IJBPAS, June, Special Issue, 2023, 12(6): 83-98

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PRECLINICAL CHRONIC ADAPTOGENIC, ANTIDEPRESSANT, ANXIOLYTIC AND ANTISTRESS ACTIVITY OF MULTIVITAMIN-MINERAL-HERBAL TABLETS

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Received 19th Jan. 2023; Revised 20th Feb. 2023; Accepted 18th March 2023; Available online 15th June 2023

https://doi.org/10.31032/IJBPAS/2023/12.6.1014

ABSTRACT

Micronutrient, macronutrient, and vitamin deficiencies can have a negative impact on both mental and physical health, thus it's important to fix them. There will be a further benefit to supplementing nutritional supplements with permitted herbs. Since there is a lack of reliable preclinical evidence about the safety and efficacy of multivitamin-mineral-herbal (MMH) tablets for depression, stress, anxiety, and physical performance, the present study intended out to address that gap. Acute toxicity effect was investigated to ensure the safety of tablets. The open-field test and the elevated plus maze were used to evaluate antianxiety property, while the immobility stress test was performed to measure antistress activity in rats. The study showed that administration of MMH in tablet form was safe up to 2000 mg/kg BW, and that doing so had a notable effect on both physical and mental health. The animal had a significant increase in body weight from week to week. Affirming that health is better after pretreatment. Compared to the control group, the experimental rats swam for significantly longer periods of time. Both the 200 mg/kg and 400 mg/kg MMH tablet groups showed significantly higher locomotor activity than the control group of rats ($p<0.001$), with a mean increase of 271.17±65.98 and 352±90.77, respectively. Time spent in the centre, time spent in the open arm, number of open arm entries all increased significantly, as did the total time spent in the open arm throughout the elevated plus maze test. Rats that were pretreated with MMH showed a substantial reduction in depressive symptoms ($p<0.001$). In an open field test, rats given the maximal dose of the tablet preparation spent less time in the corner block and more time in the centre block, and there was no significant rearing or other negative effects. The number of ulcers, ulcer scores, gastrointestinal volume, and percentage of

ulcer inhibition were all significantly reduced across all dosages of tablet formulations, suggesting considerable antistress effect. We draw the conclusion from these findings that, at an adequate dose, MMH pills are safe, improve physical performance, and assist overcome depression, stress, and anxiety.

Keywords: Animal study, Lotomotor activity, Overall health, Mental health, Physical health, Physical endurance

INTRODUCTION

There has been a global epidemic of anxiety, depression, and stress in recent decades, brought on by modern luxuries, increased demands at work, and a general lack of emotional moderation **[1]**. Anxiety, depression, and stress are at an all-time high, and the recent pandemic COVID-19 and its aftereffects are a major contributing factor **[2-3]**. It's no surprise that this has led to widespread stress and even widespread panic. Anxiety, depression, and stress afflict over 25% of the world's population, according to a report, and this number has increased significantly in the post-COVID era **[4]**.

We see malnutrition across all age groups and geographical locations. It is estimated that more than 3.1 million fatalities a year, and over 45% mortality in the under-five age group, are attributable to poor nutritional conditions. The lack of variety in one's food is thought to have negative effects on both one's physical and mental health **[5]**. Research in India has shown that many school-aged children and adolescents suffer from micronutrient deficiencies that have a negative effect on several mental processes **[6]**. Low intakes and inadequate status of critical micronutrients such as vitamins D, B2, B12, folate, and calcium are cited as causes of malnutrition among Europe's older population, posing a barrier to healthy ageing **[7]**. Reduced immunity, greater risk of illness, diminished physical function, prolonged recoveries, and higher medical costs are all consequences of undernutrition in the elderly **[8]**. One of the major reasons identified for stunting, increased morbidity and mortality, developmental disorders including cognitive, motoric, language, loss of productivity, and increased economic burden for the cost of caring for and treating sick children is malnutrition, especially chronic or recurrent undernutrition during pregnancy **[9]**. Foetal growth restriction, premature birth, low birthweight, and small-for-gestational-age are all signs of maternal complications **[10]**. Anorexia, melancholy, social isolation, and loneliness have all been associated to clinical undernutrition along with immunological dysfunction, anaemia, impaired cognitive function, poor wound healing, delayed surgery recovery, increased hospital readmission rates, and severe morbidity and mortality. Therefore, the nutritional quality of persons of all ages is a

crucial factor in their overall physical and mental health **[11]**.

