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COMPARATIVE STUDY OF VARIOUS MARKETED BRANDS OF INDIAN CHYAWANPRASH FOR THEIR ANTI-ANXIETY AND ANTI-OXIDANT POTENTIAL

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Abstract

In the present study an effort has been made to investigate anti anxiety and antioxidant potential of different marketed formulations of Indian chyawanprash. The brand names of different formulations were unrevealed. Behavioral assessments were done by studying loco motor activity, elevated plus maze test, and mirror chamber test in experimental animals. Later animals were sacrificed to estimate different oxidative stress parameters. Results of the study showed significant differences in different marketed formulations in terms of their anti anxiety and antioxidant potential

INTRODUCTION

Ayurveda gives enough emphasis on promotion of health—a concept of strengthening host defenses against different diseases. Rasayana's are a group of non-toxic herbal drug preparations which are used to improve the general health by stimulating the body's own immunity[1]. In ayurveda, Chyawanprash is classified under the category of Rasayana, which aims at maintaining physique, vigor and vitality, while delaying the ageing process[2]. Chyawanprash is a household remedy all over India, and is popular for its nutritional value. Chyawanprash is made in anwala base (Indian gooseberry, *Emblica officinalis*), which is one of the richest sources of vitamin C (ascorbic acid). The rejuvenating and tonic properties of 'Chyawanprash' are considered majorly due to their antioxidant principles, which in turn are due to the presence of phenolic compounds[3]. Experimental and clinical evidence are, however lacking. Recent studies have shown that polyphenols possess potential neuroprotective and antioxidant properties[4].

Currently, chyawanprash is being prepared by several manufacturers, and claim to have its beneficial effect on immunity and strength, physical and mental health, antioxidant activity, increased metabolic activity, detoxifying and cleansing properties. However, there preparations are not fully validated or scientifically tested in terms of modern tools and techniques. Besides, their claims are based on its traditional use. Some of the clinical trial reports do suggest adaptogenic effect of chyawanprash on normal and depressive patients and its antioxidant effect[5].

So based on above findings, the present study was undertaken to explore and compare the various claims of their preparation (anxiolytic effect and antioxidant activity). Such studies are important to substantiate the claims made with regard to the traditional preparations/formulations documented in ancient Ayurvedic texts.

MATERIALS & METHODS

Animals

Laca mice of either sex weighing between 22–30 g bred in Central University Animal House facility were used. The animals were housed under standard laboratory conditions and maintained on natural light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. All the experiments were carried out between 0900 and 1500 hours. The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) of Panjab University and conducted according to the CPCSEA guidelines on the use and care of experimental animals.

Chyawanprash preparations

The following marketed chyawanprash preparations were used in the present study. Brand D (Batch No. PN0964), Brand B (Batch No. 86), Brand Z (Batch No. MCH-9020), Brand Dh (Batch No. BN.CPS.94) and, Brand A (Batch No. JVPF8085SJ). All brands of the Chyawanprash were procured from the local market.

Drugs and Treatment Schedule

Each group consisted of 6 animals. Entire study was conducted in multiple phases. The entire drug treatment group has been shown in Table 1.

Immobilization Stress

The animals were immobilized for 1 hour by taping all four limbs to board after placing them on their backs using zinc oxide hospital tape[6]. Release was affected by unraveling the tape after moistening with acetone in order to minimize pain or discomfort. In unstressed group, the mice were kept in animal cages with soft bedding in the experimental room

Behavioral Assessments

Loco motor Activity

The loco motor activity was assessed by using an actophotometer[7]. (IMCORP, Ambala, India). The motor activity was detected by infrared beams above the floor of the testing area. Animals were placed individually in the activity chamber for a 3-minute acclimatization period before performing actual activity task. Each animal was observed over a period of 5 minutes and was expressed as counts per 5 min.

