

## Preclinical Evaluation of the Effect of Antioxidant N-acetyl-D-Glucosamine on Haematonic Potentials of Lauha Bhasm and Mandura Bhasm

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The effect of antioxidant N-acetyl-D-Glucosamine on haematonic activity of two ayurvedic haematonic preparations Lauha bhasm and mandura bhasm was studied in albino rats. Anaemia was induced by administration of phenyl hydrazine (80 mg/kg for 24 hrs) by intra-peritoneal injection. Changes in body weight, red blood cell count (RBC), white blood cell count (WBC), haemoglobin (HB) concentration and haematocrit (HCT) were analysed as indices of anaemia. The mean cell haemoglobin (MCH), mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) which are indicators of macrocytosis were calculated accordingly. Phenyl hydrazine induced a significant decrease ( $P < 0.05$ ) in the blood parameters indicating anaemia and also resulted to significant increase ( $P < 0.05$ ) in the macrocytosis parameter. Combination of bhasm with antioxidant induced a significant ( $P < 0.05$ ) increase in the RBC count, HGB concentration, and HCT% which had been originally decreased by phenylhydrazine administration. The macrocytosis also turned towards normal as the animals recovered from anaemic condition. The results obtained suggested that combination of antioxidant N-acetyl-D-Glucosamine with Lauha bhasm & mandura bhasm shows improved haematonic activity.

**Keywords:** Anaemia; Haematonic Activity; Lauha Bhasm; Mandura Bhasm; N-Acetyl-D-Glucosamine; Phenylhydrazine.

Anaemia is a global health problem affecting persons of all the age and socio-economic groups worldwide. More than 20% of the world's population suffers from iron deficiency.<sup>1,2</sup> It has been an major health problem in most tropical countries<sup>3,4</sup> and a common cause of weakness and related issues of poor health in young women.<sup>5</sup> Regular cycles of women life like menstruation,

pregnancy, parturition and lactation demands high physiological needs for iron<sup>6</sup> and may lead to anaemia if not met.

Ayurveda therapy is aimed at targeting the cause of disease and associated symptoms. In ayurveda, medicines like Punarnavadi Mandur, Tapyadi Loh, Arogya Vardhini and Lohasav are used to increase the RBC count in anaemic patients.

Medicines like Manjishtha (*Rubia cordifolia*), Patol (*Tricosanthe device*) Amalaki (*Emblica officinalis*), Mandukparni (*Centella asiatica*) used for anaemia act on the “Rakta” (blood) and “Majja”(Nervous) dhatu (tissues) and bone marrow of the body<sup>7</sup>.

Lauha Bhasm and Mandura Bhasm, the most frequently used Ayurvedic preparation<sup>8,9</sup>. The effect of both the Bhasms in the management of iron deficiency anaemia in animal model as well as human were found to be very effective<sup>8-13</sup>.

N-Acetyl D Glucosamine<sup>14</sup> has proven antioxidant activities and can be a vital adjuvant in treatment of anaemia. The antioxidants neutralized reactive oxygen species (ROS) by reacting with O<sub>2</sub><sup>-</sup>, NO and peroxy nitrite<sup>15-19</sup> and can be very effective in improving anaemic conditions. The study evaluates the effect of antioxidant N-Acetyl D Glucosamine on haematinic activity of Lauha Bhasm and Mandura Bhasm .

Phenyl hydrazine (PHZ) is a strong oxidant and is very potent anemia inducing agent.<sup>20-23</sup>

## MATERIALS AND METHODS

### Materials

#### Experimental animals

The albino rats (Wistar strain) of both the sex with a weight of about 150-200 g and age of 8 to 10 weeks were used for study. Rats were placed in polypropylene cages with maximum of 5 animals per cage. The animals were fed with a standard animal feed and water “*ad libitum*” during the complete experimental period. Temperature of  $25 \pm 2^\circ\text{C}$  and relative humidity of 40-60 % with 12 hrs light dark cycle was maintained in the animal housing area. The study was approved