The United Nations has set a deadline of 2030 to put an end to all types of malnutrition as one of its sustainable development goals. The developing problem of simultaneous appearance of multiple forms of malnutrition, requires multifactorial intervention to address the problem **[12]**, despite significant progress over the last decades to reduce undernutrition. Artificial supplements are losing popularity as people turn to more natural methods of bettering their health **[13]**. These supplements should contain the maximum safe intake of each vitamin, mineral, macronutrient, and amino acid **[14]**. The majority of vitamins can be found in foods like fruits and vegetables, but whether or not these sources provide the recommended dosage and purity remains an open question **[15]**. Therefore, external dietary supplements in the form of pills, capsules, and powder satisfying daily dose requirements are necessary **[16]**. Though there are countless brands of multivitamins and minerals on the market, studies have shown that the majority of them do are not evidence based and little to help people deal with mental health issues like stress, anxiety, or depression or build muscle mass and strength **[17-18]**. Many research have been conducted on multivitamin and multimineral tablets and capsules, and they have been shown to be effective in treating a wide range of mental health issues **[19]**.

This study aims to develop evidence-based tablets with all the necessary daily doses of vitamins, minerals, and micronutrients, plus the FSSAI (Food Safety and Standards Authority of India) -approved doses of extract from ashwagandha, valerian, safed muesli, mucuna pruriens, ginseng, tribulus terrestris, grape seed, lutein, and lycopene, and then test them out on rats and mice for safety and efficacy against depression, anxiety, stress and physical performance.

MATERIALS AND METHODS

Experimental animals-

Ten Swiss albino laca mice and 30 adult Swiss albino wistar male rats were used in the study. Mice weighed between 24 and 28 g, and rats weighed between 153 and 375 g. These animals were kept in a room that was about room temperature and had a relative humidity of about 50%. The animals were given the adequate amount of food and water. All experiments were done according to the CCSEA guidelines.

Acute Toxicity study-

According to OECD guideline 423, the study was conducted using the down-titration method with 3 mice at a time. The study was done with a first oral dose of 2000 mg/kg in a volume of 5 mL/kg. For 24 hours, the animals were watched for signs of toxicity and death. If any of the mice die, the experiment will be done again with three

more mice, and so on. But there were no signs of toxicity or animal deaths. So, the LD_{50} was considered to be 2000 mg/kg when given orally. Using this data, we chose doses of 200, 400, and 500 mg/kg BW for pharmacological testing.

Animal Group-

The animals were divided into five groups of six each. The group 1, 2, 3, 4, and 5 were given normal saline and doses of paroxetine 30 mg/kg, MMH tablet 200, 400, and 500 mg/kg orally for 28 days. Each animal was given test drugs orally 30 minutes before the experiment began. For a study on acute toxicity and pharmacology, MMH tablets were grinded and mixed with acacia and water to make up to 7ml for administration of 2 ml/kg **[20]**.

Elevated Plus maze Test-

The anxiolytic performance was evaluated using this fabricated device. Each rat was put in the middle of the device and watched for ten minutes. Total time in the centre, number of entries in open and closed arm, total time in open and closed arm. Entry of rats were only counted when all the paws were inside the arm **[21]**.

Open field test-

As was done in an earlier study, anxiolytic and exploratory effects of MMH tablets were tested with wooden equipment. The animals were each put in the centre of the equipment for 20 minutes. During that time, their behaviour was observed for things like rearing and grooming themselves, as well as the number of times they went into the black and white squares and how long they stayed there **[22]**.

Locomotor Test-

Using an actophotometer, each rat's movement was measured. Over 10 minutes, the basal activity was written down. Drug was given to all four groups except the control groups 30 minutes before the photoactometer experiment. The counts of animal movement were recorded so that they can be compared **[23]**.

Swimming endurance Test-

The adaptogenic effect of MMH tablets was tested by putting them through a swimming endurance test in a 45 cm x 20 cm cylinder that was filled with water up to a height of 36 cm. The water in the cylinder was kept at a steady 25±2°C. During their moment of inactivity, rats stopped swimming and stopped making the movements required to raise their heads above the water. The duration of swimming was counted from the time of insertion in water to movement of inactivity **[24]**.

