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Anti-anemic potential of *Moringa oleifera* flower extract against phenylhydrazine-induced anemia in rats

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ABSTRACT

The present study was focused to evaluate the anti-anemic activity in hydro-alcoholic extract of *Moringa oleifera* flower extract against phenylhydrazine induced anemic rats. Anemia was induced by administration of (40 mg/kg b.w.) intraperitoneally in rats for two days. The animals were divided into 6 groups containing 6 animals each. Group 1 received normal saline as a control, and all other groups received phenylhydrazine for two days in order to cause anaemia. Group 3 served as the standard group and received ferrous sulphate treatment (100 mg/kg). The remaining groups 4, 5, 6 received *M. oleifera* flower extract at three different doses (200 mg/kg, 400 mg/kg, and 800 mg/kg) orally for 14 days. On 15th day blood was withdrawn, through cardiac puncture and subjected to the estimation of RBC, Hb and HCT. The anemic parameters were reversed after treatment with *Moringa oleifera* flower at different doses for 15 days when compared with phenylhydrazine-induced anemic rats.

Keywords: anemia, phenylhydrazine, *Moringa oleifera* flower extract, ferrous sulphate

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INTRODUCTION

A common public health issue known as anaemia is defined as a decrease in haemoglobin concentration or erythrocyte mass in the blood, which results in a reduction in the blood's ability to carry oxygen[1]. When haemoglobin levels in the blood fall below the normal range of less than 12 g/dL for female adults and less than 13 g/dL for male adults, anaemia has occurred[2]. Anaemia tends to be three to four times more common in developing nations than it is in developed nations. Anaemia has an impact on a person's physical and mental growth, which reduces their capacity for work and has an impact on the nation's development[3]. The behavioural effects of anaemia are extremely relevant because a nation's technological and economic development heavily depends on its trained human resources. Consequently, a nation's intellectual and economic potential may be significantly hampered if anaemia is highly prevalent there[4]. Although synthetic drugs are frequently used to treat a particular disease, due to their high cost and unwanted side effects, attention is now being paid to the use of medicinal plant products to manage or prevent various diseases or ailments in both humans and animals [5]. For the treatment of various types of anaemia, medicinal plants are used around the world, particularly in the tropics [6].

At the end of the 19th century, phenylhydrazine (PHZ) and its derivatives were first prescribed as antipyretics, but their use was hazardous due to their toxic effects on red blood cells. Rats were given injections of phenylhydrazine (40 mg/kg), which caused anaemia[7]. Haemoglobin experiences oxidative denaturation when oxygen is present, and the first step in this process is a bimolecular reaction, likely a two-electron transfer from phenylhydrazine to oxyhemoglobin; the reaction product is neither methemoglobin nor deoxyhemoglobin[8]. Red blood cells (RBC) membrane skeleton experiences oxidative degradation of spectrin, peroxidation of lipids, and the formation of reactive oxygen species as a result of PHZ. The red blood cell proteins appear to undergo oxidative changes that cause the PHZ-induced hemolytic injury [9].

Moringa oleifera Lam (*M. oleifera*) is a cruciferous plant in the *Moringaceae* family. *M. oleifera*, also known as the horseradish tree or drumstick tree by locals, is well-liked throughout the world[10]. Because of its many applications, exceptional nutritional value, and potential for use in nutraceutical, *M. oleifera* is known as "The Miracle Tree". This plant has been recognized as the "Botanical of the Year-2007" by the National Institutes of Health (NIH)[11]. *Moringa* flower is a rich reservoir of bioactive phytochemical and crude flower extracts showed promising antibacterial, antifungal, anti-larval, antioxidant, antiinflammatory and anticancer properties[12]. It contains A, B, C, D, E, and

