

Effect of Ashwagandharishta on the Red Blood Cell (RBC) indices of male and female Albino rats

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Abstract

RBC indices are part of a complete blood count (CBC) that classify red blood cells by size and hemoglobin content to diagnose types of anemia (e.g., iron deficiency, thalassemia) and other conditions. In this study RBC indices are analyzed. After 51 days of chronic administration of the Ashwagandharishta liquid preparation at three different dose level, the Male and Female rats, showed no statistically significant change in MCV, MCH and RDW level than their corresponding control group. A highly statistically significant decrease in the total numbers of the red blood cells (RBC, [14.575 % decrease ($p=0.006^{**}$)], Hemoglobin, [12.122 % decrease ($p=0.021^{*}$)] and Haematocrit [15.112 % decrease ($p=0.001^{***}$)] of the male rat high dose group was noticed. But the female rats showed no statistically significant change in Hemoglobin and Haematocrit level. Only the female rat medium dose group showed a statistically significant increase [7.771 % ($p=0.038^{*}$)] than their corresponding control group. There was a statistically significant decrease in the Mean corpuscular hemoglobin concentration (MCHC), a red cell index of the female rat medium dose group [1.991 % decrease ($p=0.005^{*}$)] and high dose group [1.670% decrease ($p=0.019^{*}$)]. But the male rats showed no statistically significant change for this parameter.

Keywords: Ashwagandharishta (ASG), RBC indices, chronic administration, Sprague-Dawley rats

INTRODUCTION

RBC indices are part of a complete blood count (CBC) that classify red blood cells by size and hemoglobin content to diagnose types of anemia (e.g., iron deficiency, thalassemia) and other conditions. Key indices include MCV (size), MCH (hemoglobin amount), MCHC (hemoglobin concentration), and RDW (size variation). The core RBC Indices: Mean Corpuscular Volume (MCV): Measures the average size of red blood cells. Mean Corpuscular Hemoglobin (MCH): Measures the average amount of hemoglobin in each red blood cell. Mean Corpuscular Hemoglobin Concentration (MCHC): Measures the average concentration of hemoglobin in a volume of red blood cells. Red Cell Distribution Width (RDW): Measures the variation in red blood cell size (anisocytosis). Abnormal RBC indices are crucial for identifying the root cause of anemia such as Anemia diagnosis: Differentiates between iron deficiency, thalassemia, and hemolytic anemia. Nutritional deficiencies: High RDW/MCV can indicate B12/folate deficiency; low MCV can indicate iron deficiency. Chronic diseases: Associated with chronic inflammation, liver disease, or hypothyroidism (National Institutes of Health, An official website of the Department of Health and Human Services, 9000 Roc).

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Table 1. Botanical name of ingredients along with the amount used to prepare Ashwagandharishta (ASG)

No	Sanskrit Name	Botanical Name	Amount of ingredient
1	Ashwagandha	<i>Withania somnifera</i>	2.400 kg
2	Sweta Musli	<i>Asparagus adscendens</i>	960 g
3	Manjishtha	<i>Rubia cordifolia</i>	480g
4	Hareetaki	<i>Terminalia chebula</i>	480g
5	Haridra	<i>Curcuma longa</i>	480g
6	Daruharidra	<i>Berberis aristata</i>	480g
7	Yashtimadhu	<i>Glycyrrhiza glabra</i>	480g
8	Rasna	<i>Pluchea lanceolata</i>	480g
9	Vidarikanda	<i>Pueraria tuberosa</i>	480g
10	Arjun Tvak	<i>Terminalia arjuna</i>	480g
11	Mustaka	<i>Cyperus rotundus</i>	480g
12	Trivrit	<i>Ipomoea turpethum</i>	480g
13	Anantamool	<i>Hemidesmus indicus</i>	384 g
14	Krishna Sariva	<i>Cryptolepis buchanan</i>	384 g
15	Rakta Chandan	<i>Pterocarpus santalinus</i>	384 g
16	Chandan	<i>Santalum album</i>	384 g
17	Vacha	<i>Acorus calamu</i>	384 g
18	Chitrak Mool	<i>Plumbago zeylanica</i>	384 g
19	Water for decoction	98.304 L reduced to	12.288L
20	Dhataki puspa	<i>Woodfordia fruticosa</i>	768 g
21	Madhu	Honey	14.400 kg
22	Shunthi	<i>Zingiber officinale</i>	96 g
23	Maricha	<i>Piper nigrum</i>	96 g
24	Pippali	<i>Piper longum</i>	96 g
25	Tvak	<i>Cinnamomum zeylanicum</i>	192 g.
26	Tejpata	<i>Cinnamomum tamala</i>	192 g.
27	Elach	<i>Elettaria cardamomum</i>	192 g.
28	Priyangu	<i>Callicarpa macrophylla</i>	192 g.
29	Nagakeshar	<i>Mesua ferrea</i>	96g.