Tail Suspension test-

The antidepressant effect on rats was tested by hanging them by their tails for a maximum of 5 minutes, 50 cm above the floor, with an adhesive tape in a front-opening chamber, so they couldn't be stopped by their surroundings. It was made

sure that there were no other animals nearby while the experiment was being done **[25]**.

Immobility stress test-

For 16 hours prior to the stress exercise, the rats were starved. For the purpose of applying stress, rats were confined in an immobile phase for eight hours in a small chamber made of plastic. After that, all of the rats were sacrificed by dislocating their cervical, and their stomachs were removed so that the number of ulcers, ulcer score, gastric volume, and ulcer inhibition could be measured **[26-27]**.

Statistical Analysis-

All results were compiled, collated and analysed by GaphPad prism. All results are presented as mean ± standard deviation. We used paired 't' test for continuous data and unpaired 't' test for discrete data followed by one way ANOVA. The p value $p<0.05$ was considered statistically significant.

RESULT

Acute Toxicity study-

During 24 hours of observation, there were no signs of toxicity, and no mice died. So, the LD50 was assumed to be 2000 mg/kg body weight (po).

Effect of MMH tablets on body weight of rats-

It is seen in **Figure 1** that long term treatment of MMH tablets significantly gained body weight consistently over the period of 28 days. The body weight of normal control rats increased from 176.33±46.2 g to 220.67±21.04 g whereas paroxetine, 200, 400 and 500 mg/kg BW of MMH tablets from 212.5±51.15, 232.5±24.47, 238.17±25.42, 243.67±24.05 to 245±30.23, 242±18.11, 278±40.6 and 252.83±30.28 respectively. Results were highly significant week on week. Indicating that pre-treatment improves the health overall.

Adaptogenic activity of MMH tablets using swimming endurance test-

Figure 2 illustrates that long term i.e. 28 days oral pre-treatment of rats with MMH tablets and paroxetine had increase the duration of swimming significantly from 6.33±2.42 sec in control to 11±5.14, 14.17±6.18, 20.00±9.34, and 25.00±8.05 sec with paroxetine, tablets of 200, 400 and 500 mg/kg BW respectively.

Effect of MMH tablet on locomotor activity-

As demonstrated in **Figure 3** the locomotor activity of MMH tablets at 200 and 400 mg/kg dose significantly ($p<0.001$) increased to 271.17±65.98 and 352±90.77 respectively compared to normal control group of rats 253.5±49.15. However, tablets at 500 mg/kg BW reduced locomotor activity to 210.17±39.63 significantly ($p<0.001$).

Effect of the Multivitamin Tablet on Elevated Plus Maze-

In elevated plus maze test, it was found that in all the parameters such as time spent in

centre, time spent in close arm, time spent in open arm, open arm entry, and close arm entry **(Figure 4)**. Surprisingly, paroxetine treatment group insignificantly increased the time sent in open arm compared to normal control group of rats. Maximum number of entries 36.17±5.53 was spent by 200 mg/kg BW treated group of rats; group 5 shown highest number of entries in closed arm and open arm 6±1.79 and 3.33±1.21 respectively, similarly reduced the time spent in closed arm whereas significantly increased the duration of stay in open arm.

Effect of multivitamin tablets on tail suspension test-

As shown in **Figure 5**, tail suspension method, group 2 i.e. paroxetine treated animals shown highly significant ($p<0.001$) effect in rats and proves that partoxetin treatment for 28 days will help in overcoming from depression. Similar results were recorded with tablets of 400 mg/kg, BW 30.17±10.89 compared to 39.83±13.15 of the control group of rats. Group 3 and group 5 also shown antidepressant effect significantly $p<0.05$ and $p<0.01$ respectively.

Effect of the Multivitamin tablet on Open Field Test-

As given in **Figure 6** the maximum dose of the tablet preparation had shown the best effect with respect to time spent by rats in white block, number of entries in white block, time spent in centre, non-significant rearing and significantly (<0.01) lesser duration of stay was observed in corner block. The effect of MMH tablets 400 mg/kg BW on rats shows that the anti-anxiety effect matches with the effect of paroxetine. Overall the dose dependent, statistically significant anxiolytic effect of MMH tablets are seen in pre-treated rats when exposed to open field. Rats treated with doses 400 mg/kg (group 3) of multivitamin tablets showed a significance value $p<0.001$ in terms of number of entries in white square. At dose of 500 mg/kg BW has level of significance $p<0.001$ in parameters such as time spent in centre, entries in white and time spent in white.