Elevated plus Maze

Briefly, EPM consists of two open arms (16 cm x 5 cm) and two closed arms (16 cm x 5 cm x 12 cm) extended from a central platform (5 cm x 5 cm), and the maze is elevated to a height of 25 cm from the floor. Experiments were conducted in a quiet room. Each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time (in seconds) taken by the animal to move from the open arm into one of the closed arms with all its four legs. The mouse was allowed to explore the maze for another 2 min and then returned to its home cage. Retention of this learned task (memory) was examined 24 h after the learning trial. Percent retention of memory was calculated by the formula[8].

$$\% \text{ Retention of Memory} = \frac{\text{Transfer latency (day7 - day8)}}{\text{Transfer latency (day7)}} \times 100$$

Mirror Chamber

The mirror chamber consists of a wooden chamber having a mirror chamber enclosed within it. During the 5 min test session, following parameters were noted:

a) Latency to enter the mirror chamber,

b) Total time spent in mirror chamber,

c) Number of entries in mirror chamber.

Animals were placed individually at the distal corner of the mirror chamber at the beginning of the test. An anxiogenic response was defined as decreased number of entries and time spent in the mirror chamber[9].

Dissection and homogenization

On day 8, after behavioral quantification, the animals were sacrificed by cervical dislocation immediately. The whole brains were removed and 10% (w/v) tissue homogenates were prepared in chilled 0.1 M phosphate buffer (*p*H 7.4). The homogenates were centrifuged for 20 min, 4ºC at 15000 rpm. The post mitochondrial supernatants so obtained were used for further enzymatic analysis.

Measurement of oxidative stress parameters

Lipid Peroxidation Assay

The amount of malionaldehyde (MDA) concentration formed was measured by the reaction with thiobarbituric acid at 532 nm using Perkin-Elmer lambda 20 spectrophotometer (Norwalk, CT, USA). The results were expressed as nanomole of

MDA per mg protein using the molar extinction coefficient of chromophore (1.56 × 10 $M^{-1}cm^{-1}$).

Estimation of Reduced Glutathione

A 1.0 ml of the homogenate was precipitated with 1.0 ml of 4% sulfosalicylic acid by keeping the mixture at 4^0C for 1 h and the samples were immediately centrifuged at 1,200×g for 15 min at 4^0C. The assay mixture contains 0.1 ml of supernatant, 2.7 ml of phosphate buffer of pH 8.0 and 0.2 ml of 0.01 M dithiobisnitrobenzoic acid (DTNB). The yellow color developed was read immediately at 412 nm using Perkin-Elmer lambda 20 spectrophotometer (Norwalk, CT, USA). The results were expressed as nanomole GSH per mg protein.

Estimation of Nitrite

Nitrite is the stable end product of nitric oxide (NO) in living system. Accumulation of nitrite was measured in cell free supernatants from brain homogenates by spectrophotometer assay based on Greiss reagent (1% sulphanilamide / 0.1% napthylethylenediamine dihydrochloride / 2.5% phosphoric acid) and incubated at room temperature for 10 min to yield a chromophore, absorbance was read at 543 nm spectrophotometrically. The nitrite concentration was calculated from a standard curve using sodium nitrite as standard and expressed as micro molar nitrite per ml protein content.

Estimation of Catalase

Briefly, the assay mixture consisted of 3 ml of H_2O_2 phosphate buffer and 0.05 ml of supernatant of tissue homogenate (10% w/v), and the change in absorbance was recorded at 240 nm. The results were expressed as micromole H_2O_2 decomposed per mg of protein/min.

Statistical Analysis

Graph Pad Prism (Graph Pad Software, San Diego, CA) was used for all statistical analysis. All values are expressed as mean ± SEM. The data were analyzed using analysis of variance (ANOVA) followed by the Tukey's test. In all the test criteria, statistical significance was $P<0.05$.

