E ISSN 2350-0204

# IJAPC

VOLUME 11 ISSUE 3 2019

www.ijapc.com
Greentree Group Publishers (GGP))

Int J Ayu Pharm Chem

RESEARCH ARTICLE

www.ijapc.com

e-ISSN 2350-0204

# Effect of *Kushamanda* (Benincasa hispida), *Swarasa* along with Jeeraka (Cuminum cyminum) and *Sharkara* on Arsenic Trisulfide (Haratala) Induced Polycythemia: Experimental Evaluation in Wister Albino Rats.

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## ABSTRACT

Today Arsenic is commonest source of heavy metal toxicity. Especially in India, the most common source of accidental arsenic poisoning is seen by consumption of well water. Also, in medicinal fields, improper *shodhana*, over dosage & prolong use of formulations which contain *Haratala* (Arsenic trisulfide) may produce symptoms like burning sensation, fainting, cardiac pain, convulsions and even death. So it is very essential to develop an effective antidote in arsenic related hazards.

**Materials and methods**: The experimental study was conducted on Wister Albino rats and the trial group was administered with *Haratala* + *Kushamanda* (Benincasa hispida) *Swarasa*, *Jeeraka* (Cuminum cyminum) and *Sharkara* in TED for 28 days. The rats were then assessed for hematological, histopathological and ponderal changes and body weight, food Water ratio, food intake etc.

All the values were expressed as MEAN±SEM (Standard error of Mean). The data was analyzed by one way ANOVA followed by Dunnet's multiple 't' test. A level of $P<0.05$ was considered as statistically significant.

**Results**: The study showed mild to moderate effect on all Ponderal, hematological, biochemical histopathological changes and body weight in Arsenic trisulfide (Haratala) induced toxicity on polycythemia condition.

**Conclusion**: Aqueous of Benincasa hispida (*Kushamanda Swarasa)* along with *Jeeraka* (Cuminum cyminum) and *Sharkara* has got protective mechanism in the Arsenic trisulfide (*Haratala*) induced toxicity on polycythemia condition.

## KEYWORDS

*Polycythemia, Haratala, Arsenic, Kushmanda*

Greentree Group Publishers

Received 03/08/19 Accepted 19/09/19 Published 10/11/19

## INTRODUCTION

Arsenic is the commonest type of heavy metal toxicity in India. The most common source of accidental arsenic poisoning is due to consumption of "well water". Also due to improper *Shodhana*, over dosage & prolonged use of formulations containing *Haratala* (Arsenic trisulfide), toxicity produces symptoms like burning sensation, fainting, cardiac pain, convulsions and even death. So it is very essential to develop an effective antidote in arsenic related hazards.[1]

Polycythemia Vera is a slow-growing blood cancer in which your bone marrow makes too many red blood cells.[2] Polycythemia Vera (PV) is a clonal disorder involving a multipotent hematopoietic progenitor cell in which phenotypically normal red cells, granulocytes, and platelets accumulate in the absence of a recognizable physiologic stimulus. The most common of the chronic myeloproliferative disorders, PV occurs in 2 per 100,000 persons, sparing no adult age group and increasing with age to rates as high as 18/100,000. Familial transmission occurs but is infrequent and women are predominating among sporadic cases.

**Etiology:**

The etiology of PV is unknown. Although non- random chromosome abnormalities such as 20q and trisomy 8 and 9 have been documented in up to 30% of untreated PV patients, unlike CML, no consistent cytogenetic abnormality has been associated with the disorder. However, a mutation in the auto inhibitory, pseudo kinase domain of the tyrosine kinase JAK2—that replaces valine with phenylalanine (V617F), causing constitutive activation of the kinase—appears to have a central role in the pathogenesis of PV.

**Symptoms:**

Uncontrolled erythrocytosis causes hyper viscosity, leading to neurological symptoms such as vertigo, tinnitus, headache, visual disturbances, and transient ischemic attacks (TIAs). Systolic hypertension is also a feature of the red cell mass elevation. In some patients, venous or arterial thrombosis may be the presenting manifestation of PV. Any vessel can be affected; but cerebral, cardiac or mesenteric vessels are most commonly involved. Intraabdominal venous thrombosis is particularly common in young women and may be catastrophic if a sudden and complete obstruction of the hepatic vein occurs.

