Experimental Evaluation of Hiṅgvādi Ghṛta in Behavioral Despair Using Animal Models

Abstract

Context: Depression, a sustained mood disorder caused by selective diminution of specialized cells in brain is increasing at an alarming rate. It will be the second largest morbid illness by next decade and is the leading cause of suicidal deaths. The available antidepressant medications benefit only a third of its recipients and have many side effects. Hence, it is imperative to search in Ayurveda for leads. Aim: To evaluate Anti- depressant activity of Hiṅgvādi Ghṛta in vivo. Settings and Design: Comparative preclinical study. Materials and Methods: Hiṅgvādi Ghṛta (HG) was prepared using standard operating procedure, physicochemically analyzed and assessed. Tail Suspension Test (TST) model with Swiss albino mice and Forced Swim Test (FST) model with Wistar albino rats were used to assess anti-depressant activity. Imipramine hydrochloride in dose of 15 mg/kg for TST and 10 mg/kg for FST, was the standard drug and Ghee as vehicle control in dose of 0.1g/20g for TST and 0.72g/200g for FST orally. Hiṅgvādi Ghṛta in doses of 0.05g/20g (x/2), 0.1g/20g (x) and 0.2 g/20g (2x) for TST and 0.36g/200g (x/2), 0.72g/200g (x) and 1.44g/200g (2x) for FST was administered to 3 test groups for 21 days orally except Plain control group which received only distilled water. Duration of immobility in seconds for TST and number of rotations for FST were noted for assessment. Statistical Analysis Used: One way ANOVA followed by Dunnets test and Paired t test. Results: HG was significantly effective at dose of 0.1gm/20gm for TST (P = 0.0037; P < 0.01) and 0.72g/200g for FST (P = 0.0055, P < 0.01) comparable to Imipramine hydrochloride. Conclusions: HG displayed potent anti depressant activity comparable to standard drug Imipramine Hydrochloride.

Keywords: Anxiety, ghee, neurotransmitters, psychosis, unmad

Introduction

Depression, a mood disorder increasing at an alarming rate impairs cognitive, emotional and behavioral functions of an individual. It affects one out of every five women and 12 men with suicidal tendency accounting for 60% of deaths, which is a cause of concern.[1] The available antidepressant medications benefit only a third of its recipients and have many side effects. Hiṅgvādi Ghṛta[2] (HG), a unique lipophilic formulation from Ayurveda repertoire is claimed to pacify both Unmāda (psychosis) and Apasmār (epilepsy). Viṣāda (dejection)[3] is a premonitory symptom of Unmāda, synonymous with depression, which may be pacified by HG and hence the hypothesis.

Aim

To evaluate Anti- depressant activity of Hiṅgvādi Ghṛta.

Materials and Methods

Study drug Hiṅgvādi Ghṛta

The raw materials, Hiṅgu (Ferula narthex) resin (10.42 g), Šoṣṭhi (Zingiber officinale) rhizome, Márica (Piper nigrum) fruit, Pipppali (Piper longum) fruit (10.42 g- trikatu) and Sauvarcala lavana (Black salt - 10.42 g) were obtained from a standard authentic source. Cow ghee (1 kg) and cow urine (4 lt) were procured from Kenjal, Wai, (Satara) an

Objectives

Primary

Significant increase in number of rotations and decrease in duration of immobility in Forced Swim Test (FST)[4] and Tail Suspension Test (TST)[5] respectively.

Secondary

To take note of adverse effects, if any, during the studies.

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organic farm wherein both these products were obtained from cows reared using organic means. Physicochemical tests were conducted on all raw materials in an accredited laboratory and matched with standards [Table 1].

Cow urine was analyzed for parameters viz. pH (7.3), specific gravity (1.02), viscosity (1.178), turbidity (NA) and total solid content (3%). Cow ghee was analyzed for pH (4), Specific gravity (0.92), Free fatty acids (0.55%), Burtorefractometer reading (42.1), Wt/ml (0.82 g), Saponification value (249.62), Iodine value (44.80), Peroxide value (absent), Acid value (2.53) and Moisture (0.15%). All values complied with standards.

