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Original Research Article

Preclinical Hemotoxicological Profile Investigations of an Ayurvedic Medication Following Long-Term Administration to Male Sprague-Dawley Rats

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Abstract: Kutajarista (KTJ) is an Ayurvedic preparation used as a traditional medicine in ulcerative colitis in the rural population. A self-made alcoholic preparation derived from the Indian Medical System known as Kutajarishta. The main ingredient in this polyherbal combination is Holarrhena antidysenterica Wall. Ex DC (Family: Apocynaceae). It is used to treat gastrointestinal disorders, diarrhea, and amebic dysentery. Ayurvedic practitioners have been using Kutaja formulations for centuries, as they have been found to be quite helpful clinically in certain diseases. This study was conducted using mainly old literature as a basis method. At a dose of 40 milliliters per kilogram, KTJ was given chronically to male Sprague-Dawley rats in order to determine its toxicological characteristics. Following a continuous 28-day KTJ preparation administration, the following toxicological alterations were observed. Several erythrocytic parameters were found in this experiment. The anemia panel studies' findings are as follows: The total amount of red blood cells in the male rat has decreased by [7.98%]. This drop was noticeable (p=0.083) even if it was not statistically significant. There is a [8.08 %] decrease in the Hemoglobin content of the blood of the male rat, the decrease though not significant yet it was prominent (p=0.097). There is a [8.03 %] decrease in the Hematocrit level of the blood of the male rat, the decrease though not significant yet it was prominent (p=0.072). There is a statistically insignificant (p=0.882) [0.19 %] increase in the Mean corpuscular volume, a red cell index of the male rat. There is a statistically insignificant (p=0.420) [3.02 %] decrease in the Mean corpuscular hemoglobin, a red cell index of the male rat. A statistically insignificant (p=0.203) [8.50 %] increase in the red cell volume distribution width, a red cell index of the male rat, is observed, along with a negligible [3.01 %] decrease in the Mean corpuscular hemoglobin concentration, a red cell index of the male rat, which was statistically not at all significant (p=0.561).

Keywords: Ayurvedic, medicinal, hemoglobin, kutajarista, rat etc.

1. INTRODUCTION

The world's oldest medicinal system, Ayurveda, has its roots in India and dates back around 3,000 years. Ayurvedic medicine inspired most ancient traditional medical systems, including Tibetan, Chinese, and Greek medicine. Approximately 80% of people in the Indian subcontinent today use medicinal plants and ayurvedic products to address their primary level of illness recovery. The use of ayurvedic and herbal origin extracts is growing daily, and the World Health Organization advocated at the beginning of the 20th century that ayurvedic medicine be used more widely in underdeveloped nations as an alternative medical system (Mohammed *et al*., 2020; Pehlivan and Sevindik, 2018; Sevindik *et al*., 2017; "WHO, 2002). Bangladesh National Formulary of Ayurvedic Medicine included Kutajarista (KTJ) as herbal medicine in 1992 (Bangladesh National Formulary of Ayurvedic Medicine, 1992). As a liquid Ayurvedic medication, kutajarista (KTJ) is prepared and taken once or twice daily at a dose of 12 to 24 mL for the treatment of various ailments

such as sprue syndrome, ulcerative colitis, dysentery or blood dysentery fever, irritable bowel syndrome, diarrhea, Croon's disease, etc. (Verma, 1991; Mishra, 2010).

According to Ernst (2002), ayurvedic medications have a solid safety record. However, recent research has shown that heavy metals (such as lead and arsenic) in ayurvedic preparations are harmful (Keen *et al*., 1994; Olujohungbe *et al*., 1994). Medicinal plants are widely available in nature and their use increasing with scientific evidence (Majedul Hoque *et al*., 2023). Most ayurvedic medications' safety profile has not yet been thoroughly studied. In light of this problem, a study has been conducted to determine the degree of hemotoxicity following long-term use of the commercially available ayurvedic medication formulation, KTJ.

The striking advantages of Ayurvedic medicines are cost effectiveness and wide acceptability. Ayurvedic medicines have become very popular especially in rural areas of the country. People of lower income generally prefer Ayurvedic medicines to other types of medicines which are expensive. In addition to its excellent efficacy in treating diseases these drugs have excellent safety & tolerability profile as well. Many herbal medicines are now strongly established worldwide for the treatment of a number of diseases. The World Health Organization (WHO) has recently approved the use of herbal medicines like Unani & Ayurvedic medicines in rural areas of the developing countries to ensure health care service at the root level of population. WHO estimated that around 1.5 billion people are being treated with these medications worldwide. This research work is about the toxicological effects of an Ayurvedic medicine named Kutajarista (KTJ) on rat model. The objective of the study is to acquire a better understanding of the potential toxicological aspects of the drug and the justification of its usage under stated conditions. However, further investigation is required to have more clear idea.