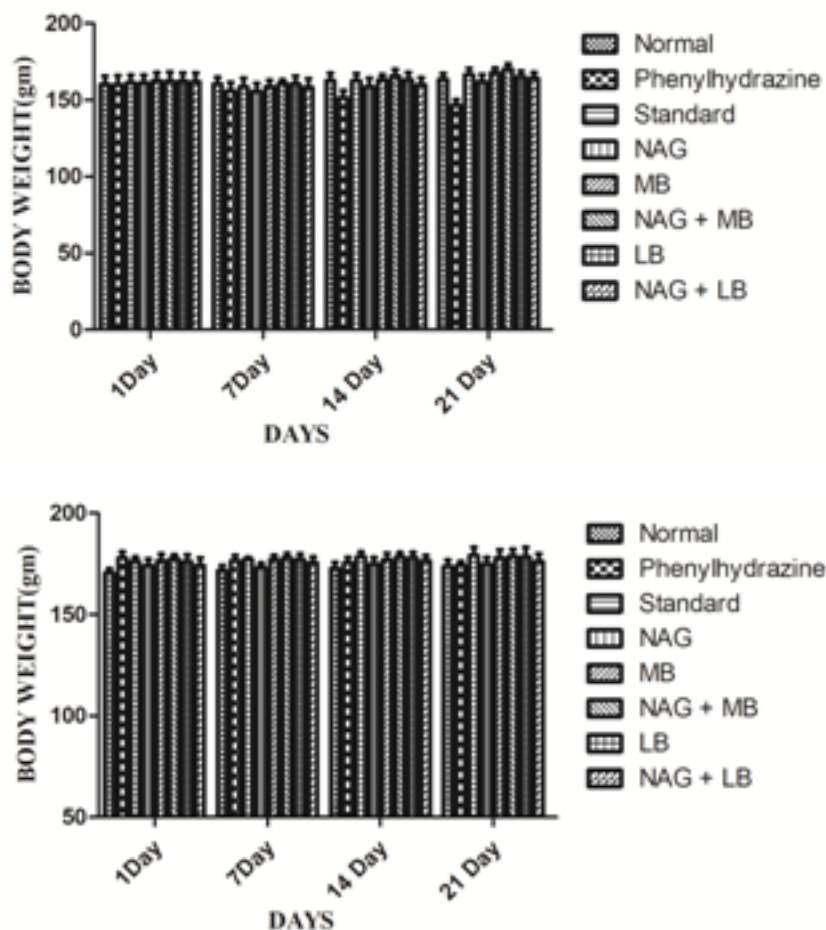


Fig. 1. Changes in body weight of rats during experimental period

by the Institutional Animal Ethical Committee (Registration No. 535/02/a/CPCSEA/Jan2002).

**Chemicals/ Material**

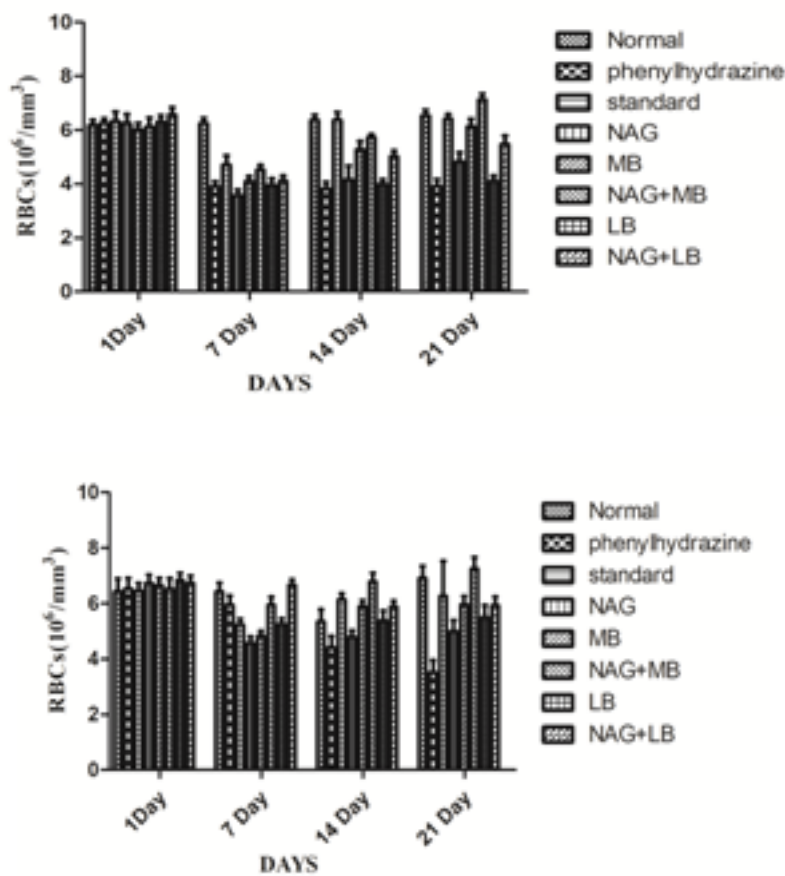
The Lauha Bhasm and Mandura Bhasm were procured from the local ayurvedic pharmacy. The chemicals N-acetyl D glucosamine, EDTA disodium, phenylhydrazine, ferrous sulphate were purchased from S.D. Fine chemicals India. All ingredients used were of AR grade and used as received.