Preparation of HG

Raw Hiṅgu was pounded in an iron mortar and pestle into small pieces of the size of green gram and deep fried in an iron fry pan [pālīkāyantra] using equal quantity of cow ghee. It was fried till it turned light weight and dark brown in color and kept aside for some time. Śuṇṭhi, Mārīca, Pippalī, Hiṅgu and Sauvarcala lavaṇa were then powdered individually and sieved (80 mesh size). They were then homogenously mixed into a fine bolus (kalka) in a porcelain mortar and pestle. Cow ghee was taken in a thick bottom vessel and heated on low flame. The bolus was added to ghee on appearance of slight fumes, followed by cow urine and further stirred to obtain a homogenous form. The mixture was boiled on low to medium flame with intermittent stirring till it complied with the testing criteria. With fulfillment of both wick and flame tests, HG was filtered through a muslin cloth and stored in an airtight glass jar. The final yield was 890g. from 1kg which matched with general yield pattern in in-house pharmacy.

The prepared HG was dark greenish, oily, bitter to taste and had a strong urinous smell. The samples were sent to analysis to an accredited lab [Table 2].

Materials for experimental study

Animals

Swiss Albino mice, of either sex, weighing 18 – 30 g, for TST and Wistar Albino rats, of either sex, weighing 160 – 200 g for FST were obtained from CPCSEA (258) approved Central Animal House. [Proposal number: 1/2013 Ref: BVDUMC/1559/2013-2014].

There were 36 animals in each experimental model with 6 animals in each group. The animals were placed in polypropylene cages and provided rodent pellet diet supplied by Amrut animal laboratory, manufactured by Pranav Agro Industry feed, Sangli. and water ad libitum. Standard conditions were maintained regarding environment, temperature and housing.

Models

The standard models of both FST and TST were procured from Department of Pharmacology, BVDU Medical College, Pune.

### Table 1: Analytical values of herbal drugs

| Parameters                        | Hiṅgu | Pippalī | Mārīca | Śuṇṭhi | Sauvarcala lavaṇa |
|-----------------------------------|------|--------|--------|--------|-------------------|
| Foreign matter (%w/w)             | 1.7  | 1      | 1.5    | 0.4    | 0.3               |
| Total ash (%w/w)                  | 4    | 4.8    | 4.5    | 4      | NA                |
| Alcohol soluble extractive (%w/v) | 44.6 | 32     | 25     | 7      | NA                |
| Water soluble extractive (%w/v)   | 64   | 35     | 27     | 13     | NA                |
| Moisture content                  | 5.6  | 4      | 4      | 2.8    | 4.8               |
| pH                                | 5    | 6.4    | 6.1    | 3.8    | 9.5               |
| NA: Not available                 |      |        |        |        |                   |

### Table 2: Analytical values of Hiṅgvādi Ghṛta

| Parameters                        | HG   |
|-----------------------------------|------|
| Free fatty acid (%)               | 0.43 |
| Moisture (%)                      | 0.10 |
| Burtorefractometer reading (%)    | 42.9 |
| pH                                | 5    |
| Specific gravity                  | 0.9458|
| Weight/ml (g)                     | 0.82 |
| Saponification value              | 275.12|
| Iodine value                      | 31.3 |
| Peroxide value                    | Absent|
| Acid value                        | 1.42 |

All values complied with standard. HG: Hiṅgvādi Ghṛta

Drug

Tablet Depranil [25mg - Imipramine Hydrochloride (standard drug)], of Manas Pharma was acquired and diluted in distilled water to prepare a desired concentration.