Indeed, PV should be suspected in any patient who develops hepatic vein thrombosis. Digital ischemia, easy bruising, epistaxis, acid-peptic disease, or gastrointestinal hemorrhage may occur due

to vascular stasis or thrombocytosis. Erythromelalgia is another complication of the thrombocytosis of PV due to increased platelet stickiness.

Given the large turnover of hematopoietic cells, hyperuricemia with secondary gout, uric acid stones, and symptoms due to hyper metabolism can also complicate the disorder.[3]

Affected individuals may also have an enlarged spleen (splenomegaly) and an increased risk for heart disease, and there is a small chance that PV may progress to cause leukemia (cancer of the blood).

In rare cases, the mutation to a gene that causes PV does occur in the egg or sperm cells, which increases the risk that a person with PV will pass the mutation on to their children. In these cases, the condition appears to have an autosomal dominant pattern of inheritance. This means that only one altered copy of a gene is enough to give a person an increased risk for PV. However, not every person who has a mutation in *JAK2* or *TET2* will necessarily develop PV. Rather, if person has a mutation in one of these genes, he or she has an increased risk to develop PV during his or her lifetime.[4]

## AIMS & OBJECTIVES

1. To determine the toxicity profile of *Haratala*.
2. To assess the *Haratala* induced toxicity in polycythemia.
3. To experimentally assess the effect of the Antidotal drugs *(Kushmanda Swarasa, Sharkara and Jeeraka*) with *Haratala* induced toxicity in polycythemia.
4. To assess the effect of Antidotal drugs (*Kushmanda Swarasa, Sharkara and Jeeraka).*

## MATERIALS AND METHODS

Raw materials were collected from the SDM Ayurveda Pharmacy Udupi and identified by the authenticated sources. The *Kushamanda Swarasa* along with *Jeeraka* and *Sharkara* was prepared in the practical Hall of Dept. of Rasashatra and Bhaishajya Kalpana SDMCA Udupi.

**Animals:**

Wister albino rats of either sex weighing between 200±50 gm were used for experimental study with the following conditions.

- The animals were obtained from animal house attached to the Pharmacology Laboratory SDM centre for Research in Ayurveda and Allied Sciences.
- Ethical committee approval was obtained with reference number **CPCSEA/IAEC/SDM-AT-02 DATED 11/04/2016.**

- They were fed with Saidurga feeds, Bangalore rat pellets and tap water ad libtium
- They were exposed to natural day and night cycles with ideal laboratory condition in terms of ambient temperature, humidity, etc.

**Antido1te effect assessment:**

**Group 1 –** Normal group

**Group 2nd –** *Haratala* (1/5 th of $LD_{50}$ as per requirement)

**Group 3rd** –*Haratala* + *Kushamanda Swarasa*, *Jeeraka* and *Sharkara* in TED. Also called as Antidote group.

**Group 4th** – *Kushamanda Swarasa*, *Jeeraka* and *Sharkara* in TED

The dose of *Kushamanda Swarasa* along with *Jeeraka* and *Sharkara* by found using (Paget & Barnes formula) by considering human dose.

**EXPERIMENTAL PROTOCOL**

Animals were kept on acclimatization for 7 days. The test formulation was administered orally once a day for 28 consecutive days.

On 28th day all animals were kept for overnight fasting. Next day blood was collected by supra-orbital puncture with the help of micro capillary tubes under mild ether anesthesia for estimation of serum biochemical parameters followed by sacrifice with over dose of ether anesthesia.'h01

The abdomen was opened through midline incision to record the autopsy changes followed by dissecting out the important organs as mentioned below and extraneous tissues was removed and weighed. The tissues were transferred to bottles containing 10% formalin for the purpose of Histopathological study.

Assessment body weight gain. Assessment criteria of "gain in body weight was done weekly. Both the wet and dry fecal matter weight, fecal water content was assessed weekly.

Food conversion Ratio was calculated for each week.

Fecal Water =Fecal Wet – Fecal Dry

Food Conversion Ratio = Food Intake/ Fecal Dry Weight

All these were calculated in each week for 28 days.

**Assessment of the Antidotal Study**:

A-**Assessment of Haematological :**

Blood samples were taken using capillary tubes from orbital plexus, under anaesthesia and different haematological parameters were measured using automatic cell counter and auto analyser.