RESULTS AND DISCUSSION

Effect of different marketed preparations of chyawanprash on loco motor activity

One hour immobilization stress (IS) significantly reduced loco motor activity. 7 days pretreatment with different brands of chyawanprash and Vit E (100 mg/kg, p.o.) significantly improved the loco motor activity as compared to control group (IS). Lower doses of all the preparations of chyawanprash did not show any significant effect as compared to control group (IS) (Fig. 1). Values are expressed as Mean± SEM. $^{a}P<0.05$ as compared to naïve, $^{b}P<0.05$ as compared to control, $^{c}P<0.05$ as compared to D (100), $^{d}P<0.05$ as compared to B (100), $^{e}P<0.05$ as compared to Z (100), $^{f}P<0.05$ as compared to Dh (100), $^{g}P<0.05$ as compared to A (100). One way ANOVA followed by Tukey's test. IS= immobilization Stress, NS= Non-significant.

2. Effect of different marketed preparations of chyawanprash on anxiety in mirror chamber

One hour immobilization stress significantly caused anxiety like behavior in animals. 7 days pretreatment with different brands of chyawanprash and Vit E (100 mg/kg, p.o.) significantly alleviated the anxiety levels and showed anti anxiety like behavior (shortened latency to enter mirror chamber and increased number of entries in mirror chamber) in mirrored chamber test as compared to the control group (IS). Lower dose of both brand J (100 mg/kg, p.o.) and D (100 mg/kg, p.o.) did not show any significant effect as compared to control group (IS) (Fig.2). Brand D (200 mg/kg, p.o.), B (200 mg/kg, p.o.), Z (200 mg/kg, p.o.), Dh (200 mg/kg, p.o.) and J (200 mg/kg, p.o.) per se treatment did not show any significant effect as compared to naive group (Fig. 2).

In Figure 2 Values are expressed as Mean± SEM. $^{a}P<0.05$ as compared to naïve, $^{b}P<0.05$ as compared to control, $^{c}P<0.05$ as compared to D (100), $^{d}P<0.05$ as compared to B (100), $^{e}P<0.05$ as compared to Z (100), $^{f}P<0.05$ as compared to Dh (100), $^{g}P<0.05$ as compared to A (100). One way ANOVA followed by Tukey's Test. IS= Immobilization stress, NS= Non significant.

3. Effect of different marketed preparations of chyawanprash on Plus maze performance test

One hour immobilization stress significantly impaired cognitive performance (increased transfer latency) and shortened memory retention as compared to naïve group. 7 days pretreatment with different brands of chyawanprash and Vit E (100 mg/kg, p.o.) significantly improved memory performance (decreased transfer latency) and retention time as compared to control group (IS). Lower doses of all the preparations of chyawanprash did not show any significant effect on memory retention as compared to control group (IS) (Fig. 3). Brand D (200 mg/kg, p.o.), B (200 mg/kg, p.o.), Z (200 mg/kg, p.o.), Dh (200 mg/kg, p.o.) and J (200 mg/kg, p.o.) per se treatment did not show any significant effect as compared to naive group.

In Figure 3 Values are expressed as percentage of Mean± SEM. [a]P<0.05 as compared to naïve, [b]P<0.05 as compared to control, [c]P<0.05 as compared to D (100), [d]P<0.05 as compared to B (100), [e]P<0.05 as compared to Z (100), [f]P<0.05 as compared to Dh (100), [g]P<0.05 as compared to A (100). One way ANOVA followed by Tukey's Test. IS= Immobilization stress, NS= Non significant

4. Effect of different marketed preparation of chyawanprash on oxidative stress

One hour acute immobilization stress significantly increased malondialdehyde (MDA) level, nitrite concentration and depletion of reduced glutathione (GSH) levels and catalase activity as compared to naïve group. Pretreatment with different brands of chyawanprash and Vit E (100 mg/kg, p.o.) for seven days alleviated oxidative stress as indicated by decrease in MDA, nitrite concentration and restoration of reduced glutathione and catalase levels) as compared to the control group (IS) and their effect was comparable to Vit E (100 mg/kg, p.o.). Lower dose of both brand J (100 mg/kg, p.o.) and brand Dh Chyawanprash (100 mg/kg, p.o.) did not show any significant effect on the oxidative stress parameters as compared to control group (IS) (Fig.4 and Fig.5). Brand D (200 mg/kg, p.o.), B (200 mg/kg, p.o.), Z (200 mg/kg, p.o.), Dh (200 mg/kg, p.o.) and J (200 mg/kg, p.o.) per se treatment did not show any significant effect as compared to naive group.