Experimental study methodology

Permission was obtained from Institutional Animal Ethics Committee (IAEC) prior to start of experiment. Animals were divided into 6 groups; with Plain control group receiving only distilled water; Vehicle control group (ghee) at dose of 40 g and Standard control (Imipramine hydrochloride) group at dose of 15mg/kg for mice and 10mg/kg for rats. HG was divided into 3 test groups; X dose (similar to ghee), X/2 (half the dose) and 2X (double the dose). The doses were extrapolated by using extrapolation factor, 0.0026 for mice and 0.018 for rats.[6]

In TST, the mice, one at a time, were suspended from the end of the metal stand, with an adhesive tape wound at a distance of about 1 cm from their tail tip. Immediately the stop watch was started and the mice were observed for a period of 5 minutes. They would try to escape initially by moving vigorously and then give up, hanging passively and immobile. The duration of immobility of each mouse was noted during a period of 5 minutes and evaluated.

In FST, the rats, one at a time, were placed in the water tank and forced to swim. Once placed in the tank, the stop watch was started and the rats were observed for a period.
of 10 minutes. They would try to search for a way out but end up rotating the wheel. The number of rotations completed by each rat were displayed on the digital counter and noted for duration of 10 minutes and evaluated. The water in the tank, of similar temperature, was replaced after every rat procedure. The test drug was administered for 21 consecutive days in both the tests.

The evaluation for duration of immobility in TST and number of rotations in FST was done on day 1 prior to dosing and on 21st day post one hour of dosing. On the rest of the days, only dosing was carried out.

One-way ANOVA test followed by Dunnett’s test \((P < 0.01)\) was applied for comparison of above parameters between groups and to evaluate use of multiple treatments with a single control group. Drug dose efficacy was elicited using Paired \(t\) test. Graph Pad Prism Version 6.0 was used for result evaluation.

**Results**

**Tail suspension test**

The results were interpreted considering the duration of immobility displayed by the mice in each group when subjected to TST. On the 21st day, there was a decrease in duration of immobility in all groups except plain group. HG at X dose (0.1g/20g) showed significant result similar to standard drug Imipramine when compared with vehicle control group. The X/2 (0.05g/20g) and 2X (0.2g/20g) dose levels of HG reduced the duration of immobility but were not statistically significant when compared with vehicle (ghee) control group [Figure 1].

**Forced swim test**

The results were interpreted considering the number of rotations performed by the rats in each group in 10 minutes, when subjected to FST. On the 21st day, there was increase in number of rotations in all groups except plain group. HG at X dose (0.72g/200g) showed significant result similar to standard drug Imipramine when compared with vehicle (ghee) control group. The groups X/2 (0.36g/200g) and 2X (1.44g/200g) dose levels of HG increased the number of rotations but were not statistically significant when compared with vehicle (ghee) control group [Figure 2].

**Discussion**

It is evident from the results that, HG is significantly effective at its human therapeutic dose of 40g (i.e 0.1g in TST and 0.72g in FST), which is comparable to standard drug Imipramine Hydrochloride. The dose, though was equivalent to Ghee, results of Ghee were significant only on comparison with plain control group [Figures 1 and 2]. This particular differential effect of HG may be attributed to the active components extracted in it during preparatory stage. Also, on comparing the X/2 dose of HG i.e., 20 g with Ghee in both experimental studies, it was seen that, the results were approximately similar. This underlines the potential of extracted active components in HG, which display a better effect on the assessing parameters even at half dose (20 g) compared to full dose (40 g) of Ghee [Figures 1 and 2].

HG is composed of simple drugs; but potent enough to pacify Unmāda and Apasmāra. The available allopathic anti depressant medications are associated with a number of side effects thus limiting the number of patients seeking treatment. Ayurveda has described HG to be beneficial in Unmāda and Apasmāra which correlate with Psychosis and Epilepsy respectively. Its role as an antipsychotic and antiepileptic formulation has been shown in previous works \([7,8]\). As depression marks the beginning of psychosis, the conditions explained as ‘viśāda’ \([9]\) (dejection), which correlates with premonitory symptoms of psychosis; HG may prove worthy in the treatment of depression too.
The neurotransmitters, Noradrenaline and 5 Hydroxy tryptamine (Serotonin)\(^{[10]}\) are normally stored in the neurons and get released following nervous stimuli. They are mainly responsible for stimulatory drives and a steady behavioral pattern in an individual. The active amines thus liberated on the receptors in the cells, do not accumulate as they are immediately metabolized by enzyme Mono Amine Oxidase present in brain. The depletion of above mentioned neurotransmitters leads to a state like depression. The antidepressant drugs block this reuptake mechanism of the enzymes and allow free flow of the neurotransmitters.