**Experimental Design**

Animal Group: Albino rats were divided into twelve groups of six animals each. The details of animal groups are given in Table no.1. Phenyl hydrazine at 80 mg/kg for 24 hrs by the intra-peritoneal route was injected to eleven groups 2 to 11 for inducing anaemia.. Group 1 was administered normal saline solution.

**Haematological investigation<sup>9,24</sup>**

Blood samples were collected (1-1.5 ml) in EDTA from the retro-orbital plexus under slight chloroform anaesthesia. Sampling was done on day 0 before phenyl hydrazine administration, and on days 1, 7, 14 and 21, after phenyl hydrazine administration. Red blood cell count (RBC), white blood cells (WBC), haemoglobin (HB) concentration and haematocrit (HCT) were determined using Auto cell counter 920<sup>E</sup>, SWELAB Ltd. Mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were estimated based on above determined parameters. The average increase in body weights was also evaluated as a sign of improvement in overall health anaemia.



**Fig. 2.** Changes in RBC (10<sup>6</sup>/mm<sup>3</sup>) in rats by administering drugs and bhasm

**Statistical analysis**<sup>12,13</sup>

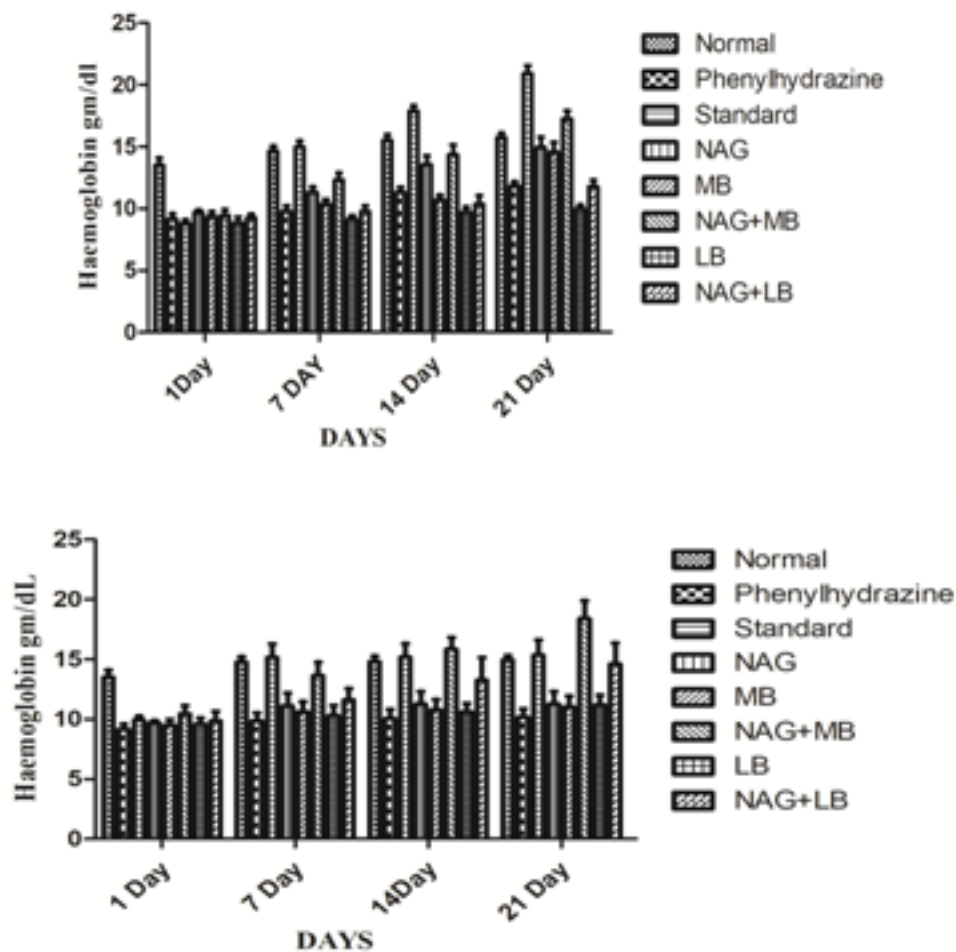
One-way ANOVA followed by Dunnett test was used as statistical tool. Results are expressed as the mean  $\pm$  SEM, n=6. The statistical significance of results was determined at a level of  $p < 0.05$ .