In Figure 4 Values are expressed as percentage of Mean± SEM. [a]P<0.05 as compared to naïve, [b]P<0.05 as compared to

control, [c]P<0.05 as compared to D (100), [d]P<0.05 as compared to B (100), [e]P<0.05 as compared to Z (100), [f]P<0.05 as compared to Dh (100), [g]P<0.05 as compared to A (100). One way ANOVA followed by Tukey's test. IS= Immobilization Stress, NS= Non significant.

In Figure 5 Values are expressed as percentage of Mean± SEM. [a]P<0.05 as compared to naïve, [b]P<0.05 as compared to control, [c]P<0.05 as compared to D (100), [d]P<0.05 as compared to B (100), [e]P<0.05 as compared to Z (100), [f]P<0.05 as compared to Dh (100), [g]P<0.05 as compared to A (100). One way ANOVA followed by Tukey's Test. IS= Immobilization Stress, NS= Non significant.

DISCUSSION

Chyawanprash, a household remedy all over India, is popular for its nutritional value and has been relished as a health food since ancient times with the same enthusiasm for the past 4000 years. Chyawanprash had been one of the most respected Ayurvedic health tonics, long before the clinical importance of vitamins, minerals and antioxidants was appreciated[2]. It possesses promising antioxidant, cardio tonic, cholesterol lowering and anti-inflammatory properties[2]. Anwala as well as ascorbic acid has been shown to be effective as memory enhancers in many studies[10].

In the present study acute immobilization stress significantly caused memory impairment, impaired locomotor activity, and anxiety like behavior which was reversed by different brands of chyawanprash pretreatment. The potential health benefits associated with chyawanprash have been partially attributed to its antioxidant property. Different brands of chyawanprash significantly attenuated rise in malondialdehyde, nitrite concentration and restored depleted reduced glutathione and catalase activity as compared to stressed animals (immobilized), thereby proving the antioxidant potential of chyawanprash. Physical immobilization for 1 h lead to development of anxiety (significant decrease in ambulation and rearing in actophotometer and significant reduction in the latency to enter, total time spent in mirrored chamber) in stressed mice as compared to unstressed mice. The results obtained after chyawanprash administration were suggestive of decreased fear or anxiety. One hour

immobilization stress significantly impaired the memory of mice as per results obtained in Elevated plus Maze (EPM). This was evident by increase in the transfer latency of animals post stress. Pretreatment with chyawanprash resulted in significant improvement in memory. Results obtained can be attributed to Nagkesar, Guduchi, nagarmotha, vidarikand, kanwal, agar, ashwagandha, shalparni, prishparni and amalaki which are known to help sharpen the CNS. Several of these ingredients also possess antioxidant and anti-inflammatory properties[2]. There were significant differences observed in different marketed formulations in terms of their anti anxiety and antioxidant potential.

CONCLUSION

All the parameters studied above, together can be used successfully for quality control of different marketed brands of chyawanprash preparation. Although most of chyawanprash preparations were well within the biological activity limit but there is significant difference among chyawanprash preparations of different brands. Hence there is a need to make more stringent quality control parameters in order to reduce variation among chyawanprash preparations.