In this study, Ashwagandharishta was used which is a classical ayurvedic formulation used in the treatment of *Murchha* (syncope), *Mandagni* (poor digestive power), etc. *Ashwagandha* (*Withania somnifera* D.), the key component of *Ashwagandharishta* (Table 1), (Anonymous, 1992), has anti-stress and anxiolytic activities. Further, it also affects brain-derived neurotrophic factor (BDNF) that boosts synaptic plasticity, delivers neuro-protection, augments neurotransmission, and has antidepressant effects (Tanna I. R. *et al.*, 2012). It also has many actions on body like anti-ageing, adaptogenic, immune-modulatory, cardiovascular protection, hypothyroidism etc. (Kushwaha, R. & Karanjekar, S. 2011). *Withania somnifera*, also recognized as Indian ginseng, or winter cherry, has been an important herb in the Ayurvedic and indigenous medical systems. The roots of the plant are categorised as *Rasayanas* which promote health and longevity by enhancing defenses against disease, arresting the ageing process, revitalizing the body in debilitated conditions. It contains alkaloids (withanine, withasomnine), steroidal lactones and glycosides (withanolides and sitoindosides). It is also used as a general tonic, to increase energy and

improve health and longevity. Some studies suggest that, it may promote growth in children and improve hemoglobin level, red blood cell count, and physical performance in adults (Sandhu J. S., *et al.*, 2010). It also increases heart weight and glycogen in myocardium and liver representing intensification of the anabolic process and enhances the duration of contractility as well as coagulation time (Kushwaha S., *et al.*, 2012). World Health Organization (WHO) reported that a substantial number of world population use herbal medicines for some aspect of primary health care. Because of the perception of low side effect profiles, traditional medicines now a days is of special research interest. To execute further clinical studies with this liquid preparation, this study was performed to evaluate the effects of ASG on the RBC indices utilizing laboratory animals of both sexes.

MATERIALS AND METHODS

Drugs, chemicals and reagents: For this study, *Ashwagandharishta* was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong. Ketamine injection was obtained from ACI Pharmaceuticals Limited, Bangladesh. All other reagents, assay kits and chemicals used in this research work were obtained from Human GmbH, Wiesbaden, Germany.

Experimental Animals: Albino rats (*Rattus norvegicus* : Sprague-Dawley strain, 48 weeks old, 70-80 g) of both sexes bred and maintained at the animal house of the Department of Pharmacy, Jahangirnagar University, were used in this toxicological experiment. They were kept in a well-ventilated hygienic experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. They were fed with rat chow which was prepared according to the formula developed by Bangladesh Council of Scientific and Industrial Research (BCSIR). Water was given *ad libitum* and the animals maintained at 12 hours day and 12 hours night cycle. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals approved by Biosafety, Biosecurity and Ethical Committee of Faculty of Biological Sciences, Jahangirnagar University.

Experimental design

Chronic toxicity studies: A total of 40 males and 40 female rats were randomly assigned to the four groups, namely group I (Control: water), group II-Low Dose (0.625 ml/kg BW of ASG), group III-Medium Dose (5.0 ml/kg BW of ASG), and group IV-High Dose (40.0 ml/kg BW of ASG) consisting of 10 males and 10 females in each group. The animals of control group were administered with distilled water only as per the same volume as the drug treated group for 51 days. Ayurvedic medicinal preparation was administered to the rats by intra-gastric syringe at fixed time daily soon after acclimatization. All the experiments were carried out in absolute compliance with the ethical guideline for care and use of laboratory animals.

Blood samples collection and preparation of serum: At the end of treatment period (51 days), after 18 hours fasting, blood samples were collected from post vena cava of the rats anaesthetizing with Ketamine (500 mg/Kg body, intra peritoneal). Blood samples were transferred into plain sample tubes immediately for serum generation. It was then

centrifuged at 4,000 g for 10 minutes using Bench top centrifuge (MSE Minor, England). The supernatant plasma samples were collected using dry Pasteur pipette and stored in the refrigerator for further analyses. All analyses were completed within 12 hours of sample collection.

RBC Indices studies: Whole blood samples were analyzed for RBC count, Hemoglobin, HCT, MCV, MCH, MCHC and RDW using Abbott's CELL-DYN 3700 System (USA).

Statistical analysis: Data were expressed as Mean $\pm$ SEM (Standard Error of the Mean). One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this data set. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ was considered statistically significant, highly significant and very highly significant respectively. Statistical programs used were SPSS (version 16, IBM software Inc, USA).

RESULTS AND DISCUSSION

Acute toxicity study: When the rats were treated with a high dose of *Ashwagandharishta* (4000 ml/kg body weight), the mortality rate was found to be zero. This suggests that LD50 value of ASG is greater than 4000 mg/kg body weight. Thus ASG may be considered safe at this dose when given as a oral dose.