**RESULTS AND DISCUSSION**

Lauha bhasm and mandura bhasm are the two most commonly used ayurvedic preparations. Apart from haematinic and astringent, they are used in disorders of liver, spleen and kidney disease. They are also useful in treating intestinal worms, nervous disease, and neuralgia. Being a powerful

haematinic and general tonic they are very valuable in the treatment of haemolytic anaemia and jaundice.<sup>9,11</sup>

The reported acute toxicity studies as per OECD guidelines and Globally Harmonized System (GHS) for the classification of acute toxic substances, the bhasm were classified in the Category 5 with a LD<sub>50</sub> higher than 2000 mg/kg.<sup>25, 26</sup> Based on the earlier reports, a lower dose of 11 mg/kg and higher dose of 22 mg/kg was selected in the present study.<sup>9</sup> Haematinic activity of both the bhasm was evaluated in vivo in phenyl hydrazine induced anaemic albino rats. Changes in body weight, RBC, HGB, HCT, MCV, MCH, MCHC and WBC were evaluated to assess and compare the haematinic activity.



**Fig. 3.** Changes in Haemoglobin level(g/dL) of rats during by administering drugs and bhasm

**Changes in body weight**

In anaemic patients, the body weight generally decreases and does not increase easily. Improvement or at least maintenance of the body weight in anaemic patients can be a sign of better anaemic control. The study shows both the bhasm and standard drug show improvement in body weight. Mandura bhasm(MB) in combination with NAG has significant improvement in body weight as compared to other treatment groups. The results are depicted in fig. 1.

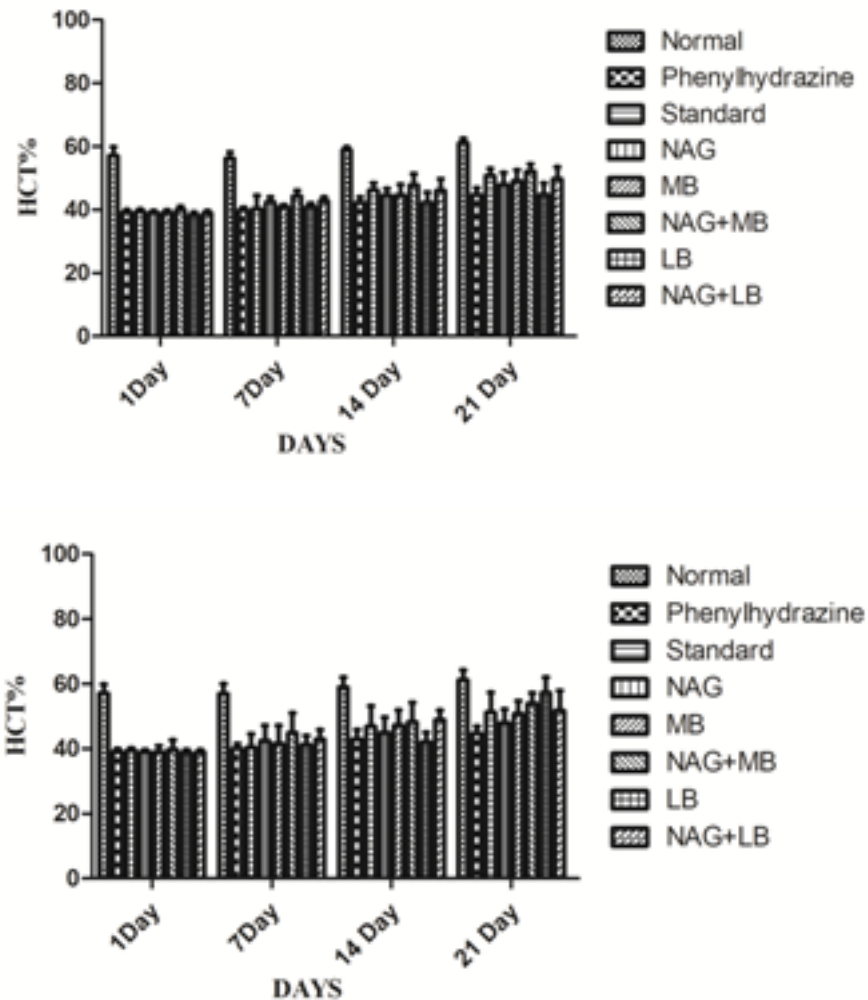
**Changes in RBC ( $10^6/mm^3$ )**

The changes in RBC count of experimental animals after treatment are given in fig.2. Increasing