K are among the vitamins that are found in the highest concentrations in this plant. Among other necessary minerals, *Moringa* contains calcium, copper, iron, potassium, magnesium, manganese, and zinc. There are several notable studies on the antioxidant properties of aqueous and ethanol extracts of flower. More than 40 natural antioxidants are reported in *Moringa oleifera* flower extract[13]. The total Antioxidant content is higher in flower than other parts of *Moringaoleifera*[14]. Traditionally, *M. oleifera* plant has been used in the treatment of diuretic, analgesic, antipyretic, vermifuge, anti-ulcer, hypoglycemic, hypolipidemic, laxative, and asthma diseases. *Moringa oleifera* also possesses antiviral, antioxidant, antimicrobial, anti-inflammatory, antipyretic, anthelmintic, antifungal, hepatoprotective, antihyperglycemic, hypolipidemic, antidiabetic, antiviral, antihyperlipidemic and cardioprotective activity[15]. The leaf of *M. oleifera* is reported for its antianemic property due to the presence of significant amount of iron and vitamin C[16]. Such contents are also found in *M. oleifera* flower. In the current study the flower of *M. oleifera* showed potent antianemic property. It might be due to the presence of iron and vitamin C[17].

METHODOLOGY

Procurement of chemicals and reagents

Phenylhydrazine was obtained from Loba Chemie Pvt. Ltd, jehangir villa, 107, Wodehouse Rd., colaba, Mumbai (India). Ferrous sulphate was obtained from s d fine-chem. limited, 1502, marathon icon, lower parel, Mumbai. Other chemicals utilized in this work are of analytical grade.

Instrumentation

Sonicator (Ana Matrix Instrument Technologies Pvt Ltd, LMUC-3, India), Water bath (Shital Scientific Industries, India), Heating mantel (Techno Scientific Products, S.S 104, India), Rotary evaporator (Rotavapor R-3, Buchi, India).

Plant material

The *Moringa oleifera* flowers were collected from surrounding area of B G Nagar, Mandya. The collected flowers were cleaned, dried in the shade, ground into a coarse powder, and then kept at room temperature in an airtight container. Dr. Pradeep, Associate Professor, Department of Dravyaguna, and Sri Dharmasthala Manjunatheshwara College of Ayurveda & Hospital verified the authenticity of the flowers. (No: SDMCAH-DG/2022/56)

Preparation of extract

In a conical flask, 1500ml of hydro alcohol (70-30%) was used to soak the fine powder (300g) for 72 hours. After 72 hours, the mixture is concentrated using simple distillation in a water bath and filtered using a fine muslin cloth followed by filter paper (Whatman No. 1)[18].

Experimental animals

Female albino Wistar strain rats weighing 100–150g were chosen for the study. Individual polypropylene cages were used to house each animal under sanitary and typical environmental conditions (22±3 °C, humidity 30–70%, 12h light/dark cycle). The animals were given free access to standard feed and water. Before being used in the experiment, they spent a week getting familiar to the surroundings. Experiments were conducted with the approval of both the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animal experimentations were approved by IAEC of SACCP, B.G Nagara. (IAEC Approval number: SACCP-IAEC/2022-02/68)

Experimental design

Induction of anemia

Phenylhydrazine (40 mg/kg) intraperitoneal injections were given once daily for two days to induce anaemia. Anemia was confirmed by the reduction of red blood cells (RBC) and hemoglobin concentration of the blood reduced by 40%[19].

Phenylhydrazine induced anemia model in rats

Rats were divided into six groups containing six rats in each group and treated for 15days.

Group 1: Normal control (received vehicle).

Group 2: Anemic group (treated with phenylhydrazine 40 mg/kg).

Group 3: Received standard drug ferrous sulfate, 100 mg/kg.

Group 4: Phenylhydrazine + Treatment with *Moringa oleifera* flower extract 200 mg/kg.

Group 5: Phenylhydrazine +Treatment with *Moringa oleifera* flower extract 400 mg/kg.

Group 6: Phenylhydrazine + Treatment with *Moringa oleifera* flower extract 800 mg/kg.

Blood collection and analysis

About (1-2 ml) of blood was collected from tail vein after the induction of anemia and blood was collected by cardiac puncher after the treatment with standard and plant extract. Blood was thoroughly mixed with EDTA to avoid coagulation and used for haematological test. The cyanomethaemoglobin method was used to estimate haemoglobin[20].

Analysis of haematological parameter

The concentrations of red blood cells (RBCs), haemoglobin (Hb), hematocrit (HCT), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular volume (MCV) in the blood were determined[21].

Histopathological analysis

For histopathological studies, the kidney and spleen were separated and preserved in a 10% formalin solution.

