WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 19, 220-229. Research Article ISSN 2277–7105

GASTRIC EMPTYING AND PSYCHOPHARMACOLOGICAL EFFECTS OF NABAYAS LOUHA ON ANIMALS

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Article Received on
14 Oct. 2018,

Revised on 04 Nov. 2018,
Accepted on 25 Nov. 2018

DOI: 10.20959/wjpr201819-13789

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ABSTRACT

Objective: The research work was carried out to characterize the gastric emptying and psychopharmacological effects of ayurvedic iron preparation "Nabayas Louha" in swiss albino mice. **Methods:** Nabayas Louha was administered by oral route to the animal model (*Swiss albino*) and the effects were determined by comparing with respect to control group which were treated with normal saline water. Nabayas Louha was used at a dose of 100 mg/kg to examine its gastric emptying effects. To investigate the psychopharmacological effects of Nabayas Louha climbing out, staircase and forced induce swim test had been carried out where 100, 200 and 400 mg/kg body weight dose (by mouth) was used for climbing out test and 100 mg/kg body weight dose (by mouth) was used for staircase and forced induce swim test. **Results:** Oral administration of Nabayas Louha was found to increase the % of gastrointestinal emptying and one of the 2nd hour results was a significant ($p<0.05$). In the forced swimming test two results were noticeable ($p<0.099$) and one result was very significant ($p<0.01$) for depressant effect of Nabayas Louha. **Conclusions:** It can be said that Nabayas Louha has significant gastric emptying effects and may have depressant activities but probably don't have any effect like anxiolytic activity. To establish these effect further studies may be required.

KEYWORDS: Nabayas Louha, Gastric emptying, Psychopharmacological, Anxiolytic, Depressant, Swiss albino mice.

INTRODUCTION

Ayurveda, the science of life, emphasizes prevention of disease, rejuvenation of our body systems and extension of life span through lifestyle interventions and natural therapies. It was placed in written form over 5,000 years ago in India.[1] ayurvedic is a traditional medicine native to the Indian subcontinent and practiced in other parts of the world as a form of alternative medicine. Laboratory and clinical studies on ayurvedic herbal preparations have shown a range of potentially beneficial effects for preventing and treating certain cancers, treating infectious disease, treating diabetes, promoting health and treating aging.[2] Mechanisms underlying these effects may include free-radical scavenging effects, immune system modulation, brain neurotransmitter modulation and hormonal effects.[3] Bangladesh is rich in biodiversity and has an abundant resource of herbs, plants and trees.

Based on its geographical and seasonal benefits, the country is a potential practitioner of Ayurveda. Indeed, Bangladesh is considered as the home of medicinal plants which have occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives of Bangladesh. This is of tremendous contemporary relevance because it can on one hand ensure health security to millions of people and on the other hand it can provide new and safe herbal drugs to the entire world. Relative to allopathic treatment, ayurvedic treatment is easy to access at affordable prices and sometimes is the only source of health care available to the poor. Huge number of the population is below the poverty line and for most people the only way to seek medication at an economical rate is by seeking ayurvedic treatment.[4] However, in light of the successful benefits of ayurvedic medicine the demand for such preparations is increasing in both developing and developed countries.[5]

Nabayas Louha is included (page 231-232) in the Bangladesh National Formulary of Ayurvedic Medicine 1992. Nabayas Louha is an ayurvedic preparation and lots of people use this drug in Bangladesh and India. It has been used for anaemia, heart disease, dermatological diseases, haemorrhoids, jaundice.[6] The objective of this current research was set to investigate the gastric emptying and psychopharmacological effects of Nabayas Louha on animal models.

MATERIALS AND METHODS

Collection of Drug

The drug, Nabayas Louha (Batch no 084) was collected from "Sree Kundeswari Aushadhalaya Ltd", Chittagong, Bangladesh.

