased non-significantly from 679±0.65 to 677±0.76 10<sup>3</sup>/μL and 654±0.63 to 652±0.62 fL respectively. These results showed that stevia aqueous extract had no adverse effect on PLT and MPV counts.

As presented in table 3, administration of the stevia extract at the dose levels of 200, 300, 400 and 500 ppm/kg b. wt caused significant effect (P<0.05) on the levels of total white blood cells (WBC) and white blood cell differentials (eosinophils, neutrophils, lymphocytes and monocytes levels) after 8 weeks study period.

**Table 3:** Effect of Stevia aqueous effect on hematology parameters (total and differential WBC) of rats after 8 weeks

Parameters	Treatments				
	G <sub>0</sub>	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>
WBC (10 <sup>3</sup> /UL)	10.28±0.002d	10.32±0.004cd	10.36±0.005c	10.41±0.003b	10.46±0.005a
Eosinophils (%)	1.20±0.01d	1.30±0.03c	1.36±0.04bc	1.40±0.03b	1.48±0.03a
Lymphocytes (%)	82.00±0.11d	82.56±0.13cd	82.80±0.14c	83.90±0.13b	84.20±0.14a
Neutrophils (%)	13.70±0.05e	14.44±0.05d	15.32±0.08c	16.05±0.05b	16.25±0.05a
Monocytes (%)	1.8±0.01	1.7±0.03	1.6±0.03	1.6±0.04	1.5±0.05

WBC = white blood cells

Values represent mean ± standard deviation (SD) (n = 10).

Values with lower case letters (a, b, c) along the column differ significantly (P < 0.05).

G<sub>0</sub>= Basal diet and distilled water

G<sub>1</sub>= Basal diet and distilled water with 200 ppm stevia leaf aqueous extract

G<sub>2</sub>= Basal diet and distilled water with 300 ppm stevia leaf aqueous extract

G<sub>3</sub>= Basal diet and distilled water with 400 ppm stevia leaf aqueous extract

G<sub>4</sub>= Basal diet and distilled water with 500 ppm stevia leaf aqueous extract

**Table 4:** Effect of stevia aqueous extract on to body weight ratios of albino rats (g/100 g body weight) after 8 weeks

Experimental groups	Liver	Pancreas	Spleen	Lungs	Heart	Right kidney	Left kidney
G <sub>0</sub>	4.00±0.06	0.61±0.007	0.35±0.02	1.13±0.01	0.33±0.007	0.42±0.07	0.42±0.03
G <sub>1</sub>	3.98±0.03	0.60±0.004	0.34±0.01	1.13±0.01	0.32±0.01	0.41±0.01	0.41±0.02
G <sub>2</sub>	3.97±0.04	0.59±0.008	0.34±0.01	1.12±0.01	0.32±0.01	0.41±0.02	0.41±0.03
G <sub>3</sub>	3.97±0.04	0.59±0.009	0.34±0.02	1.12±0.02	0.31±0.01	0.40±0.03	0.41±0.03
G <sub>4</sub>	3.96±0.05	0.58±0.008	0.33±0.02	1.11±0.02	0.31±0.01	0.40±0.05	0.40±0.04

Values represent mean ± standard deviation (SD) (n = 10).

G<sub>0</sub>= Basal diet and distilled water

G<sub>1</sub>= Basal diet and distilled water with 200 ppm stevia leaf aqueous extract

G<sub>2</sub>= Basal diet and distilled water with 300 ppm stevia leaf aqueous extract

G<sub>3</sub>= Basal diet and distilled water with 400 ppm stevia leaf aqueous extract

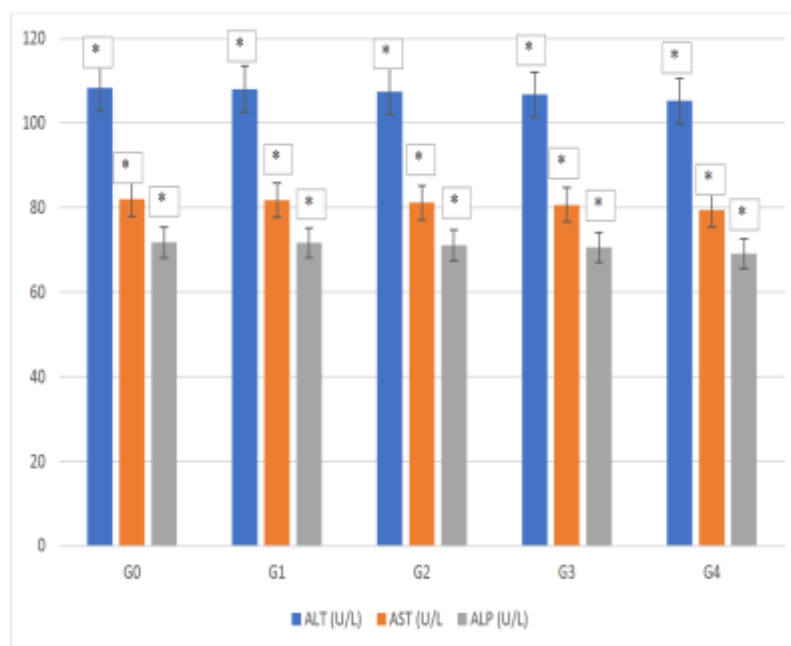
G<sub>4</sub>= Basal diet and distilled water with 500 ppm stevia leaf aqueous extract