Statistical analysis

Data were expressed as the mean ±SEM, and statistical analysis of variance (ANOVA) and Tukey's test were used to identify differences between the groups. ***P0.001 p-values were regarded as statistically significant.

RESULTS and DISCUSSION

The present study was aimed to evaluate the anti-anemic activity of *Moringa oleifera* flower extract against phenylhydrazine induced anemic rats. The ability of phenylhydrazine to induce hemolysis *in vivo* through the production of aryl and hydroxyl radicals, which have been linked to its interaction with erythrocytes, is well known. Hemolysis is largely caused by erythrocyte oxidative stress. Chronic hemolysis results in haemoglobin loss[22]. In rats, phenylhydrazine administration reduces hematocrit, red blood cell count and haemoglobin concentration[23]. The anemia which was resulted due early lysis of the blood cells was treated with hydroalcoholic extract of *Moringa oleifera* flower at different doses. This study used six groups of female rats which are consisting of six rats per group. The dose variation which is used in this study is determined to know which dose of *Moringa oleifera* flower extract that has significant effect on various parameters of anemia was observed.

Phenylhydrazine induced anemia model in rats

The aim of the current study was to evaluate the *Moringa oleifera* flowers ability to treat anaemia. The following comparisons were made between anemic induced animal groups and control animal group.

Table 1 represents the impact of *Moringa oleifera* flower extract on Wistar albino rats RCB, Hb, and HCT counts. When compared to Group I rats, Group II phenylhydrazine-intoxicated rats displayed a significant decline in RBC, Hb, and HCT count. Rats treated with regular ferrous sulphate who had consumed Group III phenyl hydrazine had higher levels of RBC, Hb, and HCT than those who had consumed Group II. Rats treated with *Moringa oleifera* flower extract at different doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg each showed a significant rise in RBC, Hb, and HCT levels compared to group II intoxicated with phenylhydrazine, group IV, group V, and group VI. The higher dose of (800 mg/kg) showed highly significant activity as compared to low doses (200 mg/kg and 400 mg/kg). Effect of *M. oleifera* flower extract might due to the presence of vitamins and iron content in them.

Table 1. Effect of hydroalcoholic extract of *Moringa oleifera* flower on blood levels of RBCs, hemoglobin, and HCT in anemia-induced rats

Groups	Complete Haemogram		
	RBCs count (10^6/µl)	Hemoglobin count (g/dl)	HCT count (%)
Normal control	5.135±0.9	13.03 ± 0.26	39.5 ± I.83
PHZ - induces	3.441 ± 0.8	9.463 ± 0.4	23 ± 0.9
Standard	4.555 ± 1.2***	13.315 ± 0.5***	34.83 ± 1.81***
PHZ + Low dose	4.123 ± 0.9*	11.465 ± 0.9*	32 ± 1.98*
PHZ + Medium dose	4.240 ± 0.8**	11.811 ± 0.6**	33.166 ± 1.57**
PHZ + High dose	4.425 ± 1.1***	12.323 ± 0.9***	35.33 ± 1.98***

The inducer control group was used as a comparison point for the haematological parameters. Data are presented as Mean + SEM, n = 6, with two-way ANOVA and Tukey's test used for statistical analysis. When compared to the inducer control, ***$p<0.001$.