Anti-stress effect of MMH tablets in restrained rats:

As projected in **Figure 7** the number of ulcers were significantly ($p<0.01$, $p<0.001$, $p<0.05$ and $p<0.05$) reduced with paroxetine, MMH tablets 200, 400, 500 mg/kg BW doses. Similarly some differences were also seen with ulcer score in pre-treated rats in which groups 3 and 5 shows significant decrease in stress with p value < 0.001where as in groups 2 and 4 has value of significance $p<0.01$. Highly significant ($p<0.001$) gastric volume reduction was seen in the patients treated with MMH tablets dose dependently. Whereas the paroxetine reduced the gastric volume very significantly ($p<0.01$). The highly significant ($p<0.001$) percentage

inhibition of ulcer was noted with all doses of MMH tablets along with paroxetine.

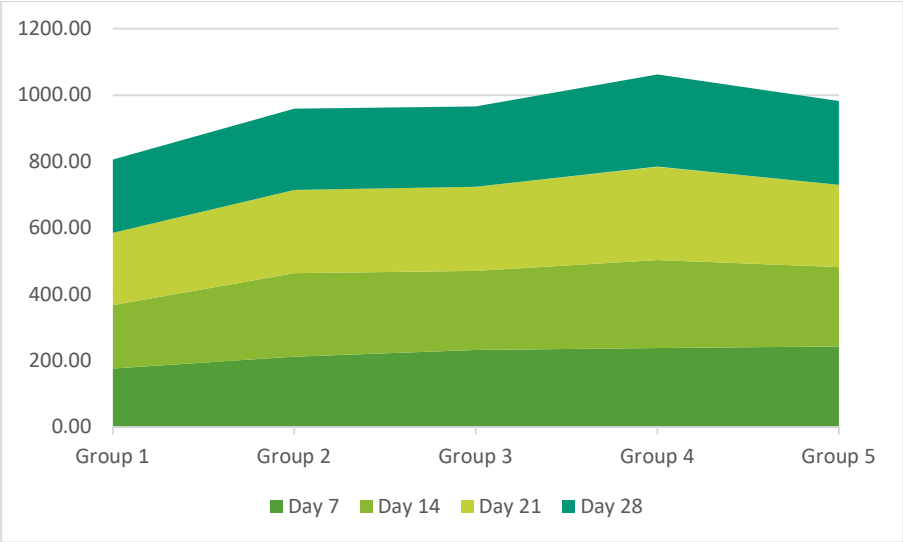

Figure 1: Effect of 28 days MMH tablets pre-treatment on body weight of rats

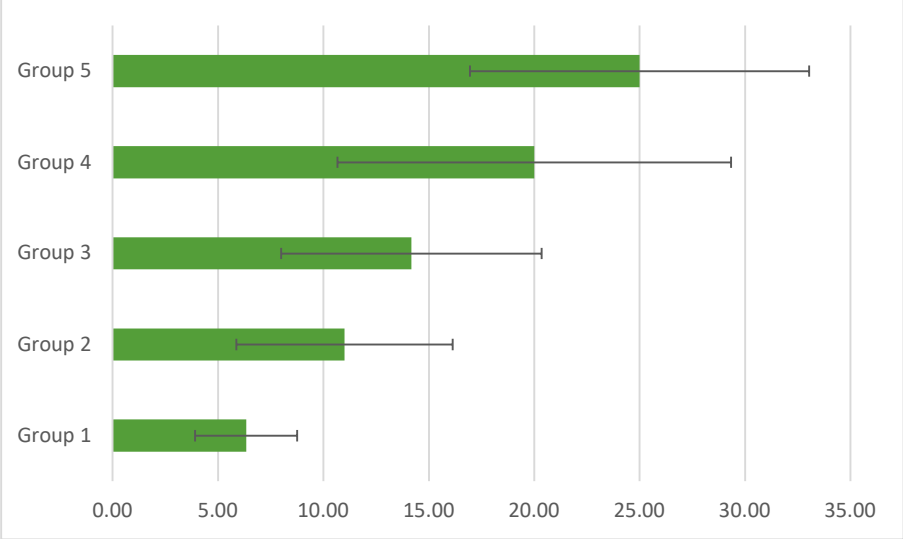

Figure 2: Effect of MMH tablets on swimming performance of rats

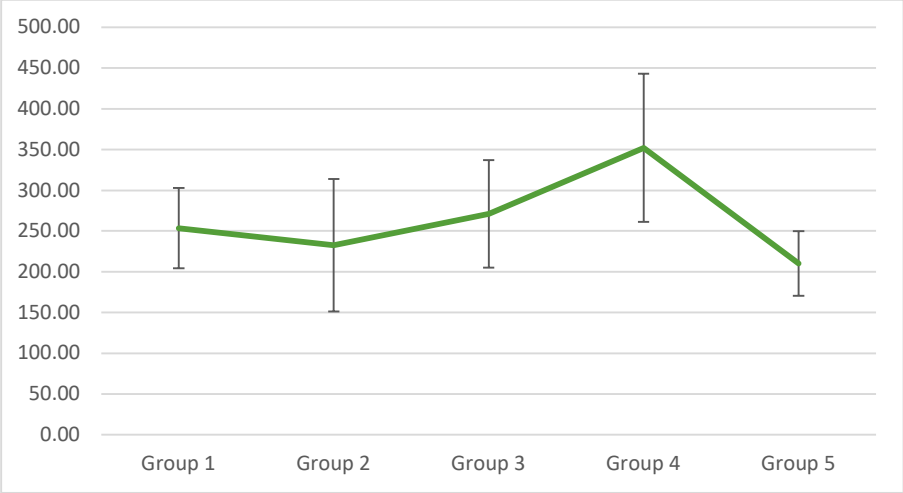

Figure 3: Effect of MMH tablets on locomotor activity of rats using actophotometer