B-**Assessment of the Ponderal changes** :

All the important organs were carefully dissected, cleaned of extraneous tissue and transferred to 10% formalin solution for

fixation. Spleen, testis, skin and bone marrow were the tissues subjected to histopathological study. After noting the weight of the organ they were transferred to 10% formaldehyde solution for fixation and sent to a commercial laboratory for preparation of slides. The slides with sections obtained were scanned in Trinocular Carl Zeiss's microscope (Germany) under different magnifications. Changes, if any in the cytoarchitecture were noted down.

C- **Statistical Analysis**

The data were analyzed by one way ANOVA followed by Dun net's multiple 't' test. A level of $P<0.05$ was considered as statistically significant. Level of significance was noted and interpreted accordingly.

## OBSERVATION & RESULTS

Effect of Arsenic trisulfide (*Haratala*) , Arsenic trisulfide + Antidote drugs and Antidote drugs in Arsenic trisulfide poisoning on parameters such as Food Intake, Fecal Wet, Fecal Dry, Food Conversion Ratio, Fecal Water Ratio, %Changes in Body Weight, Weight of Spleen and Testis. The changes observed in hematological factors HB%, RBCs, WBCs, PCV, MCV,MCH, MCHC, RDWSD, RDWCV and platelets were noted. The histopathology of Spleen, Testis and Bone-Marrow were recorded.

**1) Spleen –**

Figure 1 & 2: Group 1st showed normal *spleen* histopathology report normal tissue architecture.

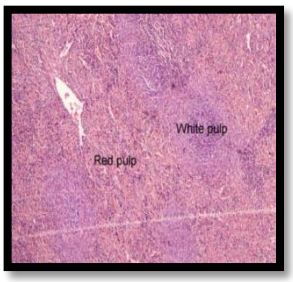


**Figure 1**

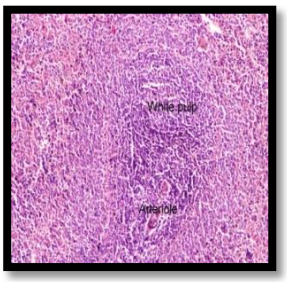


**Figure 2**

Figure3 & 4: Group 2nd showed severe degenerative changes with inflammation.

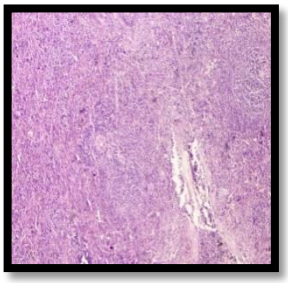

**Figure 3**

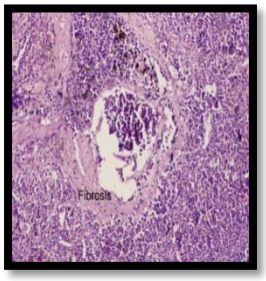


**Figure 4**

Figure 5& 6: Group 3rd showed no degeneration to moderate degenerative changes. Antidotal drugs showed protection in spleen 1against the *Haratala* poisoning.

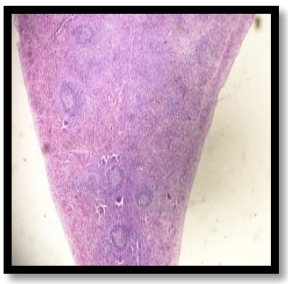

**Figure 5**

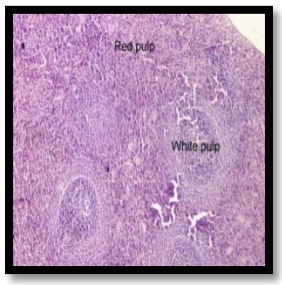


**Figure 6**

Figure 7&8: Group 4th showed no degenerative changes in spleen.

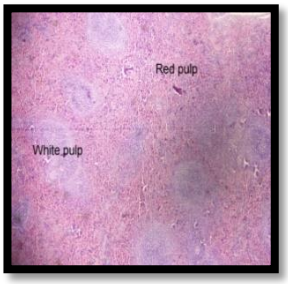


Figure 7

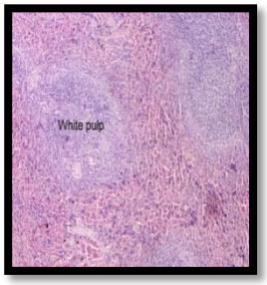


Figure 8

**2) Testis –**

Figure 9 & 10: Group 1st showed Normal testis histopathology report shows normal tissue architecture.