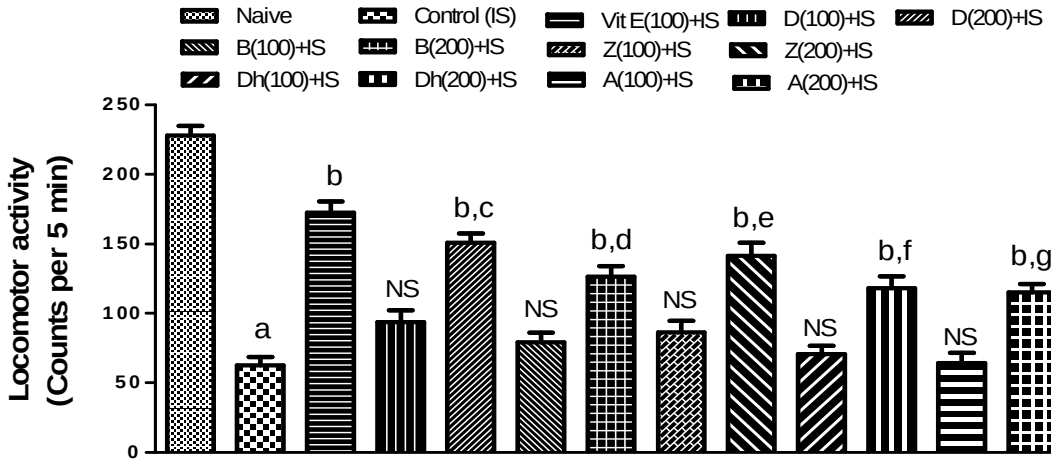

Figure 1 Effect of Chyawanprash preparation on loco motor activity

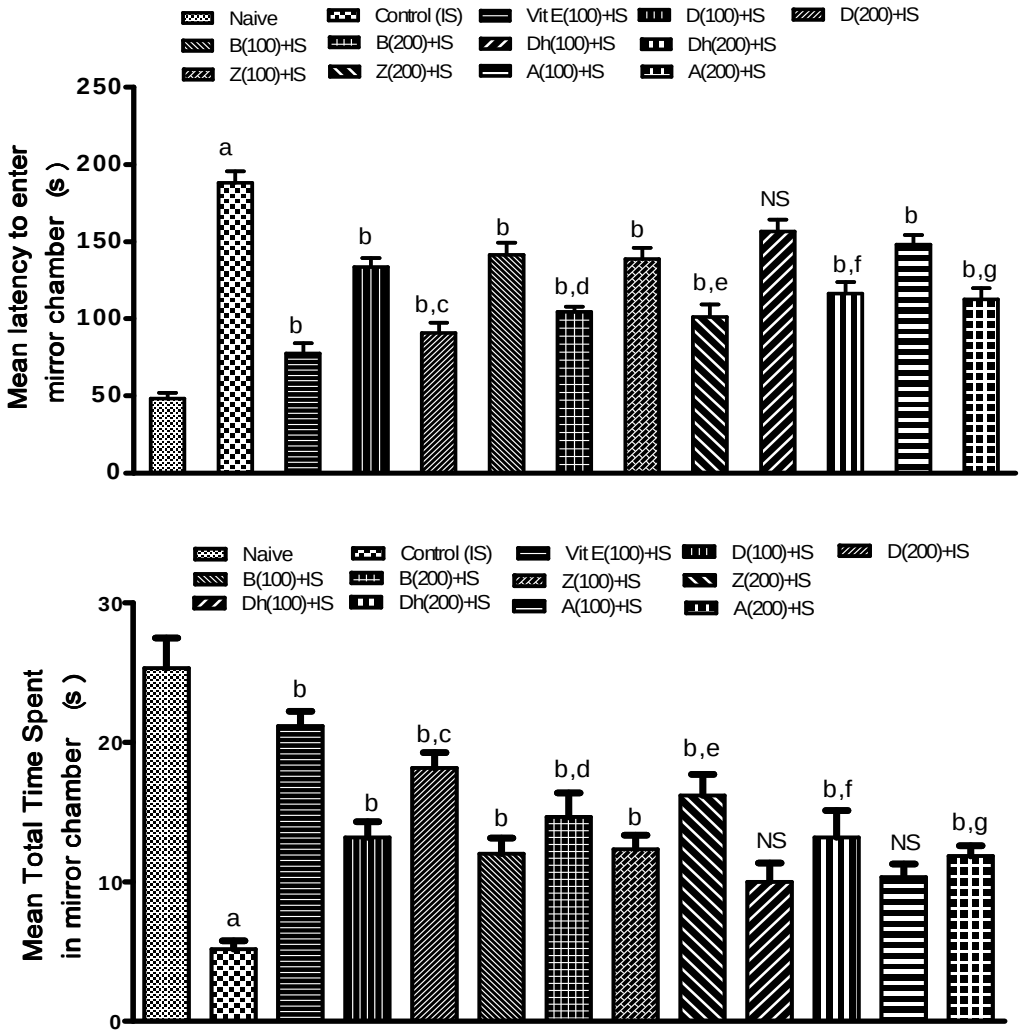

Figure 2 Effect of chyawanprash preparation on anxiety like behavior in mirror chamber (a) Mean first latency to enter mirror chamber (b) Total time spent in mirror chamber.