It is explained in Ayurveda that the causative factors for ‘Vişāda’ involves both, the tridoṣa (3 functional entities) and triguna (3 entities related to psychological characterization). Vāta doṣa channelizes almost every function of the body and its hypo function leads to manda cheṣṭhā\(^{[11]}\) (slowness in all activities), alpa vāk (dysfluent speech), loss of zest, zeal, together termed as viśāda. Similarly, hypo functioning of pitta doṣa affects digestion, appetite, intelligence. Hyper function of kapha doṣa displays symptoms like gauravā\(^{[12]}\) (heaviness) pandrā (semi conscious state), nidrā (sleep); indicative of depressive phase. Sattva, one of the trigunas, enlightens our conscience, rajas initiates all mental activities while tamas regulates or inhibits the same thereby achieving a balance. Excess inhibition by tamagūṇa affects normal functioning of rajogūṇa. Cintya (initialization of thought process), Vicārya (consideration), Īhya (reasoning, hypothesis), Dhveya (aim), Sānkalpa (determination, judgment) are the functions of mind\(^{[13]}\) which suffer a setback once a person falls prey to anger, greed, vanity, jealousy, shamelessness, subjecting himself to intellectual indiscertions (prajñāparađha). The mental faculties, dhīḥ (intellect, cognition), dhruti (retention) and smṛti (memory, recall) which prove superiority of humans over other life forms are deranged as a result. Thus, there is a need of a formulation which would take care of above mental faculties and act effectively.

HG, is a formulation consisting of four herbal drugs i.e., Hīṅgu\(^{[14]}\) (Ferula narthex boiss), Sauvarcala lavaṇa\(^{[15]}\) (Zingiber officinale), Mārīca\(^{[16]}\) (Piper nigrum), Pippāli\(^{[17]}\) (Piper longum), along with drugs from animal sources viz. Goghṛta\(^{[18]}\) (Cow ghee), Gomūtra\(^{[19]}\) (Cow urine) and mineral drug Sauvarcala lavana\(^{[20]}\) (Black salt). Except Sauvarcala, all the other contents of HG formulation are predominant in Kaṭu Rasa (pungent taste) and Usṇa Vṛgya (hot potency) which best pacify vāta and kapha doṣa, clear obstructions in srotas (channels), alert and sharpen senses.

Physicochemical analysis revealed the pH of HG to be 5. Drugs which are weakly acidic ionize faster in the presence of alkaline pH.\(^{[21]}\) The alkaline pH of Sauvarcala lavana may thus help in the ionization of Hīṅgvādi Ghṛta better, which has a weak acidic pH aiding absorption.

The dosage form Ghee (Sneha kalpanās - oleaginous medicament), further facilitates absorption according to its principle of mass transfer of aqueous and lipid-soluble active principles of all treated herbal drugs and materials of animal and mineral origin into it. It gains access through the blood brain barrier due to its lipophilic nature and attains target action. It is the most snidgha (unctuous) sneha among all sneha dravyas\(^{[22]}\) (oleaginous entities) which nourishes the brain - an entity rich in majā dhātu (body tissue resembling an unctuous mass) by principle of ‘guna sāmānya’ (similarity synergistic of bio-physicochemical properties). It is also yogavāhi, i.e., it enhances the properties of other drugs without losing its own.

Besides Ayurveda, modern organic chemistry decipher specific characteristics and actions of each drug of the formulation. Ghee is rich in Omega-3, Omega-9, fatty acids and vitamins A, D, E, and K. Deficiency of the same leads to depression and cognitive decline,\(^{[23]}\) according to a study. Each herbal source is rich in many phytochemicals, some of those are active principles. However the reviews authors have come across during the study are of whole plant extracts which goes with Ayurvedic concept of cumulative effect.