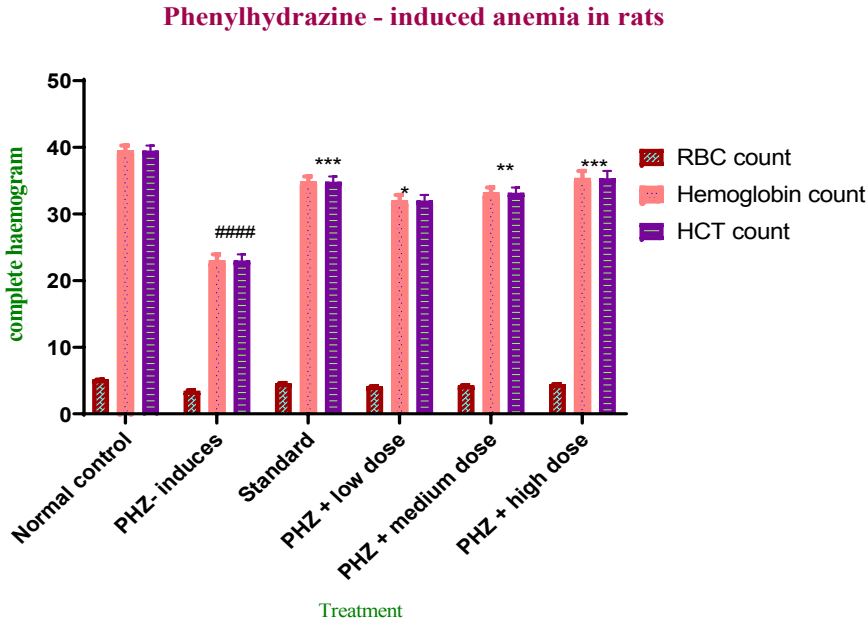

Figure 1. Effect of hydroalcoholic extract of *Moringa oleifera* flower on blood levels of RBCs, hemoglobin, and HCT in anemia-induced rats

Table 2 represents the impact of *Moringa oleifera* flower extract on Wistar albino rats PCV, MCH, MCHC, and MCV counts. Comparing Group II phenylhydrazine-intoxicated rats to Group I rats, the PCV, MCH, MCHC, and MCV count significantly decreased. When compared to Group II, rats treated with standard ferrous sulphate who had been intoxicated with phenyl hydrazine in Group III showed a significantly higher level of PCV, MCH, MCHC, and MCV. When compared to group II, group IV, group V, and group VI phenylhydrazine-intoxicated rats received doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg of *Moringa oleifera* flower extract, respectively. These doses resulted in a significant rise in PCV, MCH, MCHC, and MCV levels. The higher dose of (800 mg/kg) showed highly significant activity as compared to low doses (200 mg/kg and 400 mg/kg). Effect of *M. oleifera* flower extract might due to the presence of vitamins and iron content in them.

Table 2. Effect of hydroalcoholic extract of *Moringa oleifera* flower on PCV, MCH, MCHC, and MCV

Groups	Differential Count			
	PCV	MCH	MCHC	MCV
Normal Control	46.4±0.121	29.305±0.137	44.456±0.159	84.691±0.137
PHZ – induces	31.611±0.175	21.09±0.234	31.118±0.276	68.386±0.151
Standard	45.57 ± 0.129***	28.31±0.234***	40.308±0.146***	78.471±0.171***
PHZ + Low dose	41.66±0.137*	26.33± 0.128*	36.351± 0.117*	74.418± 0.117*
PHZ + Medium Dose	42.211±0.051**	26.68± 0.139**	37.336± 0.103**	77.203± 0.045**
PHZ + High Dose	44.306± 0.140***	27.37± 0.157***	38.51±0.151***	78.595± 0.149***

The inducer control group was used as a comparison point for the haematological parameters. Data are presented as Mean + SEM, n = 6, with two-way ANOVA and Tukey's test used for statistical analysis. When compared to the inducer control, ***p<0.001.