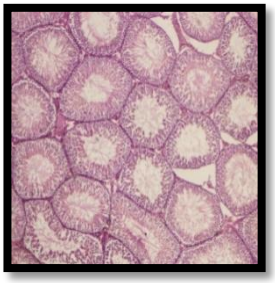

Figure 9


Figure 10

Figure 11&12: Group 2nd showed mild degenerative changes with necrosis in testis.

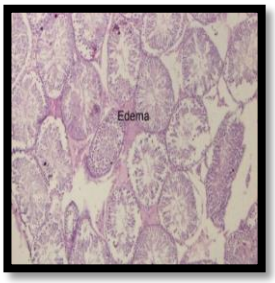


Figure 11

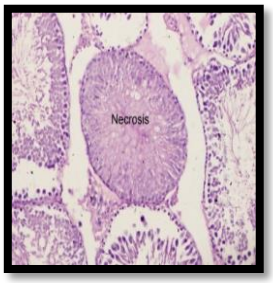


Figure 12

Figure 13 & 14: Group 3rd showed mild degenerative changes. Antidotal drugs showed protection in testis against the *Haratala* poisoning.

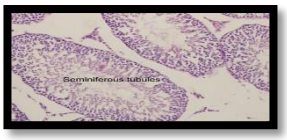


Figure 13

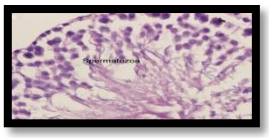

Figure 14

Figure 15 & 16: Group 4th, showed mild degeneration with increased spermatogenetic activity in testis.

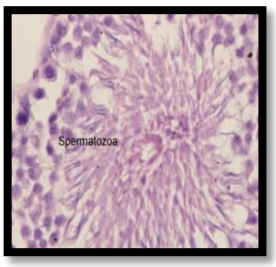


Figure 15

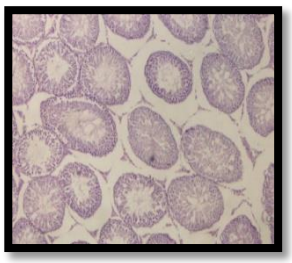

Figure 16

**3) Bone Marrow –**

Figure 17&18: Group 1st showed Normal bone marrow histopathology report normal tissue architecture.

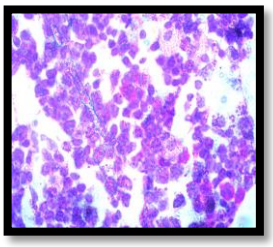

Figure 17

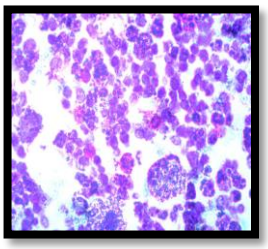

Figure 18

Figure 19&20: Group 2nd, showed reduced erythropoiesis in bone marrow.

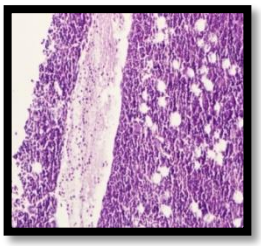

Figure 19

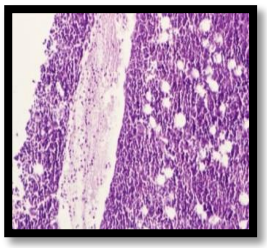

Figure 20

Figure 21&22: Group 3rd showed mild increased erythropoiesis. Antidotal drugs showed mild protection in bone marrow against the *Haratala* poisoning

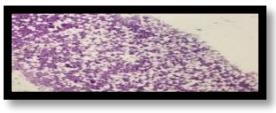

Figure 21

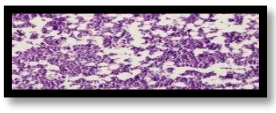

Figure 22

Figure 23&24: Group 4th, showed increased erythropoiesis.