2. METHODOLOGY

2.1 Collection of Kutajarista (KTJ)

Kutajarista had been collected for the toxicological research project from Sri Kundeswari Aushadhalaya Ltd. in Chittagong.

2.2 Dose of Administration

The liquid was given in the toxicological experiment at a volume that would allow for the best possible dosing precision but barely increasing the overall amount of bodily fluid. The medication was given orally at a dose of 40 milliliters per kilogram of body weight for the toxicological investigations.

2.3 Route of Administration

Oral administration of the suspension was employed for the toxicological studies. [By mouth (p.o.) method]. (500 mg/kg i.p), of ketamine was injected intraperitoneally.

2.4 Experimental Animal

Eight-week-old, healthy, male and female Albino rats (Rattus novergicus: Sprague-Dawley strain) were used in this toxicological study. These animals weighed between 50 and 70 grams. The Jahangirnagar University Department of Pharmacy's Animal House is home to the rats. Rats were randomly assigned to 4 groups of 10 animals per sex before to the experiment. As a result, ten rats each were used in the experimental and control groups.

2.5 Control Groups

In parallel with the drug-treated group, a control group consisting of the same number of rats was used in the experiment. For the same number of days as the drug-treated group, they received distilled water as a placebo in an equivalent volume.

2.6 Animal Care

Every rat was kept in a plastic cage of thirty by twenty by thirteen centimeters, with bedding made of soft wood shavings. Ad libitum feeding, watering, and maintenance of the animals' natural day-night cycle were practiced. An hygienic, well-ventilated experimental animal housing was employed to house the animals.

2.7 Toxicological Experiment

The Ayurvedic medication preparation has been administered by intra-gastric syringe. Medication administration has taken place between the hours of 10 AM and noon.

2.8 Doses used in Different Experiments

Dose: 40 ml/kg body wt.

2.9 Animal Treatment

The animals were starved for 18 hours at the conclusion of the 28-day treatment period and for an additional 24 hours following the last administration. The patient received 500 mg/kg i.p. of ketamine to induce anesthesia. Whole blood samples were drawn from the post vena cava and promptly placed in containers with EDTA added. Within 12 hours of the material being collected, all analyses were finished.

2.10 Statistical Analysis

The standard error of the mean, or mean ± SEM, is used to express the group data. The statistical significance tests were conducted using unpaired "t" tests. Data analysis was done using SPSS (Statistical Package for Social Science) for Windows (Ver. 11). At $p < 0.05$ for significant, $p<0.01$ for highly significant, and $p<0.001$ for very highly significant, differences between groups were deemed significant.

3. RESULT AND DISCUSSION

Kutajarista is an Ayurvedic preparation used as a traditional medicine in ulcerative colitis in the rural population. To find out the effect of KTJ on Lipid profile, it was administered chronically to the male Sprague-Dawley rats at a dose of 40ml/kg. After 28 days of chronic administration of the KTJ preparation the following biochemical changes were noted. Method and analysis were done on the basis of previous studies (Zilva *et al*., 1991; Marks *et al*., 2003; Wallach, 2006; Kaplan, 1983; Bush, 1991).

The research work has been designed based upon the following aspects:

Red Blood Count
Hemoglobin
Hematocrit

Mean Corpuscular Volume
Mean Corpuscular Hemoglobin
Mean Corpuscular Hemoglobin Concentration
Red Cell Volume Distribution Width

3.1 Effect of Kutajarista on RBC, Hemoglobin and Hematocrit in Male Rats

Following 28 days of continuous KTJ preparation administration, the male rat group's total red blood cell count was measured. The total amount of red blood cells in the male rat has decreased by [7.98%]. This drop was noticeable ($p=0.083$) even if it was not statistically significant. The male rat's blood has a [8.08%] drop in hemoglobin content; while this decrease is not statistically significant, it is noticeable ($p=0.097$). There is a [8.03 %] decrease in the Hematocrit level of the blood of the male rat, the decrease though not significant yet it was prominent ($p=0.072$), [Table 1, Figure 1-3].