Chronic toxicity study

Effect of ASG on Red blood cell (RBC/Erythrocyte) count of male & female rats: At the higher dose level, there was a **highly statistically significant** ($p=0.006^{**}$) **decrease** in the total numbers in the red blood cells of the male rat [14.575 % decrease]. There was a **statistically significant** ($p=0.038^{*}$) **increase** in the total numbers in the red blood cells of the female rat. [7.771 % increase] at the medium dose level (Table 2).

Table 2. The effect of ASG on the RBC (Erythrocyte) count utilizing of laboratory animals (Male & Female Rat) compared to their corresponding Control group after 51 days

Group	RBC (Erythrocyte) 10^6 per microlitre			
	Male Rat (N=10)		Female Rat (N=10)	
	Mean $\pm$ SEM (p value)	% of change	Mean $\pm$ SEM (p value)	% of change
Control	7.055 $\pm$ 0.098	-	6.099 $\pm$ 0.057	
ASG (0.625 ml/kg)	6.944 $\pm$ 0.136 (0.967)	$\downarrow$ 1.573%	6.370 $\pm$ 0.188 (0.337)	$\uparrow$ 4.443 %
ASG (5.0 ml/kg)	6.626 $\pm$ 0.232 (0.362)	$\downarrow$ 6.080 %	6.573 $\pm$ 0.162 (0.038*)	$\uparrow$ 7.771 %
ASG (40.0 ml/kg)	6.026 $\pm$ 0.342 (0.006**)	$\downarrow$ 14.575 %	6.195 $\pm$ 0.054 (0.916)	$\uparrow$ 1.574 %

Values are presented as mean $\pm$ SEM (n=10). One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this data set. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ was considered statistically significant, highly significant and very highly significant respectively. Values are self-explanatory when compared against control. $\uparrow$: increase, $\downarrow$: decrease.

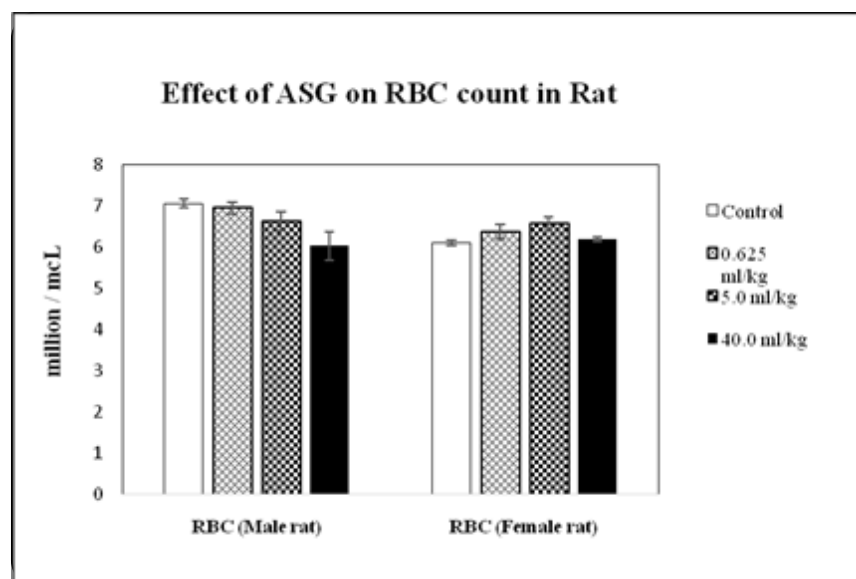


Figure of the effect of ASG on the RBC (Erythrocyte) level utilizing of laboratory animals (Male & Female Rat) compared to their corresponding Control group after 51 days

In the above-mentioned results, for all of the three-dose group of male rats, showed a decrease in the parameter. Only the high dose group result was statistically significant. But the above mentioned results for the male rats are contradictory to a previously published study. In that mice treated study, significant increases ($p < 0.05$) in red blood cell count were observed in WS-treated mice compared to untreated control mice (Mishra *et al.*, 2000). So, the study conducted by Mishra *et al.*, 2000 doesn't support the present research result.