The results of total white blood cell (WBC) count of control group rats was depicted as 10.28±0.002 10<sup>3</sup>/μL while total WBC ranged from 10.32±0.04 to 10.46±0.05 10<sup>3</sup>/μL by the addition of stevia aqueous extract at different levels (200, 300, 400 and 500 ppm/kg b. wt of albino rats) (table 3). Moreover, the levels of eosinophils (1.20±0.01 %) and neutrophils (13.7±0.05%) decreased in control group (G<sub>0</sub>) and their values notably (P<0.05) increased from 1.30±0.02 (G<sub>1</sub>) to 1.48±0.03% (G<sub>4</sub>) and 14.2±0.04 (G<sub>1</sub>) to 16.25±0.05 % (G<sub>4</sub>). Results showed that level of lymphocytes observed in control group was 82.00±0.11% while these levels increased from 82.56±0.12 to 84.20±0.14% (G<sub>1</sub>-G<sub>4</sub>) respectively in albino rats received stevia extracts. The values of monocytes for control group was 1.8±0.01 % while these values decreased from (G<sub>1</sub> to G<sub>4</sub>) 1.7±0.03 to 1.5±0.05 % respectively in the albino rats given stevia extracts (table 3).

Means values for ALT, AST and ALP levels in different groups of rats have been shown graphically in figs. 1. The results indicated that control group rats had highest levels of AST, ALT and ALP and their values were 108.32±2.22, 82.03±1.02 and 71.97±1.32 U/L

respectively. On the other hand with the addition of stevia aqueous extract at the dosage of 200, 300, 400 and 500 ppm/kg, the levels of AST, ALT and ALP decreased in G<sub>1</sub> (108.00±2.28, 81.77±1.19 and 71.67±2.32 U/L), G<sub>2</sub> (107.44±2.29, 81.22±1.34 and 71.12±1.65 U/L), G<sub>3</sub> (106.77±2.32, 80.65±1.42 and 70.66±1.28 U/L) and G<sub>4</sub> (105.22±2.42, 79.40±1.62 and 69.12±2.25 U/L) respectively as compared to the normal control group at the end of study (fig. 01).

The values of organs to body weight ratios of albino rats are shown in table 4. The results illustrated that stevia aqueous extract had non-significant effect on liver, pancreas, left and right kidneys, heart, spleen and lungs. The mean value for liver to body weight ratio of control group was 4.00±0.06 g/100g (G<sub>0</sub>). While in G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub> and G<sub>4</sub> these values were 3.98±0.03, 3.97±0.04, 3.97±0.04 and 3.96±0.05g/100 g respectively (table 4). The mean of heart to body weight ratio in G<sub>0</sub> was non-significantly varied. However, heart to body weight ratio values of albino rats that received stevia extracts ranged from 0.32±0.01 (G<sub>1</sub>) to 0.31±0.01g/100g (G<sub>4</sub>). Likewise, body to weight ratios of left and right kidneys in control group was 0.42±0.03 g/100g and 0.42±0.07 g/100g).



Results are expressed as levels of ALT (U/L), AST (U/L) and ALP (U/L) in normal albino rats (mean $\pm$  standard deviation (SD), n=10). The levels of ALT (U/L), AST (U/L) and ALP (U/L) in albino rats (G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub> and G<sub>4</sub>) received stevia aqueous extract in different concentrations (200, 300, 400 and 500 ppm) respectively significantly (P<0.05) decreased from control group rats (G<sub>0</sub>).

**Fig. 1:** Liver functioning enzymes levels in albino rats during 8 weeks

While non-momentous effect of stevia aqueous extract on the weights of left and right kidney were noted. Similarly, spleen weight of G<sub>0</sub> was non-significantly changed and these weights varied non-substantially from 0.34 $\pm$ 0.01 (G<sub>1</sub>) to 0.33 $\pm$ 0.02 g/100 g (G<sub>4</sub>) in albino rats. The mean value regarding pancreas showed that pancreas to body weight ratio was 0.61 $\pm$ 0.007 g/100 g (G<sub>0</sub>). Pancreas body to weight ratio values of albino rats received stevia ranged from 0.60 $\pm$ 0.004 (G<sub>1</sub>) to 0.58 $\pm$ 0.008 g/100 g (G<sub>4</sub>). Likewise, lungs to body weight ratio varied non-significantly in control group rats. While lungs weights diabetic rats given stevia aqueous extract ranged from 1.13 $\pm$ 0.015 (G<sub>1</sub>) to 1.11 $\pm$ 0.02 (G<sub>4</sub>) (table 4).