Table 1: Effect of Kutajarista on RBC, Haemoglobin and Haematocrit in Male Rats

	RBC	**Hemoglobin**	**HCT**
Control (Mean+SEM)	7.2624 ± 0.0898	12.30 ± 0.4865	43.84 ± 0.5094
KSTm (Mean+SEM)	6.679 ± 0.2720	11.3016 ± 0.3199	40.35 ± 1.7076
t/p	1.902 / 0.083	1.793 / 0.097	1.958 / 0.072
95% CI	-0.07732 to 1.2442	-0.34333 to 2.34253	-0.62067 to 7.59747
(%) increase/decrease	Decr 7.98 %	Decr 8.08 %	Decr 8.03 %

Note: *p<0.05, **p<0.01, ***p<0.001

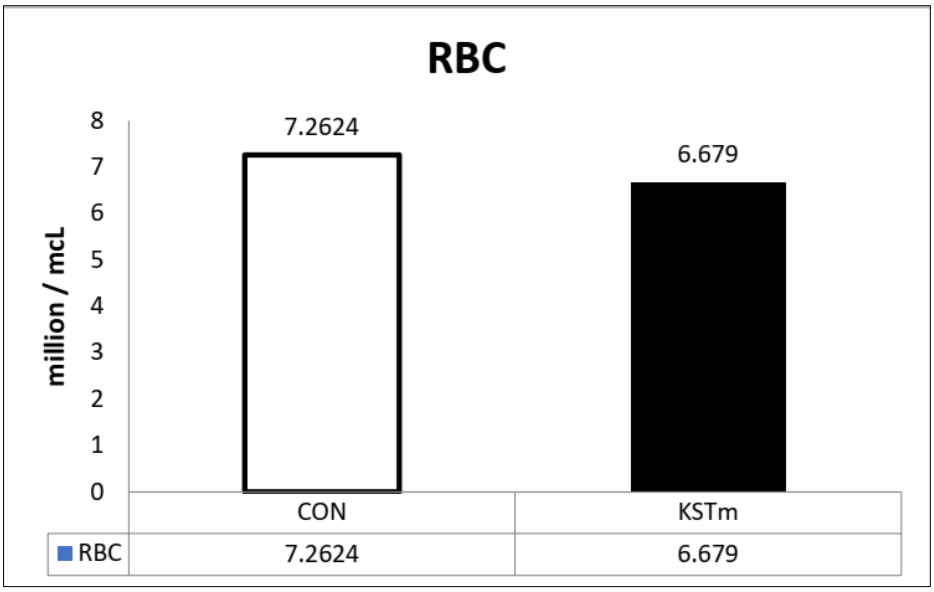

Figure 1: Impact of Kutajarista on the number of red blood cells (RBCs) in male rats