Preparation of drug solution: For the experiments, the tablets of Nabayas Louha were crushed into powder and made into a solution with distilled water. Then the solution was administered at a volume such that it would permit optimal dosage accuracy without contributing much to the total increase in the body fluid.

Table. 1: The formulation of Nabayas Louha.

Constituents	Fraction
Sunthi (Rz.)	1 part
Marica (Fr.)	1 part
Pippali (Fr.)	1 part
Haritaki (Fr.P.)	1 part
Bibhitaka (Fr.P.)	1 part
Amalaki (Fr.P.)	1 part
Musta (musta) (Rz.)	1 part
Vidanga (Fr.)	1 part
Citraka (Rt.)	1 part
Ayoraja (Lauha bhasma)	9 part

Chemicals and apparatus: Methanol and normal saline (0.9% NaCl) were collected from East West University laboratory, Dhaka, Bangladesh. To perform gastric emptying test plastic cases, electronic balance (Shimadzu, Japan), scissor, forceps were purchased. For climbing out test, a cage with dimension of 60 X 50 X 30 cm and having dark walls was collected. Cotton and staircase (the apparatus consists of a white PVC enclosure with a five-step staircase) were arranged to conduct the staircase test. To perform forced induce swim test, a large box made by glass was collected. Stopwatch and feeding needle were taken to maintain observation time accurately and administer experimental drug perfectly.

Experimental Animals: 140 male swiss albino mice (20-40 gm body weight), bred in the animal house of the Department of Pharmacy, Jahangirnagar University, Bangladesh, were used for the gastric emptying and psychopharmacological experiments. The animals were provided with standard laboratory food and tap water, maintained at natural day night cycle and kept under room temperature of (24 ± 2)°C, relative humidity of 60% - 70%.[7] They were fed with mouse chow which was prepared according to the formula developed at Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. All of the experimental processes related to animals were directed in accordance to ethical guidelines of the Faculty of Biological Science, Jahangirnagar University which were also approved by the Institutional Ethical Committee of Faculty of Biological Science, Jahangirnagar University.

Biological activities

Gastric emptying test[8]: 40 Swiss albino male mice were fasted for 18 hours prior to the experiment where 20 were randomly chosen as the test drug group and the remaining 20 as the control group. Fasted animals had free access to water and pre-weighed solid food (solid: water ratio being 60:40) for a period of 1 hour. At the end of the 1 hour period, the remaining food was weighed and adjustment for spillage was taken into consideration. The difference between the initial and final food weights gives the total food intake. Immediately after the 1 hour feeding period, test drug was orally administered to the mice of drug group at 100 mg/kg (1x doses) while their control group counterparts were fed saline water. The percentage of the gastric emptying of the ingested food was assessed 2 hours after the administration of the drug. The mice were sacrificed by cervical dislocation and the stomach removed by cutting off the cardiac and pyloric ends. The stomach was weighed in an electronic balance and opened; the gastric content was washed with tap water and the remaining gastric wall was blotted dry and weighed.

The gastric content was calculated as the difference between the total weight of the stomach with contents and the weight of the gastric wall after the contents were washed out. Percent gastric emptying (% GE) was calculated as.

$$\% \text{ of GE} = 1 - \frac{\text{Gastric}}{\text{Total Food}} \times 100$$

Psychopharmacological effects

Climbing out, stair case and forced induce swimming test were done to evaluate psychopharmacological effects of Nabayas Louha.

Climbing out test[9]

To perform the test 60 swiss albino mice (30 mice for control and 30 mice for drug) were taken. The animals were put in climbing out cage. Animals were supplied with a ladder and the time taken to climbs out of the cage was recorded for a maximum period of 10 minutes. The experiment was carried out with a dose of 100, 200 and 400 mg/kg body weight.

The observation was conducted at 30, 60, 120 and 240 minutes after oral administration of each separate dose of drug and was compared with control animal administered with normal saline.