Effect of ASG on Hemoglobin level of male & female rats: Hemoglobin is the amount of oxygen carrying protein contained within the red blood cells (Moffat, 2013). Hemoglobin concentration (Hb) is reported as grams of hemoglobin per deciliter of blood (g/dL). Since red cells are approximately 33% hemoglobin, the hemoglobin concentration of whole blood normally is about one third of the HCT (i.e., the MCHC is 33%) (French *et al.* 2013). There are benefits to packaging Hb in RBC's; it decreases Hb turnover (the half-life of free Hb is a few hours), it keeps Hb close to the metabolic mechanisms necessary to maintain a functional status (*e.g.*, maintenance of Hb iron in the Fe^{+2} state), and it eliminates the osmotic effect free Hb would have in serum. Besides oxygen transport, Hb also is the most important protein buffer in the blood (~6x the buffering capacity of serum proteins) (Travlos, 2007). From a study of Sprague-Dawley male rats ($n=2839$) the mean for Hemoglobin was found to be 15.9 gm per decilitre with a standard deviation of 1.0 gm per decilitre (Matsuzawa *et al.*, 1993). In male Sprague-Dawley rats the Hemoglobin range between 14.7 – 17.3 gm per decilitre with a mean of 15.9 gm per deciliter (Car *et al.*, 2006). From an age-wise study of 4 weeks old Sprague-Dawley male rats ($n=152$) the mean $\pm$ std. dev. for Hemoglobin was found to be 16.1 ± 0.7 gm per deciliter with a minimum value of 13.4 and a maximum value of 17.9; and with 13 weeks old Sprague-Dawley male rats

(n=160) the mean $\pm$ std. dev. for Hemoglobin was found to be 16.2 ± 0.7 gm per deciliter with a minimum value of 13.5 and a maximum value of 18.4 (Petterino and Argentino-Storino, 2006).

Effect of ASG on Hemoglobin level: Only the higher dose level, showed a statistically significant ($p=0.021^*$) **decrease** in the Hemoglobin level of the male rat [12.122 % decrease]. No change was statistically significant for the female rat group. This data is summarized in Table 3.

Table 3. The effect of ASG on the Hemoglobin level utilizing of laboratory animals (Male & Female Rat) compared to their corresponding Control group after 51 days

Group	Hemoglobin (gm/dL)			
	Male Rat (N=10)		Female Rat (N=10)	
	Mean $\pm$ SEM (p value)	% of change	Mean $\pm$ SEM (p value)	% of change
Control	12.060 $\pm$ 0.161	-	11.130 $\pm$ 0.119	
ASG (0.625 ml/kg)	11.770 $\pm$ 0.204 (0.899)	$\downarrow$ 2.463%	11.475 $\pm$ 0.335 (0.558)	$\uparrow$ 3.099 %
ASG (5.0 ml/kg)	11.251 $\pm$ 0.338 (0.289)	$\downarrow$ 6.708 %	11.780 $\pm$ 0.242 (0.111)	$\uparrow$ 5.840 %
ASG (40.0 ml/kg)	10.598 $\pm$ 0.595 (0.021*)	$\downarrow$ 12.122 %	11.290 $\pm$ 0.099 (0.920)	$\uparrow$ 1.437 %

Values are presented as mean $\pm$ SEM (n=10). One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this data set. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ was considered statistically significant, highly significant and very highly significant respectively. Values are self explanatory when compared against control. $\uparrow$: increase, $\downarrow$: decrease.

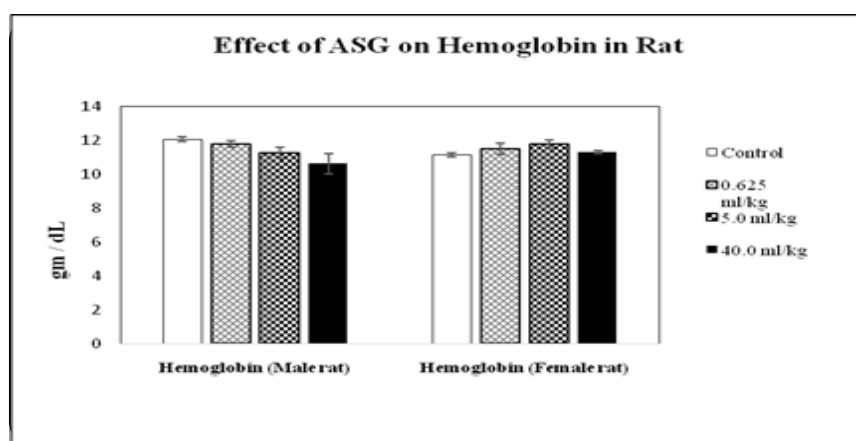


Figure of the effect of ASG on the Hemoglobin level utilizing of laboratory animals (Male & Female Rat) compared to their corresponding Control group after 51 days

Interestingly the above-mentioned results for the female rats are supportive to a previously published study. In that mice treated study, significant increases ($p < 0.05$) in hemoglobin concentration were observed in WS-treated mice compared to untreated control mice (Mishra *et al.*, 2000). So, the study conducted by Mishra *et al.*, 2000 supports the present research result.