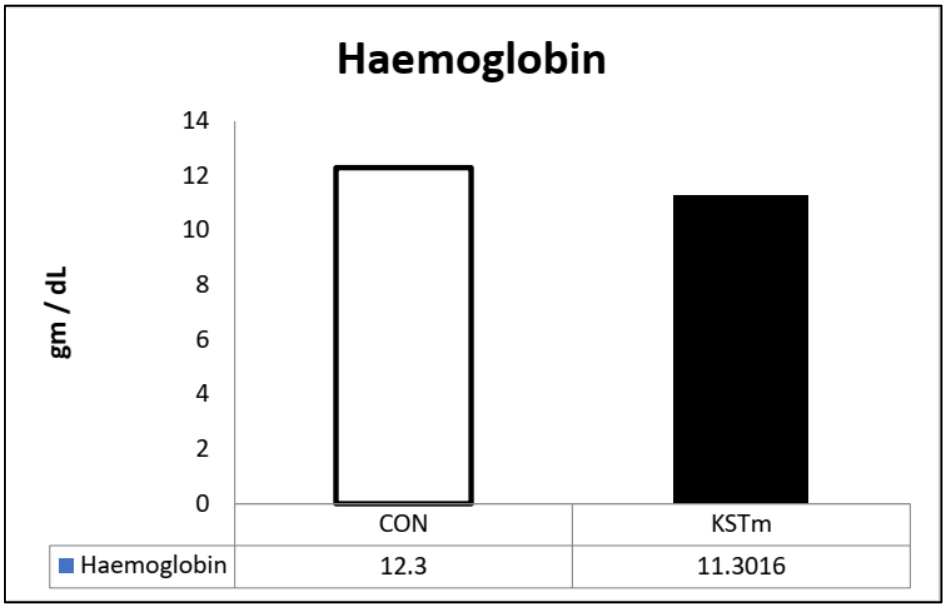

Figure 2: Effect of Kutajarista on Hemoglobin (Hb) content in male rats

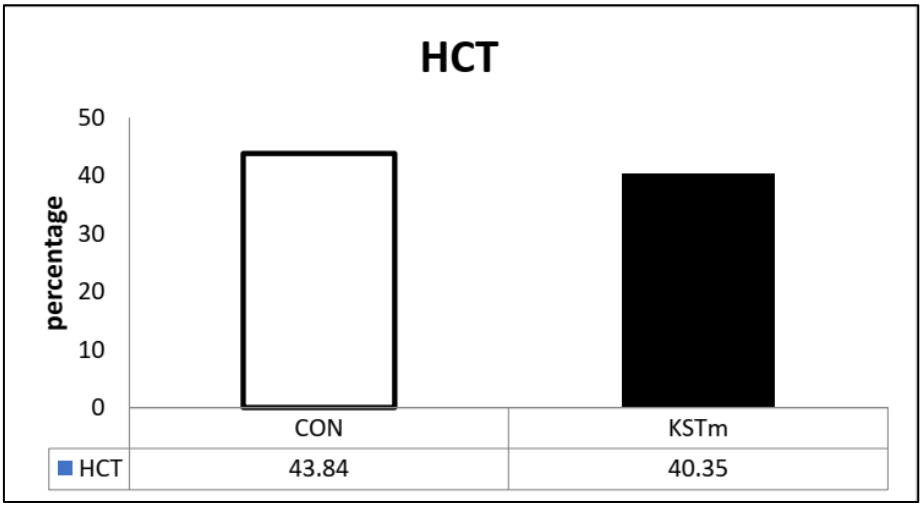

Figure 3: Effect of Kutajarista on Hematocrit (HCT) level in male rats

The mean ± standard deviation for RBC count was found to be 8.4 ± 0.4 x 106 per microliter with a minimum value of 7.1 and a maximum value of 9.1 in an age-wise previous study of 4 weeks old Sprague-Dawley male rats (n = 152). In another study, the mean ± standard deviation for RBC count was found to be 9.1 ± 0.5 x 106 per microliter with a minimum value of 7.8 and a maximum value of 10.2 for 13 weeks old Sprague-Dawley male rats [n = 160] (Petterino and Argentino-Storino, 2006).

In male Sprague-Dawley rats the Hemoglobin range between 14.7 – 17.3 gm per deciliter 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 ± standard deviation 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 ± standard deviation. 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). From a previous study of Sprague-Dawley male rats (n=2770) the mean for Hematocrit was found to be 47.0% with a standard deviation of 3.1% (Matsuzawa *et al*., 1993).

3.2 Effect of Kutajarista on the Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Red Cell Volume Distribution Width (RDW) in Male Rats

After 28 days of chronic administration of the KTJ preparation the Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Red Cell Volume Distribution Width (RDW) were determined in the male rat group. There is a negligible [0.20%] increase in the Mean Corpuscular Volume, a red cell index of the male rat, which was statistically not at all significant (p=0.91).

A statistically negligible (p=0.882) [0.19%] rise in the male rat's mean corpuscular volume, or red cell index, has been observed. The male rat's red cell index, mean corpuscular hemoglobin, has decreased, but the difference is statistically insignificant (p=0.420) [3.02 percent]. The male rat's mean corpuscular hemoglobin concentration, or red cell index, has decreased a little [3.01 percent], which was statistically not significant (p=0.561). There is a statistically insignificant (p=0.203) [8.50 %] increase in the red cell volume distribution width, a red cell index of the male rat (Table 2, Figure 4-7).

Table 2: Effect of Kutajarista on MCV, MCH, MCHC and RDW in Male Rats

	MCV	**MCH**	**MCHC**	**RDW**
Control (Mean+SEM)	61.57 ± 0.3971	17.87 ± 0.3326	29.62 ± 0.6141	10.00 ± 0.0999
KSTm (Mean+SEM)	61.69 ± 1.0811	17.34 ± 0.4891	28.74 ± 1.0616	10.87 ± 0.5029
t/p	-0.155 / 0.882	0.885 / 0.420	0.608 / 0.561	-1.328/ 0.203
95% CI	-3.07244 to 2.82764	-.83375 to 1.89455	-1.95116 to 3.70556	-2.04520 to 0.31936
(%) increase/decrease	Incr 0.19 %	Decr 3.02 %	Decr 3.01 %	Incr 8.50 %