the RBC count is one of the most important goals of the treatment of anaemia. Evaluation of RBC count in all animal groups under study indicates that as expected, the standard showed significant effect ( $p<0.05$ ) on RBCs count. MB and combination of NAG and MB also produced significant increase ( $p<0.05$  and  $p<0.01$  respectively) in RBCs count when compared with anaemic control and other groups. Though NAG, LB and combination of NAG and LB also increases RBCs count but effect was not significant when compared with anaemic control

**Changes in Haemoglobin level (g/dL)**

Depleted haemoglobin is the most



**Fig. 4.** Changes in HCT(%) in rats by administering drugs and bhasm

important reason which makes anaemia a very challenging health problem and is primarily behind the complications caused by anaemia. NAG, MB and LB when administered individually increases HGB content but effect was not significant when compared with anaemic control. The combination of NAG with MB and LB produced significant increase ( $p < 0.001$  and  $p < 0.05$  respectively) in HGB content when compared with anaemic control. Standard showed a relatively less significant effect ( $p < 0.05$ ) on HGB content as compared to the combination of NAG with MB. The results are graphically represented in fig.3.

**Changes in HCT (%)**

Hematocrit is decreased in anaemia and any viable treatment for anaemia must be able to restore hematocrit in anaemic condition. From the fig. 4 it can be observed clearly that combination of NAG and MB produced a significant increase ( $p < 0.01$ ) in HCT (%), when compared with anaemic control. Though the standard, NAG, MB, LB and combination of NAG with LB also increases HCT (%) but effect was not statistically significant when compared with anaemic control. Amongst test groups, the LB and NAG combination was better than all the other treatment groups but MB and NAG combination indicating improved efficiency of MB and NAG combination.