Effect of ASG on Haematocrit (HCT) level of male & female rats: The HCT measures the ratio of blood volume occupied by RBC's and is reported as a percentage (Travlos, 2007). Hematocrit (HCT) is actually a calculated value obtained from modern automated hematology analyzers. It is the product of the mean cell volume (MCV) and the red blood cell (RBC) count, both of which are directly measured by the analyzer. Therefore, if there are any inaccuracies in measurement of the MCV or RBC count, the HCT will reflect those inaccuracies (French *et al.*, 2013). Calculation (Shilkofski, 2005).

$$\text{Hct} = \text{Mean Corpuscular Volume} \times \text{Red Blood Cell Count}$$

In this study, at the higher dose level, there was a very highly statistically significant ($p=0.001^{***}$) decrease in the Haematocrit level of the male rat [15.112 % decrease]. But the low and medium dose group results were not statistically significant than their corresponding control group. For the female rat group no changes was statistically significant. This data is summarized in Table 4.

Table 4. The effect of ASG on the HCT level utilizing of laboratory animals (Male & Female Rat) compared to their corresponding Control group after 51 days

Group	HCT (%)			
	Male Rat (N=10)		Female Rat (N=10)	
	Mean $\pm$ SEM (p value)	% of change	Mean $\pm$ SEM (p value)	% of change
Control	41.780 $\pm$ 0.543	-	36.010 $\pm$ 0.336	
ASG (0.625 ml/kg)	41.070 $\pm$ 0.6671 (0.938)	$\downarrow$ 1.699%	37.630 $\pm$ 1.321 (0.394)	$\uparrow$ 4.498 %
ASG (5.0 ml/kg)	39.140 $\pm$ 1.146 (0.219)	$\downarrow$ 6.318 %	38.700 $\pm$ 0.915 (0.076)	$\uparrow$ 7.470 %
ASG (40.0 ml/kg)	35.466 $\pm$ 1.758 (0.001 ^{***})	$\downarrow$ 15.112 %	36.870 $\pm$ 0.351 (0.811)	$\uparrow$ 2.388 %

Values are presented as mean $\pm$ SEM (n=10). One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this data set. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ was considered statistically significant, highly significant and very highly significant respectively. Values are self explanatory when compared against control. $\uparrow$: increase, $\downarrow$: decrease.

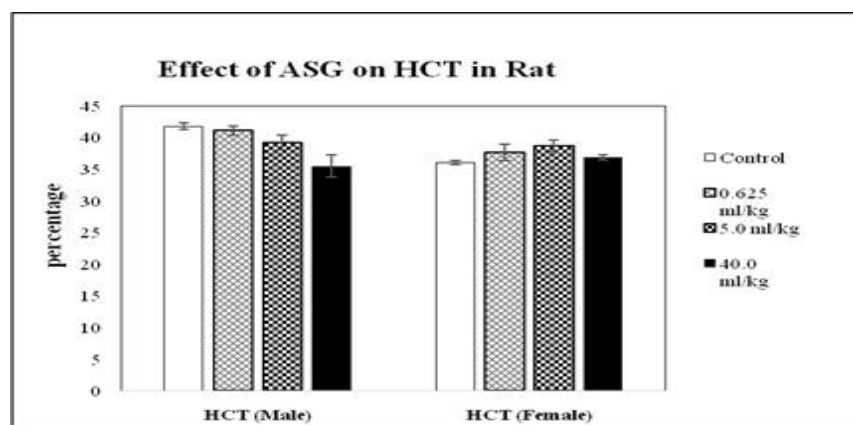


Figure of the effect of ASG on the HCT level utilizing of laboratory animals (Male & Female Rat) compared to their corresponding Control group after 51 days

Significance of MCV, MCH, MCHC and RDW parameters: After 51 days of chronic administration of the ASG preparation the Mean Corpuscular Volume (MCV), Mean

Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW) were determined in the male and female rat group. Evaluation of the red cell indices Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) are morphological measures and are useful in the classification of anemias. They are calculated values derived from the RBC count, Hct value, and Hb concentration. Therefore, the validity of the indices depends on the accuracy of the data required for calculation (Travlos, 2007).

Effect of ASG on MCV level of male & female rats: MCV expresses, in femtoliters (fL; 10^{-15} liters), the average RBC volume of a population of erythrocytes and is an indicator of “average” RBC size. MCV is increased (macrocytic cells) in anemias in which there is an increased bone marrow release of immature RBC's (reticulocytes) or as a result of deficient nucleic acid synthesis (e.g., vitamin B₁₂ and folate deficiencies). Reticulocytes are larger than mature RBC's and a reticulocytosis causes a transitory increase in MCV. Altered nucleic acid synthesis, decreases DNA production causing fewer cell divisions during development; fewer divisions' results in larger RBC's. Iron deficiency causes a decreased MCV (microcytic cells). Iron deficiency slows hemoglobin production, allowing extra cell divisions before the critical level of Hb is reached; smaller are RBC's the result (Travlos, 2007).