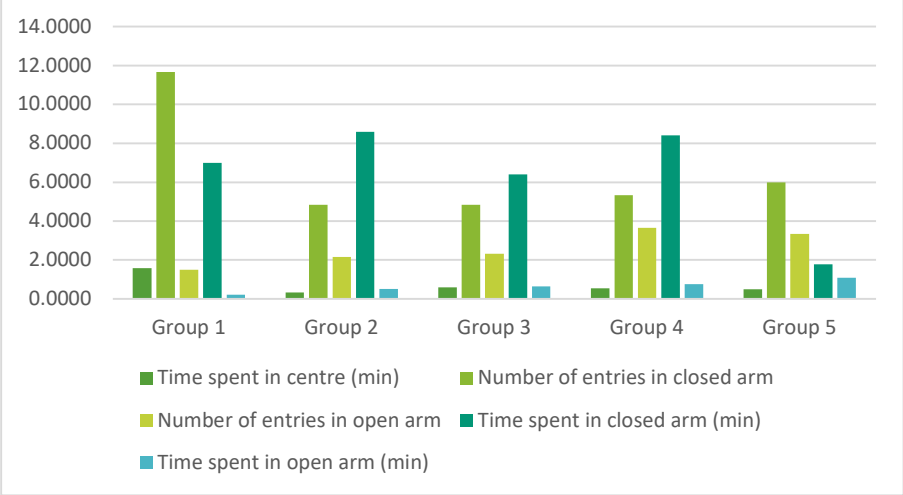

Figure 4: Anxiolytic effect of MMH tablets on rats using elevated plus maze test

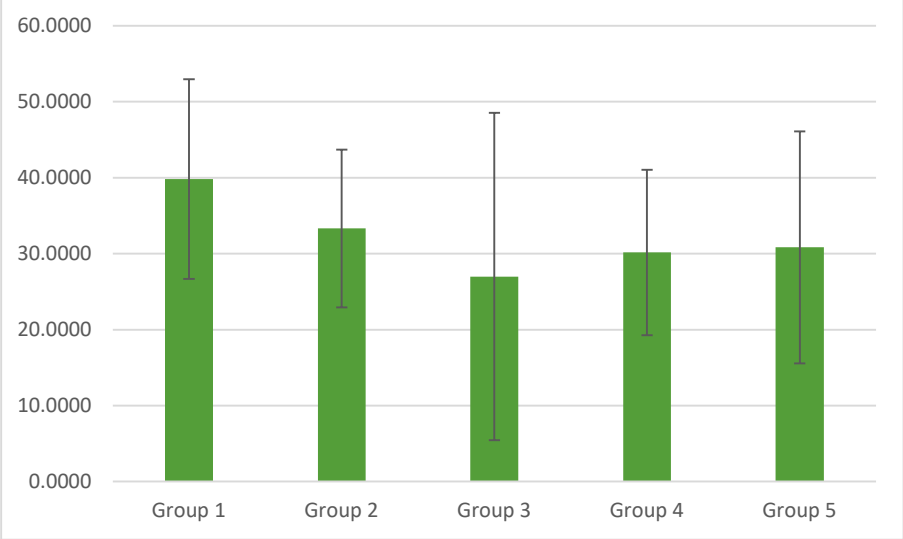

Figure 5: Antidepressant effect of MMH tablets on rats tail suspension

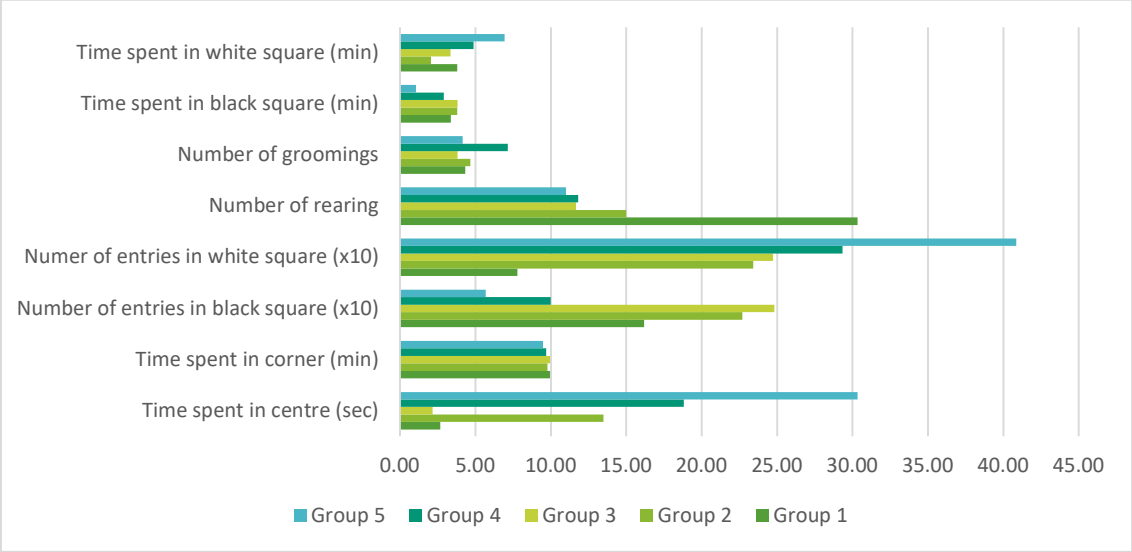

Figure 6: Anxiolytic effect of MMH tablets on rats using open field test

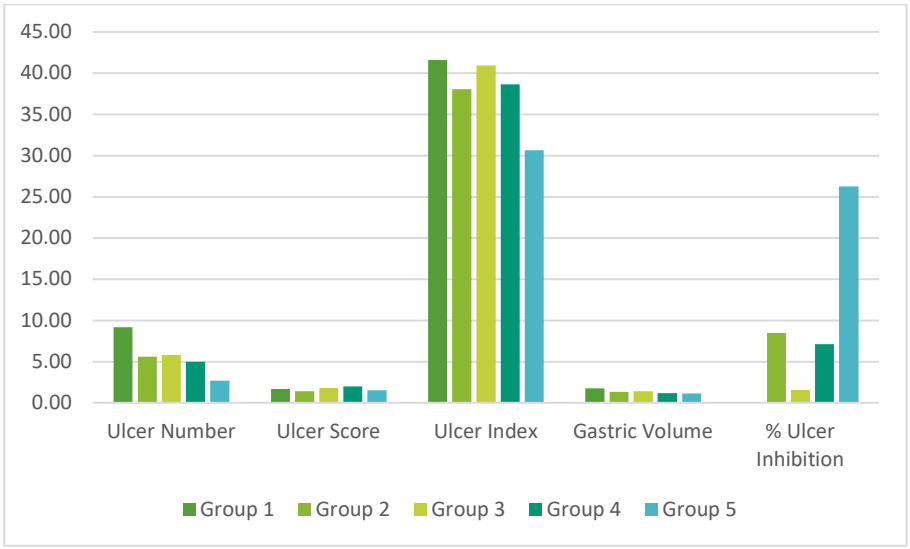

Figure 7: Anti-stress effect of MMH tablets on rats using immobility stress test

DISCUSSION

The MMH tablets are safe and work well to improve physical performance and help people get over depression, anxiety, and stress. The open-field test and the elevated plus maze have been used for a long time to measure antianxiety properties. In rats, the immobility stress test, the swimming endurance test, and the tail suspension test were used to measure antistress, adaptogenic, and antidepressant properties respectively. After a dose of 2000 mg/kg BW of MMH tablets, no sign of toxicity was seen for 24 hours. This shows that tablets are safe at this dose **[28]**. Rats given all doses of MMH tablets for 28 days showed a consistent and significant increase in body weight. This shows that the tablets have

increased levels of vitamins, minerals, micronutrients, macronutrients, and antioxidants from ginseng, grape seed, and lycopene **[29]**.

Overall, the health of each rat got better, as shown by a significant and dose-dependent increase in swimming time **[30]**. Increased physical ability to perform the function by MMH tablets extracts from ashwagandha, valerian, safed muesli, mucuna pruriens, ginseng, tribulus terrestris, grape