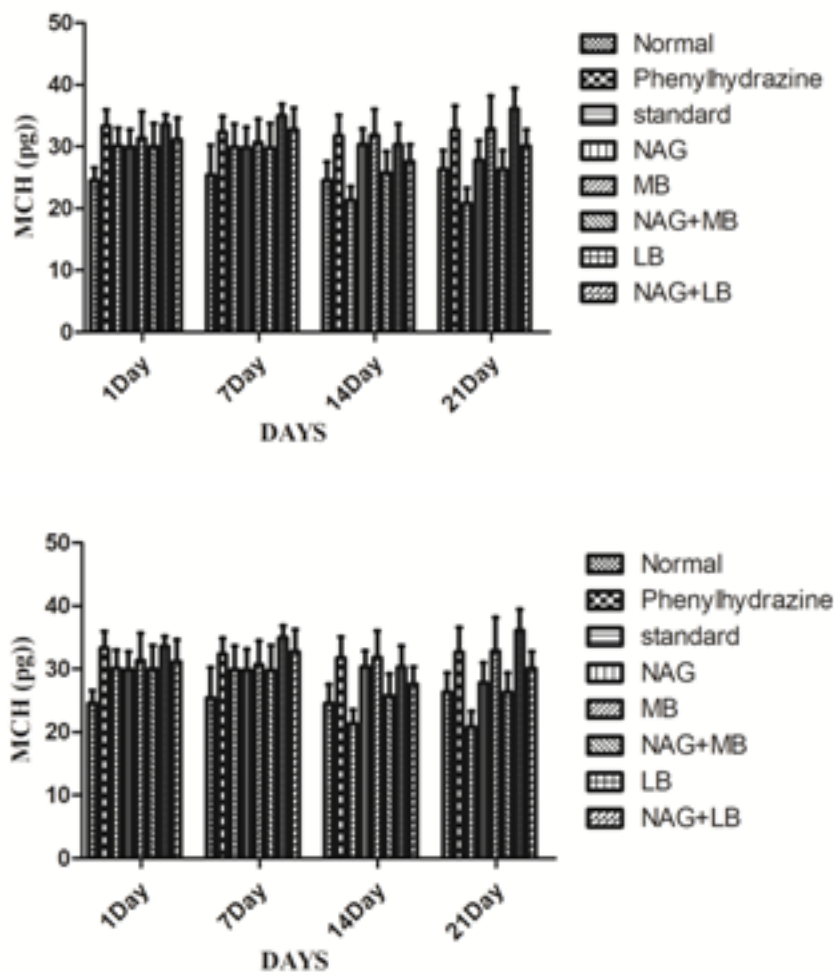


Fig. 5. Changes in MCH in rats by administering drugs and bhasm



**Changes in MCH**

Standard drug and combination of NAG with MB produced a significant decrease ( $p < 0.01$ ) in MCH as compared to anaemic control. Though, NAG, MB, LB and combination of NAG with LB when administered individually also show decreased MCH, the effect was not statistically significant when compared with anaemic control (fig. 5). The results indicate that MB-NAG combination is much superior in reducing MCH.

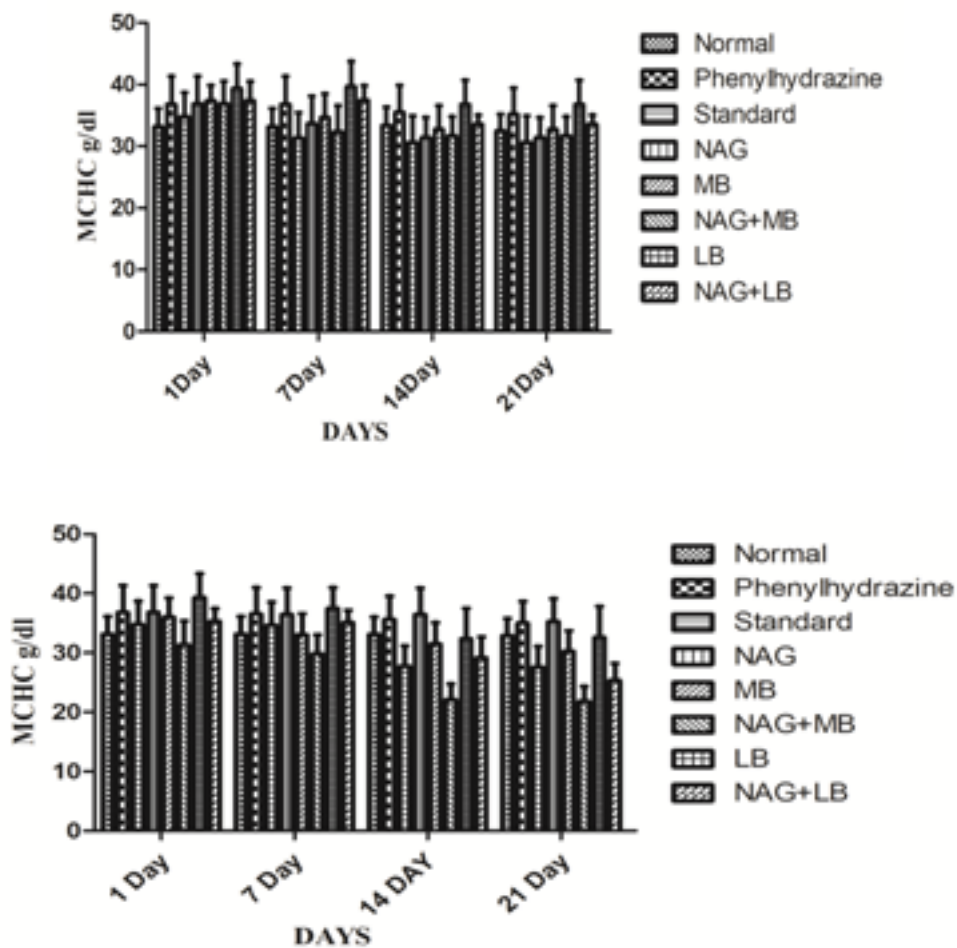
**Changes in MCHC (g/dL)**

Decreasing Mean Cell Haemoglobin Concentration indicates the recovery of anaemia. From fig. 4, It is clear that combination of NAG

with MB and LB shows a significant decrease ( $p < 0.01$  and  $p < 0.05$  respectively) in MCHC. Though, NAG, MB and LB when administered individually also decreased MCHC but the effect was not statistically significant when compared with anaemic control. The MB-NAG combination shows most significant result in reducing MCHC.