Note: *p<0.05, **p<0.01, ***p<0.001

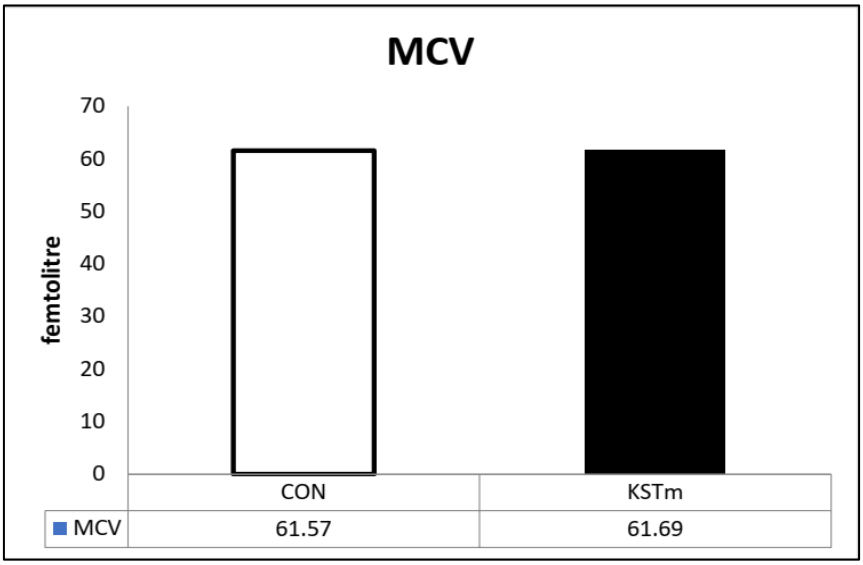

Figure 4: Impact of Kutajarista on Male Rats' Mean Corpuscular Volume (MCV)

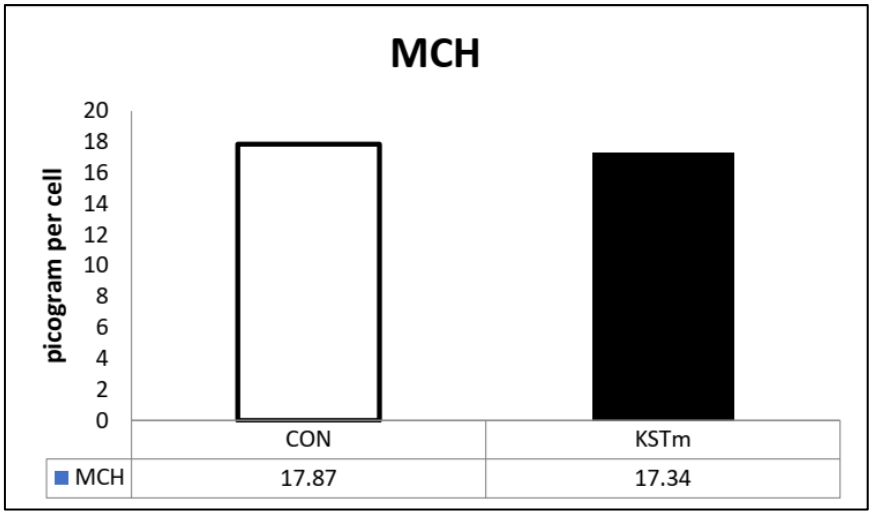

Figure 5: Impact of Kutajarista on Male Rats' Mean Corpuscular Hemoglobin (MCH)