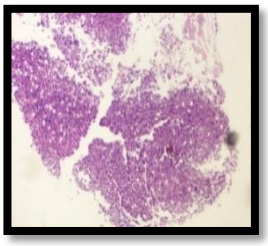

Figure 23

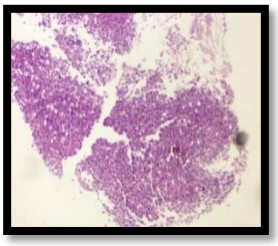

Figure 24

**4) Skin –**

Figure 25&26: Group 1st showed Normal skin histopathology report shows normal tissue architecture.

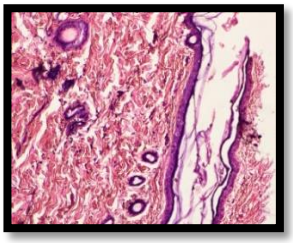

Figure 25

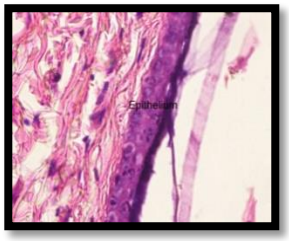

Figure 26

Figure 27&28: Group 2nd, showed Moderate to severe degenerative changes.

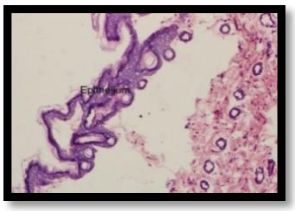

Figure 27

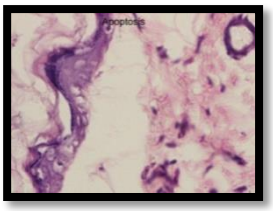

Figure 28

Figure 29&30: Group 3rd, showed mild degenerative changes with reduced inflammation. Antidotal drugs showed good protection in skin against the *Haratala* poisoning.

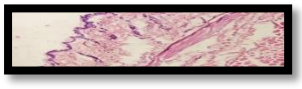

Figure 29

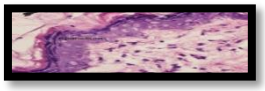

Figure 30

Figure 31&32: Group 4th, showed, no degenerative features in skin.

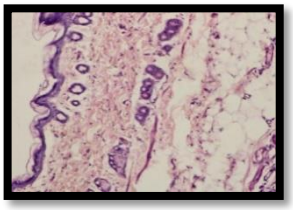

Figure 31

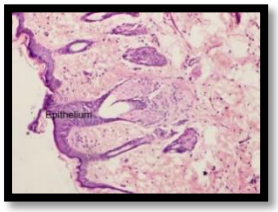

Figure 32

## MATERIALS & METHODS

Figure 33&34: Raw Material of *Haratala* (Arsenic trisulfide) & powder form of *Haratala*

Figure 33

Figure 34

Figure 35&36: Raw Material of *Jeeraka* & Powder of *Jeeraka*

Figure 35

Figure 36

Figure 37:Fruit of *Kushamanda* (Benincasa hispida)

**Figure 37**

Figure 38 Weight Machine

**Figure 38**

Figure 39: Group of rats

**Figure 39**

Figure 40: Weight Measuring of Wister albino rat

**Figure 40**

Figure 41: Administration of *Haratala* (Arsenic trisulfide) dose in wister albino rat

**Figure 41**

Figure 42: Measuring of *Haratala* along with distal water

**Figure 42**

Figure 43: Administration of *Kushamanda* (Arsenic trisulfide) Dose in Wister albino rat

**Figure 43**

Figure 44: Collection of blood from orbital artery

**Figure 44**

Figure 45: Blood collecting tube

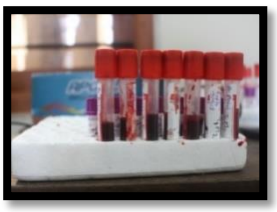

**Figure 45**

Figure 46: Ether for anesthesia

**Figure 46**

Figure 47: Midline incision

**Figure 47**

Figure 48: Removal of organs

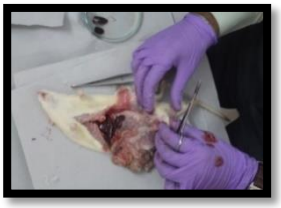

**Figure 48**

Figure 49: Removal of bone marrow

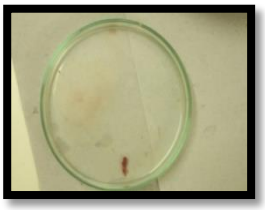

**Figure 49**

Figure 50: Removal of organs

**Figure 49**

## DISCUSSION

The experimental study on Albino rats was conducted to assess the antidote effect *Kushamanda Swarasa* along with *Jeeraka* and *Sharkara* on *Haratala* induced polycythemia based on the LD50 value of *Haratala*.