Staircase test[10]

20 swiss albino mice were taken and divided into two groups consisting of 10 mice in each group. The experimental box containing mice, methanol and staircase, was placed in a room with constant lighting, isolated from external noise and thermostatically controlled. All the mice for a single experiment where placed at the same height in the animal house. They were transferred to the laboratory at least 1 hour before the start of the test. Each animal was used only once. The animal was placed singly on the floor of the box with its back to the staircase. The number of steps climbed and the number of rears were counted over a 3 minutes period. A step was considered to be climbed only if the mouse had placed all four paws on the step. The number of steps descended was not taken into account, in order to simplify the observations. After each animal had been tested, the box was rapidly cleaned to eliminate any olfactory cue which might modify the next animal's behavior. Experimental drugs were administered orally (P.O.) at a dose of 100 mg/kg 60 minutes before the test to groups of 10 mice. In each experiment, a control group received only normal saline water. The treatments were randomized and the observer was unaware of the treatment given to each group (blind method). All studies were carried out between 8 a.m. and 5 p.m.

Forced induce swim test[11]

The most widely utilized animal model of antidepressant action is the forced swim test. For performing the test, 20 swiss albino mice were taken where 10 mice for control and 10 mice for drug.

Mice were exposed to a 15 minutes pre-swim 24 hour before a 5 minutes test exposure in 15–18 cm of 25^{o} C water. Following an initial period in which the mice produces escape-directed behaviors, it will adopt an immobile posture, which is believed to reflect either a failure to persist with escape-directed behavior or a passive behavior to cease active forms of coping to the stressful stimuli. A wide range of clinically effective antidepressants have been shown to increase the time that the rat spends in active escape behaviors.

Statistical Analysis

Data were presented as Mean ± SEM (standard error of the mean). Unpaired "t" tests were done for statistical significance tests. SPSS (Statistical Package for Social Science) for WINDOWS™ (Version 14) was applied for the analysis of data. $p \leq 0.05$ was taken to be the level of significance, $p \leq 0.01$ was taken to be the level of highly significance, $p \leq 0.001$ was taken to be the level of very highly significance.[12] P-value determines the appropriateness of

rejecting the null hypothesis in a hypothesis test. P-values range from 0 to 1. Smaller the p-value, the smaller the probability that rejecting the null hypothesis is a mistake.

RESULTS

Gastric emptying test

Table. 2: Effect of Nabayas Louha at a dose of 100 mg/kg on gastric emptying test after 2nd hour study.

Group		% GE (Mean ± SEM)
Control (n=10)		86.68 ± 2.448
Nabayas Louha (n=10)		94.22 ± 1.364
t/p		**-2.689 / 0 .018***
95% confidence interval	Lower	-13.540
	Upper	-1.527

The difference in % of gastric emptying between the Nabayas Louha treated group and the control group after 2nd hour = (94.22-86.68) = 7.54% increase.

Table. 3: Effect of Nabayas Louha at a dose of 100 mg/kg on gastric emptying test after 4th hour study.

Group		% GE (Mean ± SEM)
Control (n=10)		92.86 ± 1.055
Nabayas Louha (n=10)		91.31 ± 1.468
t/p		0.856 / 0.403
95% confidence interval	Lower	-2.251
	Upper	5.345

The difference in % of gastric emptying between the Nabayas Louha treated group and the control group after 4th hour = (91.31- 92.86) = -1.55% decrease.