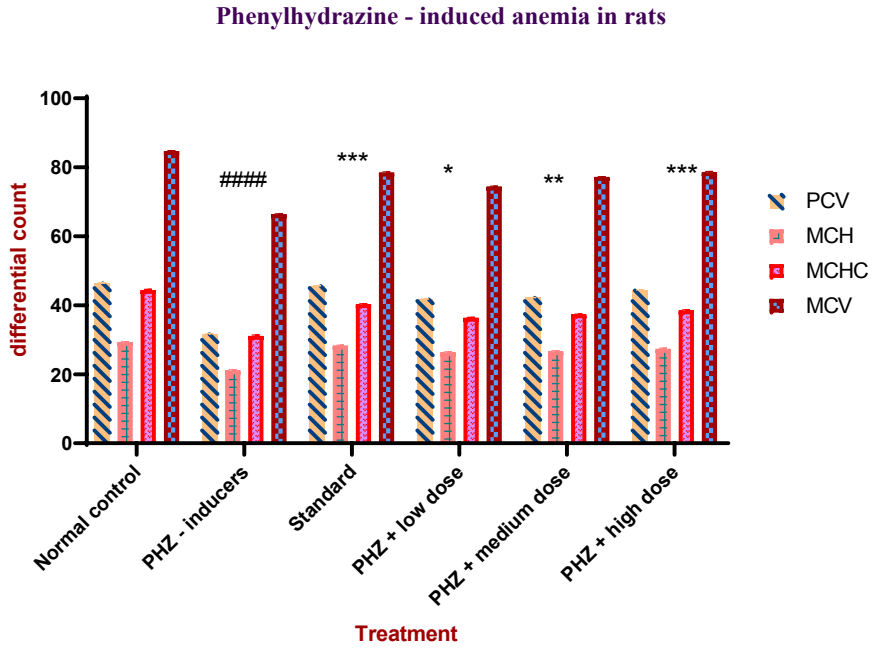

Figure 2. Effect of hydroalcoholic extract of *Moringa oleifera* flower on PCV, MCH, MCHC and MCV

Histological examination

Kidney

The kidney of the control group had intact basement membranes, normal glomeruli, and renal tubules. The renal blood vessels in the anemic group were congested, and the renal tubular epithelium had deteriorated. The groups given *Moringa oleifera* flower extract showed minimal to undetectable glomeruli or tubular degeneration in the kidneys. The renal tubular epithelial vacuolation and mild glomeruli and inter-tubular capillary congestion were seen in the ferrous sulphate group. Effect of *M. oleifera* flower extract might due to the presence of vitamins and iron content in them.

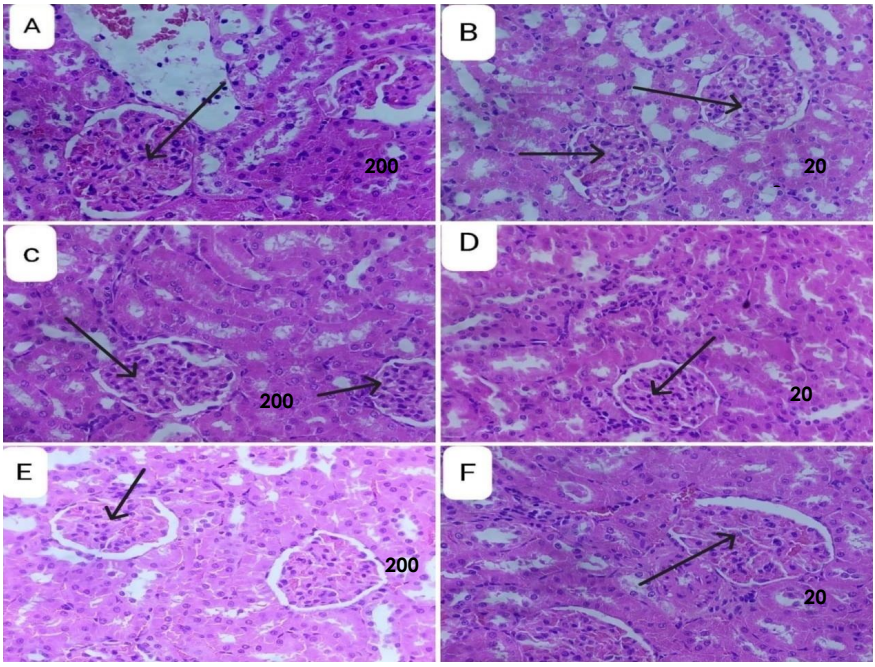

Figure 3. Histopathology of Kidney

The kidney section of histology was stained with hematoxylin and eosin (Scale bar 10X magnification).

(a) Normal control (Vehicle),

(b) Standard (Ferrous sulphate 100 mg/kg (p.o)),

(c) Inducer (Phenylhydrazine 4 0mg/kg (i.p)),

(d) Low dose (200 mg/kg (p.o)),

(e) Middle dose (400 mg/kg (p.o)),

(f) High dose (800 mg/kg (p.o)).

(g) Where A & B showed normal glomeruli and renal tubules with intact basement membranes and C showed congestion of renal blood vessels and degenerative changes of renal tubular epithelium and D, E & F showed improvement in degeneration of renal tubules or glomeruli.

Spleen

The control group's spleen displayed typical white and red pulps with a small number of megakaryocytes and minimal hemosiderin pigment deposition. The anemic group displayed hyperplasia of megakaryocytes, congested sinusoids and blood vessels, and significant hemosiderin deposition. The best outcomes were seen in the *Moringa oleifera* flower extract groups, while the other treated groups showed varying degrees of improvement as represented by a decrease in the number of megakaryocytes and deposition of hemosiderin pigments. Effect of *M. oleifera* flower extract might due to the presence of vitamins and iron content in them.