seed, lutein, and lycopene could be to held responsible **[31]**. It is well known that getting enough vitamins, minerals, micronutrients, and macronutrients will improve thier physical and mental health, which in turn will make user more active **[32]**. Similar results were observed in actophotometer test where highly significant ($p<0.001$) improvement in locomotor activity of rats.

We used the elevated plus maze and the open field tests to find out how well tablet preparations calmed rats down. In the elevated plus maze test, it was found that in all of the parameters, such as time spent in the centre, time spent in the close arm, time spent in the open arm, open arm entry, and close arm entry. These results show that MMH tablets have powerful anti-anxiety effects. This could be because the nutritional benefits of the tablets has gone up and they contain extracts of ashwagandha, valerian, safed muesli, mucuna pruriens, ginseng, tribulus terrestris, grape seed, lutein, and lycopene **[33]**. The results of the tests in the open field backed up the fact that this tablet preparation helped reduce anxiety. We observed that when rats were treated before the experiment, they spent more time in the white block, came more frequently in white block, spent more time in the centre, didn't spend much time rearing, and spent significantly ($p<0.01$) and less time in the corner block. These results back up earlier reports that ashwagandha, valerian, safed muesli, mucuna pruriens, ginseng, tribulus terrestris, grape seed, lutein, and lycopene helped people with anxiety **[34]**.