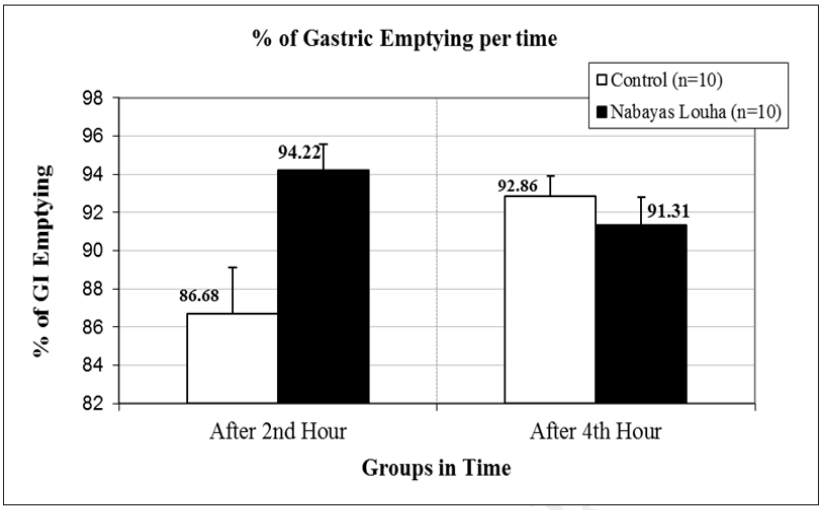

Figure. 1: Overall effect of Nabayas Louha (100mg/kg) on Gastric emptying test from 2nd hour and 4th hour study.

Psychopharmacological effects

Climbing out test

Table. 4: The effect of Nabayas Louha at a dose of 100 mg/kg in the Climbing out test.

Group		Min 0	Min 30	Min 60	Min 120	Min 180	Min 240
Control (n=10)		137.40± 27.741	167.50± 74.074	84.20± 39.197	72.20± 47.549	39.24± 23.658	111.60± 59.046
Nabayas Louha (n=10)		135.60± 59.476	130.10± 63.959	120.70± 61.357	137.40± 53.278	83.00± 43.789	31.20± 18.772
t/p		0.027/ 0.978	0.382/ 0.707	-0.806	-2.448	-2.109	1.298/ 0.211
95% confidence interval	Lower	-136.078	-168.208	-189.465	-215.228	-151.466	-56.276
	Upper	139.678	243.008	116.465	84.828	63.942	217.076

Table. 5: The effect of Nabayas Louha at a dose of 200 mg/kg in the Climbing out test.

Group		Min 0	Min 30	Min 60	Min 120	Min180	Min 240
Control (n=10)		106.20±53.742	93.00±49.232	24.00±16.199	34.00±23.104	5.50± 5.5	89.30±61.652
Nabayas Louha (n=10)		149.10±66.305	38.80±38.8	45.40±24.769	0.00±0	68.20±49.904	63.80±46.298
t/p		-0.809	0.865/0.399	-1.509	1.472/0.175	-5.139	0.331/0.745
95% confidence interval	Lower	-222.213	-77.493	-83.579	-18.264	-175.865	-136.483
	Upper	136.413	185.893	40.779	86.264	50.465	187.483

Table. 6: Effect of Nabayas Louha at a dose of 400 mg/kg in the Climbing out test.

Group		Min 0	Min 30	Min 60	Min 120	Min 180	Min 240
Control (n=10)		166.10± 63.155	105.30± 49.888	96.20± 34.106	57.90± 27.450	110.90± 57.821	0.60± 0.6
Nabayas Louha (n=10)		157.70± 45.156	177.00± 43.696	135.70± 66.324	92.50± 62.219	58.70± 58.7	0.0±0
t/p		0.108/ 0.915	-3.677	-0.879	-0.844	0.634/ 0.534	1.0/ 0.343
95% confidence interval	Lower	-154.711	-211.030	-196.186	-177.474	-120.906	-0.757
	Upper	171.511	67.630	117.186	108.274	225.306	1.957

Stair case test

Table. 7: The effect of Nabayas Louha at a dose of 100 mg/kg in the Stair case test on male mice.

Group		Steps climbed out (Locomotor)	Number of rearing
Control (n=10)		25.80±2.719	11.30±1.174
Nabayas Louha (n=10)		24.80±2.265	12.10±1.716
t/p		0.283/0.781	-0.546
95% confidence interval	Lower	-6.435	-5.168
	Upper	8.435	3.568

Forced Induced Swimming Test

Table. 8: The effect of Nabayas Louha at a dose of 100 mg/kg in the swimming test after 2 hours.