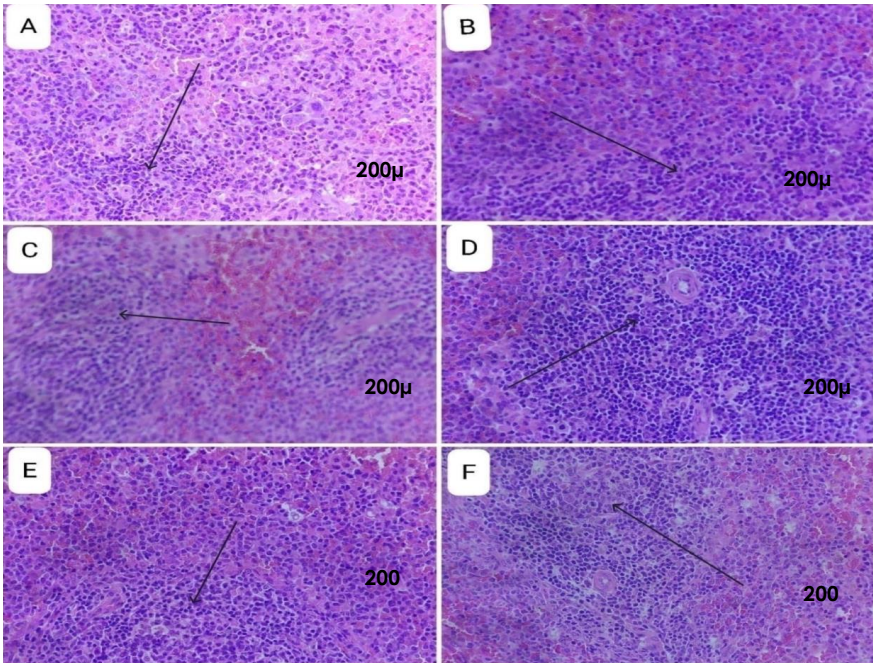

Figure 4. Histopathology of Spleen

The spleen section of histology was stained with hematoxylin and eosin (Scale bar 10X magnification).

(a) Normal control (Vehicle),

(b) Standard (Ferrous sulphate 100 mg/kg (p.o)),

(c) Inducer (Phenylhydrazine 40 mg/kg (i.p)),

(d) Low dose (200 mg/kg (p.o)),

(e) Middle dose (400 mg/kg (p.o)),

(f) High dose (800 mg/kg (p.o)).

Where A & B showed normal white and red pulps with a few numbers of megakaryocytes and deposition of hemosiderin pigments and C showed congestion of sinusoids and blood vessels and D, E & F showed improvement by reduction in the number of megakaryocytes and deposition of hemosiderin pigments.

The phenylhydrazine injections by an intraperitoneal route at the dose of 40 mg/kg for 2 consecutive days induce anemia in rats. Treatment with *Moringa oleifera* flower extract at a dose of 200 mg/kg, 400 mg/kg, and 800 mg/kg respectively, improved RBCs, Hb, HCT, MCV, MCHC, PCV, and MCH levels in phenylhydrazine induced anemic rats dose-dependently. Furthermore, it also reversed pathological changes in the spleen and kidney. The antianemic activity of *Moringa oleifera* flower extract might be due to presence of iron and vitamin C content in them. Further study is required to exactly understand how the phytoconstituents of *Moringa oleifera* flower interact with biochemical pathways in the blood to the presence of its antianemic activity.

STATEMENT OF ETHICS

The animal experimentations were approved by Institutional Animal Ethics Committee (IAEC) of Sri Adichunchanagiri College of Pharmacy, B.G Nagara. (IAEC Approval number: SACCP-IAEC/2022-02/68). Experiments were performed in accordance with the guidelines provided by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), India.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

NM: Design, acquisition of data, analysis of data, drafting of manuscript, statistical analysis. MRK: Design, acquisition of data. SSA: Design, critical review

of manuscript, supervision. AB, KS: Design, plant collection and authentication, BDR, BR: Design, critical review of manuscript, supervision.

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