Each ingredient of HG displays a certain CNS activity. Ghee enhances memory,\(^{[24]}\) Hīṅgu is a stimulant and anticonvulsant,\(^{[21]}\) Sauvarcala lavaṇa is an antidepressant,\(^{[26]}\) Mārīca is sedative and anti convulsant\(^{[27]}\) and Pippāli is a CNS stimulant.\(^{[28]}\) Sauvarcala lavana and Gomūtra have physiological roles. The sodium ions present in Sauvarcala lavana are most required for initializing nerve cell activity by generation of action potential. Influx of sodium ions, generates electric charges and triggers excitatory neurotransmitters. This stimulation is crucial to start the thought process. This is also supported by certain research studies wherein amount of salt intake determines behavioral mood changes and an estimated level is necessary for optimal neurological activity.\(^{[29]}\) Glutamine, a crucial amino acid, acquired from ghee as its dietary source, is metabolized to Glutamate which is a predominant excitatory neurotransmitter and has a critical role in synaptic maintenance i.e., nerve signal transmission. It is secreted by neurons present in the sensory pathways of CNS and cerebral cortex, which is the seat of thought processes.\(^{[30]}\) This Glutamate – Glutamine cycle is responsible for ammonia homeostasis, a major component in Cow urine. There are certain research studies of different ghees towards CNS, underlining the role of medicated ghee.\(^{[31,32]}\)

Thus, we can hypothesize that HG has a unique combination of drugs with potential active components which work in tandem and may cumulatively display anti depressant activity.

To test the activities it was essential to use appropriate models. Sir Gerhard Vogel has described 7 behavioral test models of which Forced Swim Test and Tail Suspension Test are most specific to elicit depressive behavior similar to human depression, which explains their selection.
Absorption of the drug is equally important for potentiating its efficacy. There are different theories of absorption for both food and drug, according to Ayurveda wherein there is sequential nutrient transformation at the level of tissue, termed as *Dhātupasānakrama nyāya*. According to one theory, post ingestion of food, its transformation into the last *sukra dhātu* takes 7 days (considering seven basic tissues – *dhātus*), while according to another it takes 6 days or 8 days (considering an extra day for formation of *āhāra rasa* (nutrient enriched chyme which further passes on to every single tissue). Some even mention a single day or a month, for nourishment of the same. Drug ingestion also, according to some, takes a single day, barring aphrodisiacs which show immediate effect. In our study, *Ghṛta* is the vehicle which is considered as both food and drug. Hence we considered the theory of 7 days, wherein we hypothesized that brain tissue which is synergistic with *majjā dhātu* would be nourished in a span of 6 days. Clinically it is known that therapeutic efficacy of Imipramine Hydrochloride takes approximately 3 weeks for effective results. Now in comparison with Imipramine, 3 cycles of HG would be complete with the aforementioned premise. So 21 days were finalized as duration of study.

The study was carried out at three dose levels as per standard protocol. It is clear that HG displays significant anti depressant activity at X (40g) dose. HG at double dose (2X- 80g), did display positive results as per the assessment parameters but were statistically non significant [Figures 1 and 2]. This could be attributed to development of symptoms of fullness, heaviness in the rodents similar to humans, due to excessive consumption of *ghee*. Also, the dose was very close to the actual stomach capacity of rodents, i.e., 1% of their body weight. Thus, taking into account all data regarding half, full and double doses of HG, we propose that its anti depressant activity is dose dependant.

The ingredients in the formulation are easily available. This formulation has also been studied for side effects, catalepsy *per se* which is absent, thus underlining its safe role in psychotic disorders. An interdisciplinary approach could be adapted to reduce the dose further and devise new dosage forms, without disturbing its activity profile taking into consideration palatability (dose amount/taste) constraints. Encouraging results of this preclinical study call for a well designed clinical trial.

**Conclusion**

Hingwadi Ghrita has shown anti depressant activity comparable to standard drug Imipramine Hydrochloride.

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**Conflicts of interest**

There are no conflicts of interest.

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