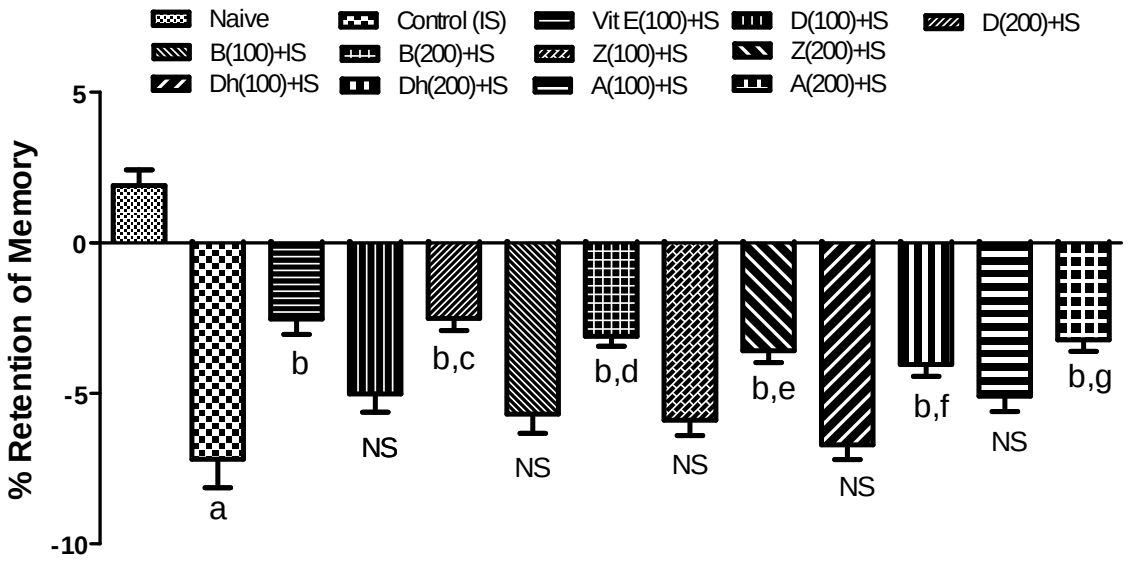

Figure 3 Effect of Chyawanprash preparation on memory