Group		1st min	2nd min	3 to 6 min
Control (n=10)		10.10±2.243	25.00±3.339	138.80±12.206
Nabayas Louha (n=10)		15.89±2.40	32.56±2.102	194.00±8.651
t/p		-18.365	-22.169	**-3.613/0.002****
95% confidence interval	Lower	-12.715	-16.220	-87.435
	Upper	1.137	1.109	-22.965

** (≤ 0.01) = Highly Significant

Table. 9: The effect of Nabayas Louha at a dose of 100 mg/kg in the Swimming Test after 24 hours.

Group		1st min	2nd min	3 to 6 Min
Control (n=10)		7.50±3.585	18.50±3.967	161.30±11.149
Nabayas Louha (n=10)		13.78±3.570	24.0±2.693	174.33±11.736
t/p		-5.309	-4.032	-1.863
95% confidence interval	Lower	-16.985	-15.852	-47.216
	Upper	4.430	4.852	21.149

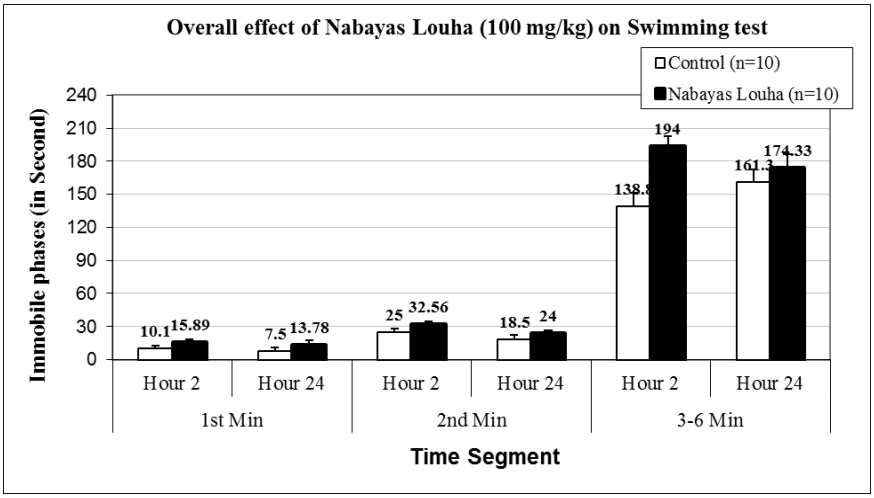

Figure. 2: Overall effect of Nabayas Louha at a dose of 100 mg/Kg in the Swimming test after 2nd and 24 hours.

DISCUSSION

In gastric emptying test, Nabayas Louha treated male mice at dose 100 mg/kg exerted increase in gastric emptying at 2nd hour compare to respective control group which is statically significant (p=0.018*). But in case of 4th hour Nabayas Louha treated mice exerted negligible decrease in gastric emptying compare to control group. But this result was not statistically significant (p<0.05).

In climbing out test, Nabayas Louha treated mice at a dose levels of 100, 200 and 400 mg /kg exerted overall increase in hole cross activity. At a dose of 100 mg/kg exerted increase in time taken to come out of the cage in minute 60, minute 120 and minute 180. The exceptions were in minute 30 and in minute 240, time required for the drug treated mice to come out the cage was decreased than the control group. At 200 mg/kg dose exerted decrease in time taken to come out of the cage in minute 30, minute 120 and minute 240. The exceptions were in minute 60 and in minute 180, time required for the drug treated mice to come out the cage was increased than the control group. At a dose of 400 mg/kg exerted increase in time taken to come out of the cage in minute 30, minute 60, minute 120 and minute 240. The exceptions was in minute 180, time required for the drug treated mice to come out the cage was decreased than the control group. But no results were statically significant in three different doses.