MCV is represented by the formula: $MCV = [Hct (\%)/RBC \text{ count (millions)}] \times 10$

In male Sprague-Dawley rats the MCV range between 55.1 – 64.2 femtolitre with a mean of 59.7 femtolitre (Car *et al.*, 2006). From an age-wise study of 4 weeks old Sprague-Dawley male rats (n=152) the mean $\pm$ std. dev. for percentage of MCV was found to be 56.7 ± 1.8 femtolitre with a minimum value of 51.1 and a maximum value of 61.1; and with 13 weeks old Sprague-Dawley male rats (n=160) the mean $\pm$ std. dev. for MCV was found to be 52.8 ± 3.0 femtolitre with a minimum value of 45.5 and a maximum value of 60.8 (Petterino and Argentino-Storino, 2006).

For the Male and Female rats, the ASG treated group showed no statistically significant change in MCV level than the corresponding control group. Data is summarized in Table 5.

Table 5. The effect of ASG on the MCV level utilizing of laboratory animals (Male & Female Rat) compared to their corresponding Control group after 51 days

Group	MCV (fL)				
	Male Rat (N=10)		Female Rat (N=10)		
	Mean $\pm$ SEM (p value)	% of change	Mean $\pm$ SEM (p value)	% of change	
Control	59.200 $\pm$ 0.142	-	58.720 $\pm$ 0.281		
ASG (0.625 ml/kg)	59.170 $\pm$ 0.320 (1.000)	↓ 0.050%	62.350 $\pm$ 3.963 (0.778)	↑ 6.181 %	
ASG (5.0 ml/kg)	59.180 $\pm$ 0.556 (1.000)	↓ 0.033 %	58.870 $\pm$ 0.226 (1.000)	↑ 0.255 %	
ASG (40.0 ml/kg)	59.288 $\pm$ 0.833 (0.999)	↑ 0.150 %	54.140 $\pm$ 5.266 (0.642)	↓ 7.799 %	

Values are presented as mean $\pm$ SEM (n=10). One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this data set. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ was considered statistically significant, highly significant and very highly significant respectively. Values are self explanatory when compared against control. ↑: increase, ↓: decrease.

Effect of ASG on Mean Corpuscular Hemoglobin (MCH) level of male & female rats:

MCH test helps diagnose the type of anemia (Moffat, 2013). MCH expresses, in picograms (pg), the weight of Hb in an average RBC in a population of cells. It is represented by the formula below and assumes all Hb measured is in RBC's. Iron deficiency causes a decreased MCH due to decreased Hb production. Reticulocytosis causes a normal to slightly increased MCH. Hemolysis (in vivo or in vitro) can cause an increased MCH due to decreased RBC numbers (Travlos, 2007).

$MCH = [Hb \text{ (g/dL)} / RBC \text{ count (millions)}] \times 10$ Calculations (Moses, 2013)

$MCH = (10 \times \text{Hemoglobin}) / (\text{Red Blood Cell Count})$

In male Sprague-Dawley rats the MCH range between 18.6 – 20.7 picogram with a mean of 19.6 picogram (Car *et al.*, 2006). From an age wise study of four weeks old Sprague-Dawley male rats (n=152) the mean $\pm$ std. dev. for percentage of MCH was found to be 19.0 ± 0.5 picogram with a minimum value of 17.8 and a maximum value of 20.4; and with 13 weeks old Sprague-Dawley male rats (n=160) the mean $\pm$ std. dev. for MCH was found to be 17.9 ± 0.8 picogram with a minimum value of 15.7 and a maximum value of 19.7 (Petterino & Argentino-Storino, 2006).

In this study, both for the Male and Female rats, the ASG treated group showed no statistically significant change in MCH level than the corresponding control group. Data is summarized in Table no 6.

Table 6. The effect of ASG on the MCH level utilizing of laboratory animals (Male & Female Rat) compared to their corresponding Control group after 51 days

Group	MCH (pg)			
	Male Rat (N=10)		Female Rat (N=10)	
	Mean $\pm$ SEM (p value)	% of change	Mean $\pm$ SEM (p value)	% of change
Control	17.090 $\pm$ 0.064	-	18.260 $\pm$ 0.134	
ASG (0.625 ml/kg)	16.920 $\pm$ 0.117 (0.799)	↓ 0.994%	18.220 $\pm$ 0.202 (0.995)	↓ 0.219%
ASG (5.0 ml/kg)	17.010 $\pm$ 0.189 (0.971)	↓ 0.468 %	17.950 $\pm$ 0.123 (0.335)	↓ 1.697 %
ASG (40.0 ml/kg)	17.000 $\pm$ 0.242 (0.963)	↓ 0.526 %	18.220 $\pm$ 0.121 (0.995)	↓ 0.219 %

Values are presented as mean $\pm$ SEM (n=10). One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this data set. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ was considered statistically significant, highly significant and very highly significant respectively. Values are self explanatory when compared against control. ↑: increase, ↓: decrease.