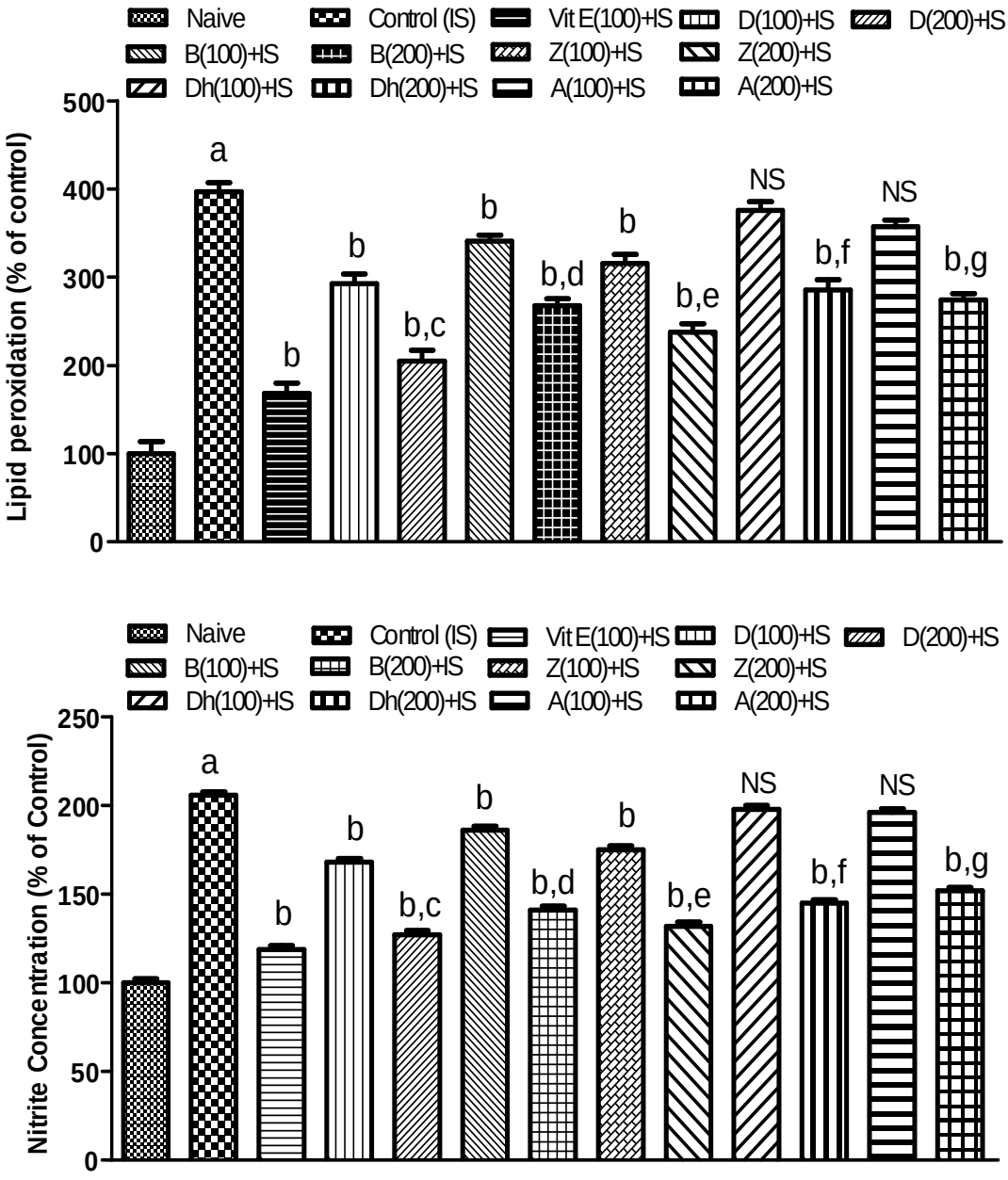

Figure 4 Effect of Chyawanprash preparation on lipid peroxidation and nitrite concentration.