**Body weight-**

After administration of *Haratala* (Arsenic trisulfide), increase (non-significant) changes were observed in body weight in *Haratala* control group. After administration of the *Haratala a*nd *Kushamanda Swarasa*, decrease in % change in the body weight (non significant) in antidotal group was observed. And after administration of *Kushamanda Swarasa* only, decrease (non significant) in % change body weight were observed in 1st and 4th week *Kushamanda* group.

This result showed that after Haratala was administered, the body weight got increased. Arsenic accumulation causes disturbances in the total body weight, food and water intake etc. The data shows relevance to the similar result- arsenic exposed rats showed decreased intake of water and food, accompanied with hinderance of growth rate and alterations in body weight gain.[5]

Increased body weight due to *Haratala* was reversed (mild to moderate) by the antidote (*Kushmanda* Swarasa) effect.This indicates that *Kushmanda* Swarasa acts as an antidote activity of *Haratala*

FOOD INTAKE-.

Administration of group 2nd and group 3rd showed decrease (non significant changes) in food intake which was decreased more (significantly decreased) in group 3rd at the end of 28 days. But, food intake wa10'hs increased (non significant) in group 3rd and group 4th. *1*

FECAL WET -

In initial, after administration of *Haratala* significant increases changes were observed in fecal wet in Group 2nd and significant decrease changes were observed in fecal wet in group 3rd and non-significant decrease changes were observed in fecal wet in group 4th.

Finally, after administration of *Haratala* non-significant decreases changes were observed in fecal wet in group 2nd and non-significant decrease changes were observed in fecal wet in group 3rd and non-significant increase changes were observed in fecal wet in group 4th.

FECAL DRY

In initial stage, after administration of *Haratala* non-significant increased changes were observed in fecal dry in group 2nd. After administration of drugs in group 3rd, non-significant increases changes were observed in fecal dry. And after administration, non-significant increases changes were observed in fecal dry in group 4th.

Finally, after administration of *Haratala* increased in fecal dry (non significant changes) in group 2nd. After administration of drugs, non-significant increased changes were observed in fecal dry in group 3rd and after administration of drugs, non-significant increased changes were observed in fecal dry in group 4th.

FOOD CONVERSION RATIO-

In initial, after administration of *Haratala* significant decreased changes were observed in food conversion ratio in group 2nd , and after administration of drug in group 3rd - significant decrease changes were observed in food conversion ratio. After administration, significant decreased changes were observed in food conversion ratio in *group 4th.*

In finally, after administration of *Haratala*, significant decreased changes were observed in food conversion ratio in group 2nd, and after administration of *drugs*, non-significant decreased changes were observed in food conversion ratio in group 3rd. After administration of *Kushamanda Swarasa*, non- significant decrease changes were observed in food conversion ratio in group 4th.

Initially, after administration of *Haratala* significant increased changes were observed in fecal water ratio in group 2nd , but after administration of drugs in group 3rd , significant decrease changes were observed in fecal water ratio. After administration, significant decreased changes were observed in fecal water ratio in group 4th.

FECAL WATER RATIO:

In finally, after administration of *Haratala*, non-significant decrease changes were observed in fecal water ratio in group 2nd. After Administration of test drugs, significant decrease changes were observed in fecal water ratio in group 3rd. After administration of *drugs,* non-significant increased changes were observed in fecal water ratio in group 4th.

**PONDERAL CHANGES**:

To understand the *Haratala* induce polycythemia, on the group 2nd organs and to assess the effect Antidote on the *Haratala* induces polycythemia.

After the administration of *Haratala* non-significant increases changes were observed in Spleen in group 2nd , Administration of test drugs, non-significant increase changes were observed

in spleen in group 3rd And administration of *Kushmanda Swarasa*, non-significant increase changes were observed in spleen in group 4th.