Effect of ASG on Mean Corpuscular Hemoglobin Content or concentration (MCHC) level of male & female rats: MCHC denotes the volume and character of the hemoglobin and is the most accurate of the red cell indices (Moffat, 2013).

MCHC expresses, in percent, the ratio of Hb weight to the volume of an average erythrocyte in a population of cells. For most mammalian species the MCHC is approximately 33%. The MCHC is the most accurate of the indices because it does not require the RBC count. MCHC is decreased in iron deficiency and reticulocytosis. MCHC is increased with in vivo and in vitro hemolysis, spherocytosis, and significant RBC shrinkage (eg., excess EDTA). High MCHC values do not occur physiologically; Hb crystallizes when a concentration of >36% is achieved. Therefore, when an increased MCHC occurs hemolysis

(in vivo or in vitro) or altered RBC count (eg., lab error) should be considered (Travlos, 2007).

MCHC = [Hb (g/dL)/Hct (%)] X 100 Calculations (Moses, 2013)

MCHC = (Hemoglobin x 100) / (Hematocrit)

In male Sprague-Dawley rats the MCHC range between 31.3 – 34.4 gm per deciliter with a mean of 32.8 gm per deciliter (Car *et al.*, 2006). From an age wise study of 4 weeks old Sprague-Dawley male rats (n=152) the mean $\pm$ std. dev. For percentage of MCHC was found to be 33.6 ± 1.1 gm per deciliter with a minimum value of 31.4 and a maximum value of 36.5; and with 13 weeks old Sprague-Dawley male rats (n=160) the mean $\pm$ std. dev. For MCHC was found to be 33.9 ± 1.1 gm per deciliter with a minimum value of 31.6 and a maximum value of 35.8 (Petterino and Argentino-Storino, 2006).

Data is summarized in Table no 7. No statistically significant change was observed in the male rat group for all of the three different doses. In the Female rat group, a highly statistically significant decrease was observed for the medium and high dose level than their corresponding control group.

Table 7. The effect of ASG on the MCHC level utilizing of laboratory animals (Male & Female Rat) compared to their corresponding Control group after 51 days

Group	MCHC (gm/dL)			
	Male Rat (N=10)		Female Rat (N=10)	
	Mean $\pm$ SEM (p value)	% of change	Mean $\pm$ SEM (p value)	% of change
Control	12.060 $\pm$ 0.161	-	11.130 $\pm$ 0.119	
ASG (0.625 ml/kg)	28.610 $\pm$ 0.120 (0.141)	$\downarrow$ 0.381%	30.880 $\pm$ 0.147 (0.389)	$\downarrow$ 0.803 %
ASG (5.0 ml/kg)	28.730 $\pm$ 0.074 (0.672)	$\downarrow$ 0.331 %	30.510 $\pm$ 0.123 (0.005**)	$\downarrow$ 1.991 %
ASG (40.0 ml/kg)	28.744 $\pm$ 0.064 (0.769)	$\downarrow$ 0.797 %	30.610 $\pm$ 0.080 (0.019*)	$\downarrow$ 1.670 %

Values are presented as mean $\pm$ SEM (n=10). One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this data set. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ was considered statistically significant, highly significant and very highly significant respectively. Values are self explanatory when compared against control. $\uparrow$: increase, $\downarrow$: decrease.

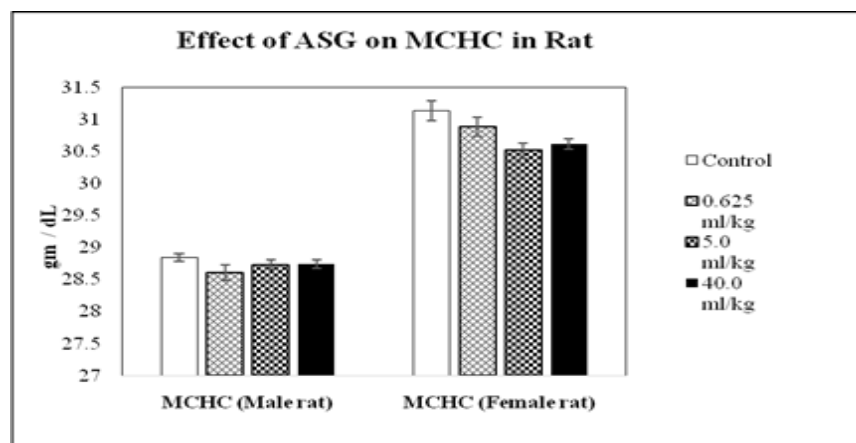


Figure of the effect of ASG on the MCHC level utilizing of laboratory animals (Male & Female Rat) compared to their corresponding Control group after 51 days