**Changes in MCV**

The Mean cell volume should decrease significantly if the treatment is good enough for anaemia. Combination of NAG with MB and LB show significant decrease ( $p < 0.001$  and  $p < 0.05$  respectively) in the mean cell volume when compared with anaemic control. The decrease is



**Fig. 6.** Changes in MCHC (g/dL) in rats by administering drugs and bhasm

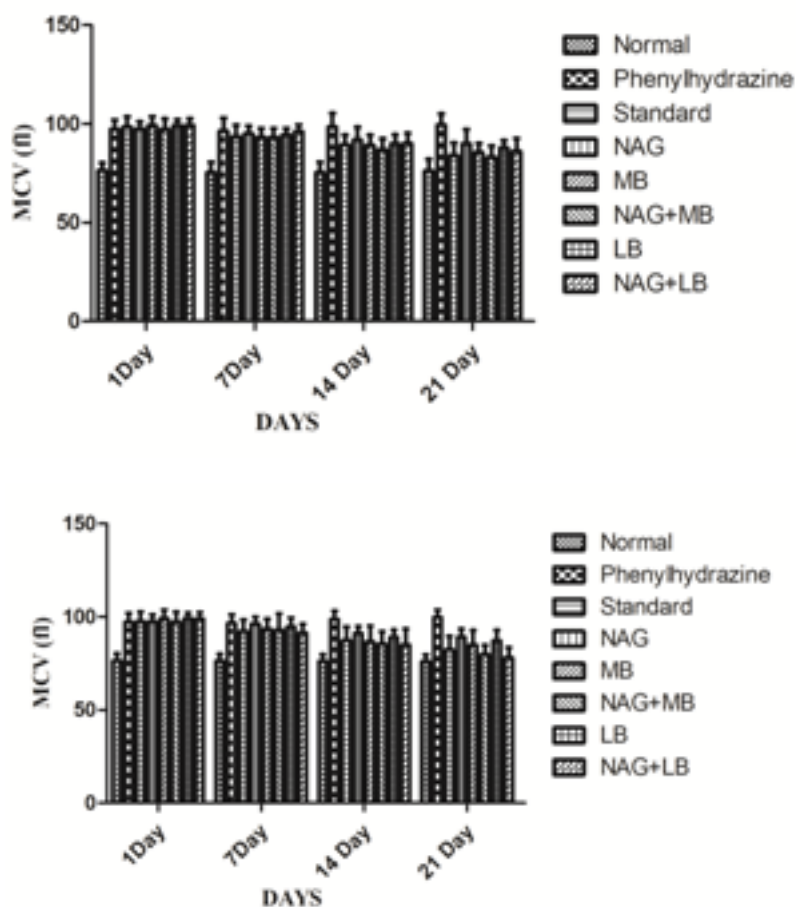


Fig. 7. Changes in MCV in rats by administering drugs and bhasm

significant compared to standard. Though NAG, MB and LB when administered individual also decreases mean cell volume but the results are not significant when compared with anaemic control (fig.6).

#### Changes in WBC $10^3/\mu\text{L}$

Most of health challenges to body including anaemia results in increased WBC count. Haematonic agent must be able to decrease WBC count in blood or at least prevent it from increasing drastically. Though NAG, MB and LB when administered individual also decrease in WBC count, the effect was not significant when compared with anaemic control. The combination of NAG with MB and LB produced a significant decrease ( $p < 0.01$  and  $p < 0.05$  respectively) in WBC compared to anaemic control comparable to standard.

The beneficial effect of antioxidant may be due to the preservation of the vascular function and preventing vascular damage by ROS and oxidant species like PHZ radicals.<sup>23, 28</sup> Another possible explanation can be protecting the membrane integrity of the RBCs<sup>29</sup> and stimulation of the erythropoiesis process<sup>24</sup>. Thus the bhasm and antioxidant in combination not only exerted its inherent anti anaemic activity but also might have neutralized the free radicals generated by PHZ by blocking lipid peroxidation and free radical generation and thus protecting RBCs haemolysis.