In stair case test, at 100 mg/kg dose, Nabayas Louha decreased (p=0.781) the number of steps and increased (p=0.705) the number of rearing in comparison to the respective control group but none of these are statically significant. Therefore, Nabayas Louha probably don't have any effect like anxiolytic activity.

In forced induced swimming test, Nabayas Louha treated mice, initially in 2nd hour, at 100 mg/kg dose treated group showed an increase in immobile phase in swimming test in 1st minute, 2nd minute and 3-6 minutes. In 1^{st} minute (p=0.096) and 2^{nd} minute (p=0.083) the increase in immobile phase is statistically noticeable and 3-6 minutes after 2 hour the increase of immobile phase was statistically highly significant (p=0.002**). At 24 hour, 100 mg/kg dose treated group showed an increase in immobile phase in swimming test in 1^{st} minute (p=0.233), 2^{nd} minute (p=0.278) and 3-6 minutes (p=0.432). But no results were statistically significant. Thus Nabayas Louha may have depressant activity.

CONCLUSION

In this research work it was tried to characterize the gastric emptying and psychopharmacological effects of ayurvedic iron preparation Nabayas Louha on animal model (swiss albino mice). In this report it was found that iron preparation Nabayas Louha increase gastrointestinal emptying rate and may have depression activity on animal trial subjected on swiss albino mice without any major side effect. In addition it should rather be emphasized that to establish these findings there is a need for a comprehensive study and large scale clinical trial to ensure the safety of the general patients/users of the country.

ACKNOWLEDGEMENTS

We greatly acknowledge the genial help of the authority of Department of Pharmacy, Jahangirnagar University for providing experimental animals. Thanks is due to Md. Shofiq (Lab assistant, Department of Pharmacy, Jahangirnagar University) for his technical support.

REFERENCES

1. Mishra LC, Singh BB; Dagenais S. Ayurveda—a historic perspective and principles of traditional health care system in India. Altern Ther Health Med., 2001; 7(2): 36-42.
2. Information regarding traditional Ayurveda alternative medicine and complementary therapies for health and illness. https://www.disabled-world.com/medical/alternative/ayurveda.
3. Chopra, AS. Medicine Across Cultures edited by Selin, Helaine & Shapiro, H. United States of America. Kluwer Academic Publishers, 2003; 75-83.
4. Poverty in Bangladesh. https://www.adb.org/countries/bangladesh/poverty.
5. Chikezie PC, Ojiako OA. Herbal Medicine: Yesterday, Today and Tomorrow. Altern Integr Med., 2015; 4(3): 1-5.
6. Navayas lauh Ingredients, Benefits and Directions to Use. https://www.bimbima.com/ayurveda/navayas-lauh-ingredients-benefits-and-directions-to-use/1232/.
7. Jaasma L, Hendriksen C. A review of the housing conditions for laboratory animals.
8. Osinski MA, Seifert TR, Cox BF, Gintant GA. An improved method of evaluation of drug-evoked changes in gastric emptying in mice. J Pharmacol Toxicol Methods. Mar-Apr; 47(2): 115-20. [PubMed].
9. JMA Hannan, Masum Shahriar, M Naimul Islam, Mafruhi Sattar, Sabera Haque, MSK Choudhuri. Neuropharmacological study of some Ayurvedic medicinal plants. Orient Pharm Exp Med., 2003; 3(1): 8-17.
10. Simiand J, Keane PE, Morre M. The staircase test in mice: A simple and efficient procedure for primary screening of anxiolytic agents. Psychopharrnacology, 1984; 84: 48-53.
11. Can, A., Dao, D.T., Arad, M., Terrillion, C.E., Piantadosi, S.C., Gould, T.D. The Mouse Forced Swim Test. J. Vis. Exp. 2012; 59: e3638, DOI: 10.3791/3638.
12. P Values. https://www.statsdirect.com/help/Default.htm#basics/p_values.htm.