Effect of ASG on RDW level of male & female rats: The RDW is an index of the variation in cell volume within the red cell population (French *et al.*, 2013). RDW test measures the different sizes and shapes of the red cell. RDW is important because the more surface area the red cell has, the better it can hook onto and transport oxygen through the system (Moffat, 2013). Some investigators feel that it is useful in differentiating thalassemia from iron deficiency anemia, but its use in this regard is far from universal acceptance. The RDW may also be useful in monitoring the results of hematinic therapy for iron-deficiency or megaloblastic anemias. As the patient's new, normally-sized cells are produced, the RDW initially increases, but then decreases as the normal cell population gains the majority (Uthman, 2001). In this study, for the Male and Female rats, the ASG treated group showed no statistically significant change in RDW level than the corresponding control group. Data shown in Table no 8.

Table 8. The effect of ASG on the RDW level utilizing of laboratory animals (Male & Female Rat) compared to their corresponding Control group after 51 days

Group	RDW (%)			
	Male Rat (N=10)		Female Rat (N=10)	
	Mean $\pm$ SEM (p value)	% of change	Mean $\pm$ SEM (p value)	% of change
Control	9.238 $\pm$ 0.117	-	7.842 $\pm$ 0.127	
ASG (0.625 ml/kg)	18.936 $\pm$ 0.108 (0.938)	↓ 3.269%	8.040 $\pm$ 0.103 (0.674)	↑ 2.524 %
ASG (5.0 ml/kg)	9.355 $\pm$ 0.652 (0.996)	↑ 1.271%	7.925 $\pm$ 0.208 (0.961)	↑ 1.058 %
ASG (40.0 ml/kg)	10.198 $\pm$ 0.630 (0.334)	↑ 10.391 %	8.118 $\pm$ 0.138 (0.430)	↑ 3.519 %

Values are presented as mean $\pm$ SEM (n=10). One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this data set. * p <0.05, ** p <0.01 and *** p <0.001 was considered statistically significant, highly significant and very highly significant respectively. Values are self-explanatory when compared against control. ↑: increase, ↓: decrease.

Effect of ASG on RBC (Erythrocyte) count level of male & female rats: *Ashwagandha* (*Withania somnifera* D.), the key component of *Ashwagandharishta* (Table 1), (Anonymous, 1992). This study was performed to evaluate the effects of *Ashwagandharishta* (ASG) on the RBC indices utilizing laboratory animals of both sexes.

In a previous study, *Ashwagandha* (*Withania somnifera*) has been studied for its effects on blood cell counts, particularly in the context of stress, immune suppression and chemotherapy induced myelosuppression. The dominant finding is that *Ashwagandha* supplementation generally increases red and white blood cell counts, hemoglobin and platelets and helps counteract reductions caused by stress or chemotherapy. Red Blood Cells (RBCs) and Hemoglobin: *Ashwagandha* supplementation leads to significant increases in total erythrocyte (RBC) count and hemoglobin levels in both animal and human studies, even under stress or drug -induced myelosuppression (Priyanka 2020, Ziauddin, 1996, Goutham, S. P., 2014).

Effect of ASG on RBC, Hemoglobin and HCT: In this study, for the following parameters, RBC, Hemoglobin and HCT level analysis, ASG caused a decrease in the low, medium and high dose result of male rat group. In the female rat group; low, medium and

high dose results showed an increase of the above-mentioned parameters than their corresponding control group.

Here, for the RBC count, the male rat high dose group ($p=0.006^{**}$) showed a highly statistically significant and the female rat medium dose group ($p=0.038^{*}$) showed a statistically significant result. For the hemoglobin study, the male rat high dose group ($p=0.021^{*}$) result showed a highly statistically significant result. And for the HCT level, the male rat high dose group ($p=0.001^{**}$) result was very highly statistically significant. And the female rat medium dose group ($p=0.076$) result was prominent but not statistically significant.

Effect on MCV and MCH level: In this study, ASG caused no statistically significant change for MCV and MCH level in the laboratory animals.

Effect on MCHC: For the MCHC level analysis; the three different doses for the male and female rat result showed a decrease in the parameter than their corresponding control group. Here the female rat medium dose group ($p=0.005^{**}$) and high dose group ($p=0.019^{**}$) showed a very highly statistically significant decrease in the parameter.

Effect on RDW: There was an increase in RDW for the male and female rats results than their corresponding control group except the male rat low dose group but no result was statistically significant.

Conclusion: The present study was limited to just 51 days period on healthy male and female rats. It carries a scope for further evaluation of Ashwagandharishta preparation and also in long term randomized placebo-controlled trial to establish its clinical use. Further studies are also required to assess whether the drug can improve other physical parameters or not.

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Conflicts of interest: No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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