In group 2nd, it showed severe degenerative changes with inflammation that implant increasing spleen weight. The weight of spleen was not changed in group 3rd .And after administration of *Kushmanda* Swarasa showed not much effect changes weight of spleen in group 4th.

TESTIS

After the administration of *Haratala* non-significant increases changes were observed in testis in group 2nd. Administration of drugs, non-significant decrease changes were observed in testis in group 3rd. Non-significant increase changes were observed in testis in group 4th.

In group 2nd, it showed mild degenerative changes with necrosis in testis that implant increasing weight of testis and in group 3rd. The weight of testis was reversible. In group 4th it showed mild degeneration with increased spermatogenetic activity in testis that implant increasing weight in testis.

**HEMATOLOGICAL PARAMETER**:

When the *Haratala* was administered, the Hb got increased; it may be due to increasing binding affinity with the hemoglobin (Hb) of rats and humans. The binding stoichiometry was consistent with the number of reactive cysteine residues in the α and β chains of Hb. Comparing the binding affinity of rat Hb and human Hb for the same trivalent arsenic species.[6]

When the *Haratala* was administered, the RBCs, WBCs, and platelets got increased, it may be due to; secondary polycythemia would more accurately be called secondary erythrocytosis or erythrocythemia as those terms specifically denote increased red blood cells. The term polycythemia is used appropriately in the myeloproliferative disorder called polycythemia Vera , in which there are elevated levels of all there peripheral blood cell lines –RBCs, WBCs, Platelets .[7]

However, since the antidote activity of the combination was studied – it is necessary to ascertain whether the antidote therapy reverses the changes brought about by *Haratala* irrespective their being desirable or undesirable. This angle Hb, MCH, and MCHC elevating effect of *Haratala* was found to be decreased at TED dose of antidote combination. This indicates reversal of the *Haratala* effect – the activity can considered as a supportive evidence for the antidote property of the combination.

RBCs elevating effects of *Haratala* was found to be decreased at TED dose of antidote Combination. This indicates reversal effects of the *Haratala* effect - the activity can considered as a supportive

evidence for the antidote property of the combination.

WBCs were little increased after the administration of antidote, we can consider this, and the effect of Antidote was not much on WBCs.

The effect of *Haratala* toxic on Platelets were nothing changed, but after the administration of Antidote the platelets were much increased .and we can considered this increased platelets will helpful in treatment of decreased platelets (like thrombocytopenia).

The platelets were much increased after administration of the Antidote. In antidote group (*Kushamanda* Swarasa).

The Toxic effect of *Haratala* were decreased the MCV, but MCV were more increased in after the administration of the antidote in antidote group. Effective reversible changes were observed in administered of antidote in antidotal group. And *Haratala* toxicity was produced increased MCH, MCHC, but after the administrated of antidote, MCH and MCHC were much decreased in antidote group. So the effective reversible changes were observed after administered of antidote in antidotal group.

So the antidotal effect of *Kushmanda* Swarasa was mild to moderate effect on the hematological parameter induced by arsenic trisulfide (*Haratala*).

No toxic effects of *Antidote* (*Kushamanda* Swarasa) on Hb%, RBC, WBC, PCV, MCH, RDWCV, RDWSD and Platelet. So this antidote may be effective in different pathological condition like Anemia, Leukemia, decrease Packed cell volume, different types of Anemia, RDW size and volume, and thrombocytopenia, dengue shock syndrome.

But moderate to high increased MCV, it may be due to effects of antidote and mild to moderate MCHC decreased due to effects of *Kushamanda* Swarasa.

## CONCLUSION

This study showed that mild to moderate effect on all Ponderal, Hematological and Histopathological changes due to *Haratala*. In *Haratala* (Arsenic trisulfide) group, polycythemia was observed which was reversed after the antidote (*Haratala* + *Kushamanda Swarasa)*. Similar observation was seen in on body weight, skin changes, bone marrow etc.

Aqueous of Benincasa along with *Jeeraka* and *Sharkara* group showed increased erythropoiesis in bone marrow and increased spermatogenetic activity in testis in compared to antidotal group. This show that *Kushamanda Swarasa* along with the *Jeeraka* and *Sharkara* having greatest

protective mechanism on the *Haratala* (Arsenic trisulfide) induced toxicity.

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