## DISCUSSION

In current research work, we evaluated the effect of aqueous extract of stevia on biochemical and safety assessment parameters of normal albino rats as previous researches proved that stevia aqueous extract contained abundant amount of stevioside (major glycoside) that is non-toxic, non-carcinogenic and can be used without any health hazards for the blood disorders.

Present study illustrated that different concentrations of stevia extracts (200, 300, 400 and 500mg/kg) for 8 weeks had a good efficacy in improving the hematological parameters of albino rats at study period of 8 weeks.

According to present research, the increase in red blood cells (RBC) counts on addition of stevia extract resulted in increase in hematocrit (HCT) and mean cell volume (MCV) levels. While increase in hemoglobin (HB) levels resulted to increase in mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) profiles. Moreover, the levels of PLT and MPV decreased non-significantly in the albino rats received stevia aqueous extract. These results showed that stevia aqueous extract had no adverse effect on PLT and MPV counts. The previous studies indicated that improvement in the hematological parameters of albino rats were due to glycosides mainly stevioside and Rebaudioside A and phytochemicals present in *Stevia rebaudiana* leaves (Nikiforov *et al.* (2008) ; Owoyele *et al.* (2011) & Salehi *et al.* (2019). According to another study performed by Abo Elnaga *et al.* (2016), who observed the effect of stevia aqueous extract on hematology of female rats and found that stevia aqueous extract increased the hematology of female rats (Rojas *et al.* (2018).

As observed in this study, the aqueous extract of stevia leaves had positive effect on the white blood cells (WBC), eosinophils, lymphocytes, neutrophil and monocytes concentrations following the oral administration of aqueous extracts of stevia to the albino rats at varying doses. This increase in WBC count and their differential cells have been due to enhancement in the rate of

entry of leucocytes into the blood from the bone marrow and a diminished rate of removal from circulation. Furthermore, it has been demonstrated that granulocyte-macrophage colony stimulating factor, macrophage colony stimulating factor, interleukins IL-2, IL-4 and IL-5 regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of white blood cells (Ganong *et al.*, 2011). While non-momentous ( $p > 0.05$ ) decrease in the monocytes levels recorded in the rats received 200, 300, 400 and 500 ppm/kg b.wt of stevia aqueous extracts as compared to the control rats proved that that stevia extract had no harmful effect on the metabolism of bone-marrow. The results of present research are in collaborations with the studies of Nikiforov *et al.* (2008); Owoyele *et al.* (2011) who found that *Stevia rebaudiana* Bertoni leaves has immune boosting properties due to glycosides such as stevioside and Rebaudioside A that may significantly increased the concentrations of white blood cells (WBC), eosinophils, lymphocytes and neutrophil. While monocytes non-significantly decreased by addition of stevioside and rebaudioside A in diets of rats.

Administration of aqueous extract of stevia orally at different dose levels decline the levels of liver functioning enzymes (ALT, AST, and ALP) in the albino rats. The lower levels of liver functioning enzymes were not considered to be adverse due to the small magnitude of difference from the control group value. The results of current research are in collaboration with works of Shivanna *et al.* (2013); Akbarzadeh *et al.* (2015) and Assaei *et al.* (2016) who found that stevioside in stevia leaves had no adverse effect on liver functioning enzymes due to its non-toxic nature.

The results of organ to body weight ratio showed that stevia aqueous extract had no harmful effect on the organs as the organ to body weight ratio values were non-significantly different than control group rats proving that stevia extract is safe to use. The results are in harmony with the research work of Nikiforov *et al.* (2008) who confirmed that organ to body weight ratios of different body organs (lungs, spleen, heart, kidneys, pancreas and brain) were non-significantly different from the control group during 90-days toxicological study of rebaudioside A. Afterwards, Awney *et al.* (2010) concluded that the liver, spleen, heart, kidneys, lungs and pancreas body to weight ratios of diabetic rats given aqueous extract of stevia were non-significantly different as compared to the control group rats. Similarly, Assaei *et al.* (2015) depicted the effect of stevia aqueous extract on histopathology of albino rats and concluded that stevia compensated for the histopathological damage in albino rats.

## CONCLUSION

The current research suggests hematological parameters such as RBC, HB, HCT, MCV, MCH, MCHC, WBC and white blood cell differentials (eosinophils,

lymphocytes and neutrophils) of diabetic rats improved and PLT, MPV and monocytes levels non-significantly decreased by using different levels of stevia aqueous extracts, compared with the control rats after eight weeks study period. Furthermore, the levels liver functioning enzymes (ALT, AST and ALP) and organ to body weight ratios of the normal albino rats showed that stevia aqueous extracts had no adverse effects. It is concluded that aqueous extract of stevia with concentration 500 ppm/kg body weight of rats showed best results of all the parameters determined. It is understood from the results that stevia extract is toxicologically safe as no health hazards were observed in the albino rats. Therefore it could be used as natural medicine for the prevention of blood disorders.

## ACKNOWLEDGEMENT

The authors are thankful to the Institute of Home and Food Sciences, Government College University Faisalabad Pakistan for providing research facilities to prepare this valuable document.

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