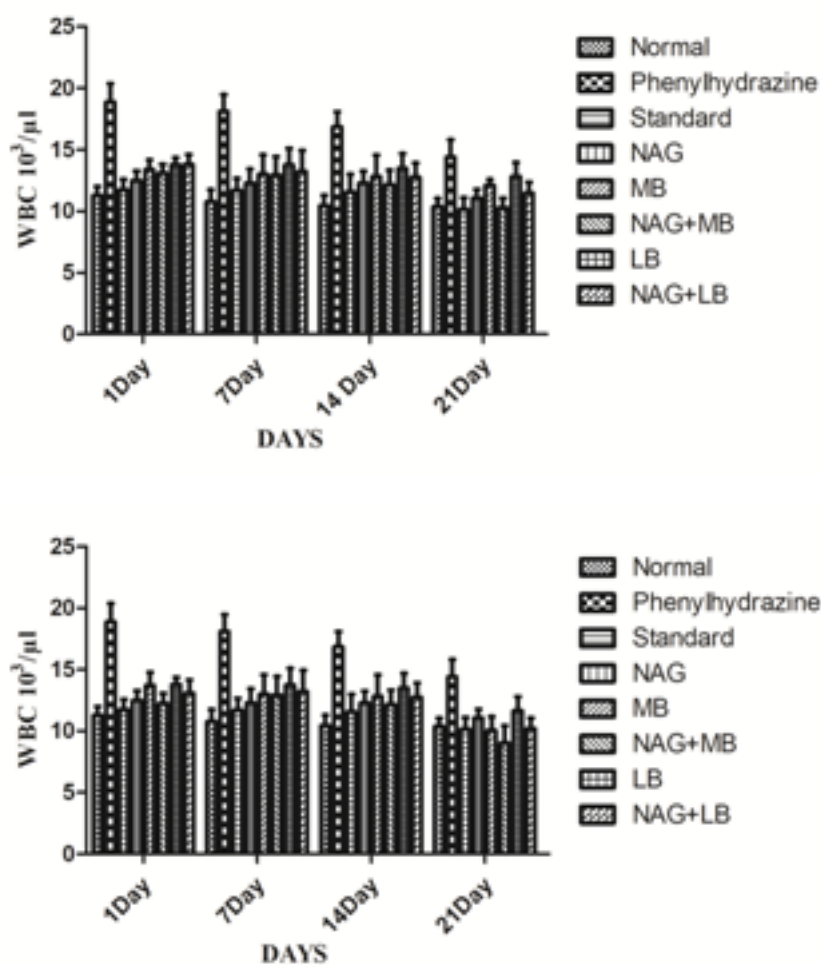
These mechanisms explain the anti-anaemic properties of the combination of bhasm and antioxidant in the current study. To summarize, the present study provides the evidence that the antioxidant and hematinic agent like bhasm could reduce oxidative stress and thus aid in prevention



**Table 1.** Animal groups for study

Animal Group	No. of Animals	Treatment
Group 1	6	Distilled water
Group 2	6	Phenyl hydrazine
Group 3	6	ferrous sulphate 40 mg/kg
Group 4	6	N-Acetyl D Glucosamine (NAG) 5mg/kg
Group 5	6	Mandura bhasm (MB) 11mg/kg
Group 6	6	NAG 5 mg/kg + MB 11mg/kg
Group 7	6	Lauha Bhasm LB 11mg/kg
Group8	6	NAG 5mg/kg + LB 11mg/kg
Group 9	6	Mandura Bhasm (MB) 22mg/kg
Group 10	6	NAG 5mg/kg+ MB 22mg/kg
Group 11	6	Lauha Bhasm (LB) 22 mg/kg
Group812	6	NAG 5 mg/kg + LB 22mg/kg

The study protocol was for 21 days.

**Fig. 8.** Changes in WBC of rats by administering drugs and bhasm

of hemolytic anemia by improvement of normal hemoglobin level, red blood cells count, and hematocrit percent and other related parameters.

In light of the results, the study indicated that co-administration of antioxidant N-Acetyl D Glucosamine & bhasm show significant haematinic activity.

### CONCLUSION

The study indicates that not only the standard drug but both Mandura Bhasm and Lauha Bhasm in combination with antioxidant N-Acetyl D Glucosamine combination significantly increased the haematinic activity with the significant increase in blood RBC, HGB and the % HCT count. Haematinics may be used in synergistic combination with antioxidant for effectively treating anaemia and anaemia related complications.

Among the Mandura Bhasm and Lauha Bhasm, Mandura bhasm when used in combination with N-acetyl D-glucosamine (5mg/kg) showed comparatively significant improved haematinic activity even at the low dosage, in anaemic rats. Further investigation and appraisal regarding the mechanism of action of antioxidants in combination with haematinics for the treatment and management of anaemia is still needed.

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#### Conflict of Interest

The authors would like to ensure that there are no conflicts of interests with anybody regarding the honorarium, grants, membership, employment, ownership of stock or any other interest or non financial interest such as personal or professional relation, affiliation, knowledge of the research topic and content and authorship of research paper.

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