The tail suspension method was used to study the antidepressant effects of MMH tablet preparations. When their tails were suspended, most rats who had taken MMH tablets before did so faster than animals in the control group. This backs up the idea that ashwagandha, valerian, safed muesli, mucuna pruriens, ginseng, tribulus terrestris, grape seed, lutein, and lycopene should be taken as supplements **[35]**. MMH tablet can help people get over depression in a satisfying way. In a similar way, antistress activity was shown in rats that were restrained by showing that ulcer score, ulcer number, gastric volume, and ulcer percent inhibition were all lower. Previous research suggested that the active ingredients in ashwagandha, valerian, safed muesli, mucuna pruriens, ginseng, tribulus terrestris,

grape seed, lutein, and lycopene were present. These results back up that theory that multivitamin, mineral, nutritional and beneficial herbs can help people successfully deal with stress **[36]**.

CONCLUSION

From this study, we can conclude that providing rats multivitamins, minerals, and micronutrients improved their overall health, and the doses chosen for this study are safe for both the short-term and long-term treatment phases. Adding ashwagandha, valerian, safed muesli, mucuna pruriens, ginseng, tribulus terrestris, grape seed, lutein, and lycopene to these nutritional agents helped rats deal with stress, anxiety, and depression. They also changed rats' physical strength. More research at the cellular and molecular level can help figure out how the added herbal supplements work.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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