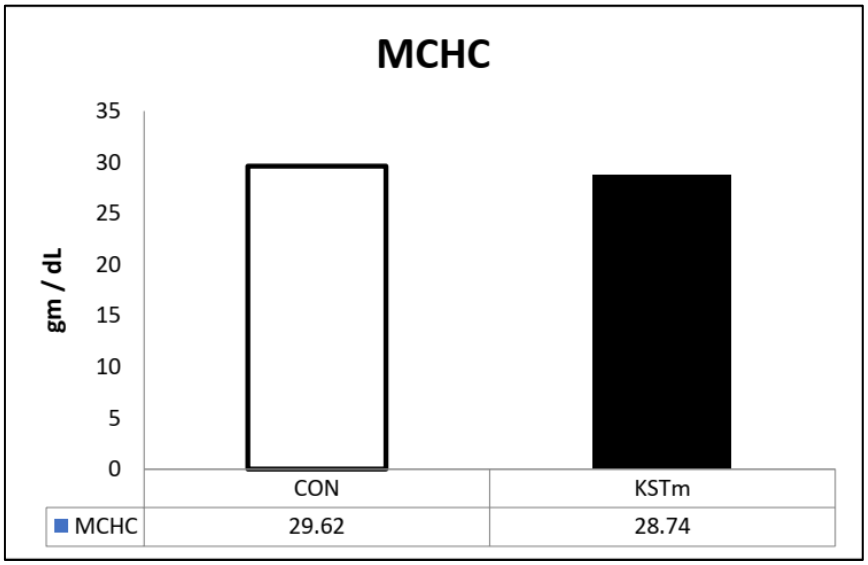

Figure 6: Kutajarista's impact on males' mean corpuscular hemoglobin concentration (MCHC)

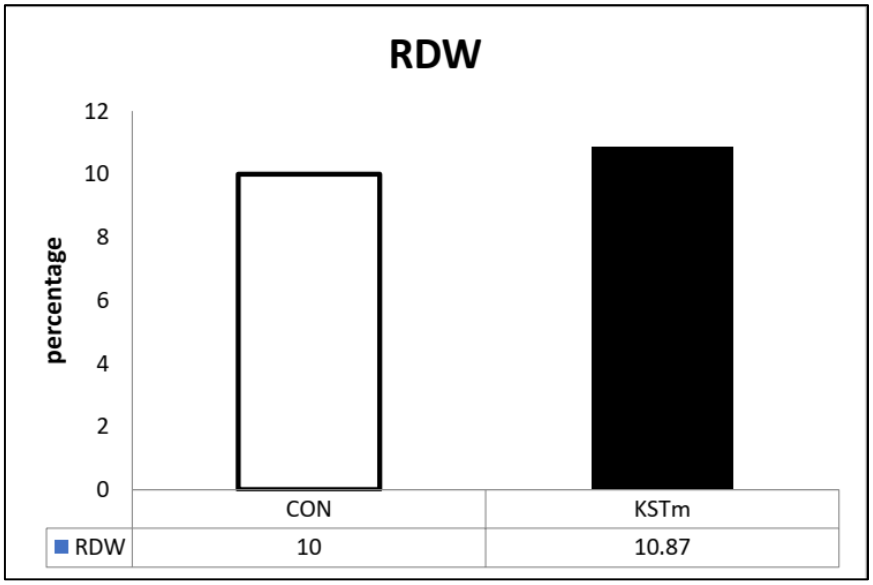

Figure 7: Effect of Kutajarista on Red Cell Volume Distribution Width (RDW) in male rats

Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) are morphological measures and are useful in the classification of anemia. 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). According to a study in male Sprague-Dawley rats the MCV range between 55.1–64.2 femtoliter with a mean of 59.7 femtoliter, MCH range between 18.6 – 20.7 picogram with a mean of 19.6 picogram, the MCHC range between 31.3 – 34.4 gm per deciliter with a mean of 32.8 gm per deciliter, the RDW range between 11.3 – 14.2% with a mean of 12.4% (Car *et al*., 2006).

4. SUMMARY

The total amount of red blood cells in the male rat has decreased by [7.98%]. This drop was noticeable (p=0.083) even if it was not statistically significant. The male rat's blood has a [8.08%] drop in hemoglobin content; while this decrease is not statistically significant, it is noticeable (p=0.097). The male rat's blood has a [8.03%], drop in hematocrit; while this decrease is not statistically significant, it is noticeable (p=0.072). There is a statistically insignificant (p=0.882) [0.19 %], increase in the Mean corpuscular volume, a red cell index of the male rat. There is a statistically insignificant (p=0.420) [3.02 %], decrease in the Mean corpuscular hemoglobin, a red cell index of the male rat. There is a negligible [3.01 %], decrease in the Mean corpuscular hemoglobin concentration, a red cell index of the male rat, which was statistically not at all significant (p=0.561). There is a statistically insignificant (p=0.203) [8.50 %], increase in the red cell volume distribution width, a red cell index of the male rat. From the experiment, it can be concluded that Kutajarista (KTJ) should not be administered chronically at a higher dose as it noticeably reduces red blood cell (RBC) count, hemoglobin, hematocrit. On the other hand there is no significant change in case of MCV, MCH, MCHC and RDW.

Disclosure of Conflict of Interest: None declared.

Author Contribution: All author contributed significantly to design and development of this work.

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