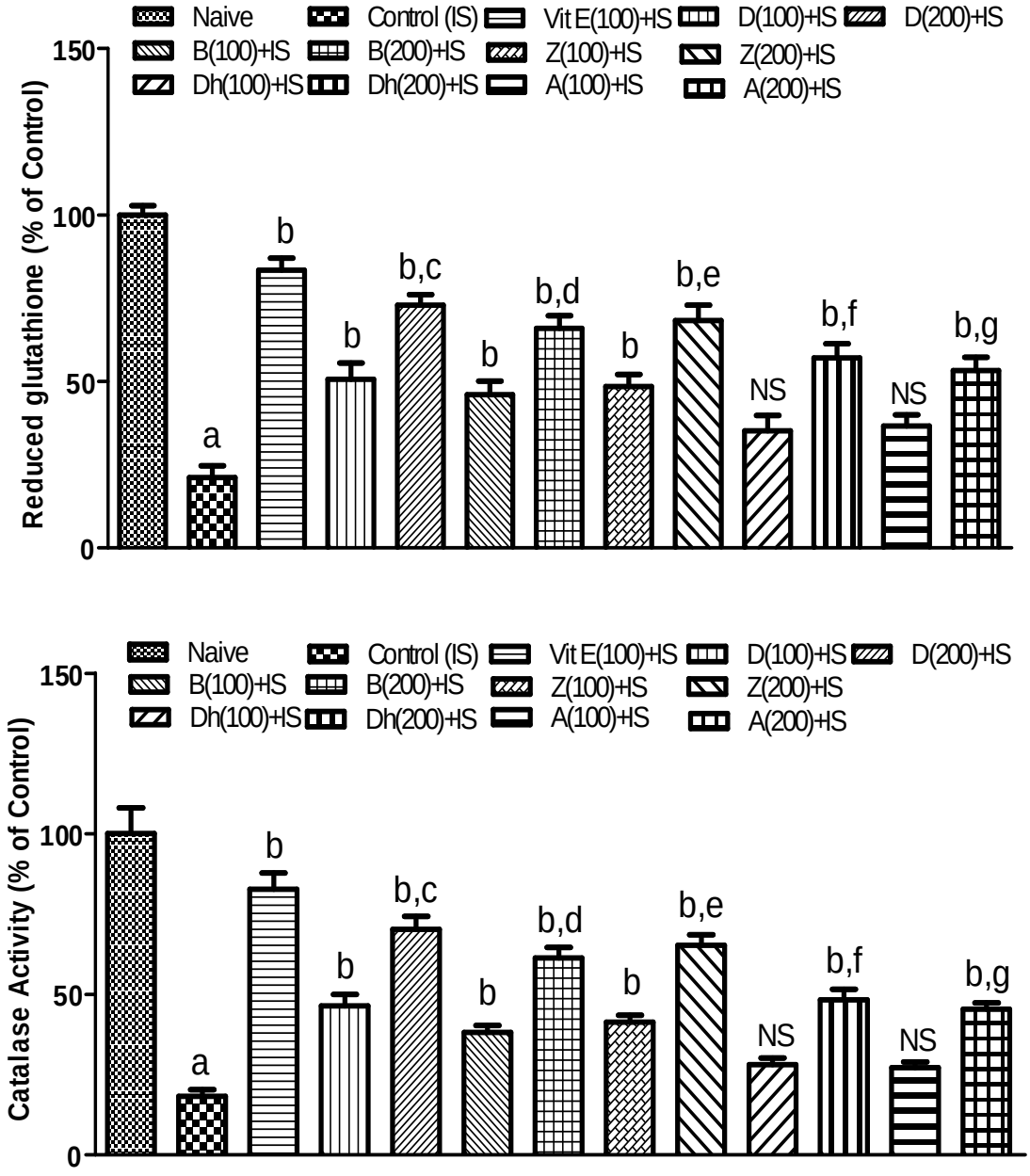

Figure 5 Effect of Chyawanprash preparation on Reduced Glutathione and Catalase levels

Table 1 Drug and treatment schedule of various preparation of chyawanprash

Sr. No.	Groups
1.	Naïve
2.	Control (IS)*
3.	Vit.E (100mg/kg, p.o.)+ IS
4.	D (100 mg/kg, p.o.) + IS
5.	D (200 mg/kg, p.o.) + IS
6.	B (100 mg/kg, p.o.) + IS
7.	B (200 mg/kg, p.o.) + IS
8.	Z (100 mg/kg, p.o.) + IS
9.	Z (200 mg/kg, p.o.) + IS
10.	Dh (100 mg/kg, p.o.)+ IS
11.	Dh (200 mg/kg, p.o.)+ IS
12.	A (100 mg/kg, p.o.)+ IS
13.	A (200 mg/kg, p.o.)+ IS

*IS=Immobilization stress

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