Research article

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Evaluation of analgesic, anti-inflammatory and antidepressant activities of Ayurvedic herbo-mineral formulations used for prophylaxis of migraine: a preliminary study.

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Abstract:

Inspite of several available modern medicines, the management of migraine remains unsatisfactory. Complimentary systems of medicines including Ayurveda are also often practiced. A combination of four herbomineral Ayurvedic formulations (HMAF) namely Godanti Mishran, Narikel Lavan, Rason Vati & Numax, has been reported to be effective in reducing the frequency and severity of migraine headache, by an Ayurvedic physician. The interim analysis of an on-going trail also corroborates this. Migraine is multifactorial disorder with associated symptoms e.g. pain, inflammation, depression, anxiety, behavioural and somatic changes. In the present study, to elucidate the mechanism of action, combination of HMAF was evaluated for its potential effect on experimental models of pain, inflammation, depression & neurobehavioral parameters including seizure threshold. No significant effect on any of the parameters at the doses tested was observed. The practicing Vaidya, consistently observed tenderness in hypochondria (personal communication), and proposes a hypothesis of gall bladder pathology, which needs scientific validation. Though the parameters studied are only surrogate model of migraine hence absence of efficacy in these experimental models warrants further exploration.

Keywords: Migraine, inflammation, pain, Godanti Mishran, Narikel Lavan, Numax, Rason Vati, Ayurvedic formulations.

Introduction:

Migraine is third common neurological disorder in world and is characterized by pulsating headache, often associated with nausea, vomiting, photophobia and phonophobia1. The prevalence of migraine is about 18% in women and 6% in men around the world 2. The exact prevalence in India is not known. In a study in India, out of 1000 patients who presented with headache, 86% had primary headaches of which 55% had migraine [3].

The current approaches for treatment of migraine are aimed to relieve pain and associated symptoms and prevent further attack. Pain-relieving medications include non-steroidal anti-inflammatory drugs (NSAIDS) e.g. aspirin, ibuprofen, naproxen, ketorolac, paracetamol. These are associated with gastrointestinal side effects including ulcers and bleed on prolonged use. The anti-emetic medications include chlorpromazine, metoclopramide or prochlorperazine are also used with above mentioned medicines to control nausea and vomiting, associated with migraine attack.

Disturbances in serotonin (5-HT) regulation have been implicated in the pathogenesis of migraine, including neuropeptide release and inflammatory responses. Drugs causing constriction of cerebral blood vessels by acting on serotonergic systems includes triptans (e.g. sumatriptan, rizatriptan, almotriptan, naratriptan, zolmitriptan, frovatriptan and eletriptan) and **e**rgot alkaloids (e.g. **e**rgotamine, dihydroergotamine). Triptans are contraindicated in patients who are at risk of stroke and heart attack and also associated with side effects e.g. nausea, drowsiness and muscle weakness.

The medications for migraine prevention include drugs acting on (a) cardiovascular system-beta blockers: e.g., propranolol, metoprolol, calcium channel blockers: e.g. verapamil, angiotensin-converting enzyme inhibitors: e.g. lisinopril (b) tricyclic antidepressants (TCA) e.g. amitriptyline and (c) anti-epileptic drugs: e.g. valproate and topiramate. These medications are also associated with several limiting side effects.

The efficacy of anti-migraine therapy shows high inter-individual variability [4]. These limitations led to a large number of people exploring complementary and alternative medicines (CAM) like acupuncture, chiropractic therapy, massage, relaxation therapy, homeopathy, vitamin or mineral supplementation and Ayurvedic medicines etc [5.]

An Ayurvedic clinic reported nearly 70% complete relief among well diagnosed as per International Headache Society Criteria (IHSDC) [6] migraine patients, who were treated for 120 days by prescribing Herbomineral Ayurvedic formulations (HMAF) named Narikel Lavan, Numax, Rason Vati and Godanti Mishran[7-11]. The on-going randomized controlled clinical study in refractory/ chronic migraine patients at Department of Neurology, AIIMS, New Delhi, also gives an early indication that the aforesaid formulations could be effective in the management of refractory/chronic migraine. These HMAF were subjected to acute, sub-acute and sub-chronic toxicological studies in mice and rats following OECD guidelines at a leading institute at Mumbai and proved to be safe in the prescribed doses [12]. However, the mechanism of action of these formulations in migraine is unknown. The present study was the first step to understand the possible mode of action of HMAF in the management of migraine and to investigate its analgesic, anti-inflammatory, anti-depressant and other potential CNS activities including effect on neurobehavioral parameters and seizure threshold in experimental models.

Materials & Methods:

Experimental Animals: Male Wistar adult rats (200-250 g) were obtained from the Central Animal Facility of All India Institute of Medical Sciences, New Delhi and stock bred in the departmental animal house. The rats were group housed in poly acrylic cages (38x23x10 cm) with not more than four animals per cage and maintained under standard laboratory conditions with natural dark and light cycle. Rats were allowed free access to standard dry rat

diet (Ashirwad, Punjab, India) and tap water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC No: 544/IAEC/10).

Drugs and chemicals

All drugs (Narikel Lavan, Rason Vati, Godanti Mishran and Numax) were manufactured at Bharat Bhaishajaya Shala Private Limited, Dehradun, and provided by IPCA laboratories, Mumbai. The standard drugs morphine, indomethacin fluoxetine valproate phenytoin, pentylenetetrazole were purchased from Sigma Chemical Company USA. All the reagents used in study were procured from Merck, India.

Composition of Herbomineral Ayurvedic formulations (HMAF)

Godanti Mishran consist of *Gypsum* (8 parts), *Serpentine* (2 parts), *Rasadi vati* (2 parts). **Numax** *is* mixture of ***Sootshekhar rasa*** *(250 mg) and* ***Sitopaladi (****400 mg).*

Sootshekhar rasa consist of *Suddhaparada* (1 Part), *Suddha gandhaka* (1 Part), *Cinnamomum zeylanica* (1 Part), *Elleteria cardamomum* (1 Part),*Cinnamomum tamala* (1 Part), *Mesuajerrea* (1 Part),*Turbinella pyrum* (1 Part), *Chalco pyrite* (1 Part), *Argentum* (1 Part), *Cuprum*(1 Part), *Datura metel* (1 Part), *Suhaga* (1 Part), *Zingiber officinale* (1 Part), *Piper nigrum* (1 Part), *Piper longum* (1 Part), *Eclipta Alba (Q.S for mardana).*

Sitopaladi churna consist of *Mishri* (16 parts), *Bambusa arumdimaceo* (8 parts), *Piper longum* (4 parts), *Ellettaria cardamomum* (2 parts), *Cinnamomum zeylanica* (1 part).

Narikel Lavana consists of *Cocus nucifera* and *Saindhava lavana*.

Rason Vati consist of *Allium sativum* (1 Part), *Cuminum cyminum* (1 Part), *Zingiber officinale* (1 Part), *Piper nigrum* (1 Part), *Piper longum* (1 Part), *Ferula foetida* (1 Part), *Saindhava lavana* (1 Part), *Gandhaka-suddha* (1 Part), *and Citrus medica* juice (q.s for bhavana).

Drugs preparation and dose calculation: HMAF (Narikel Lavan, Rason Vati, Godanti Mishran and Numax*)* were suspended in 0.1% carboxy methyl cellulose. Morphine,

Indomethacin, Fluoxetine, Valproate and Phenytoin were prepared in normal saline. Rat doses were calculated from human dose per day according to the method followed by Centre for Drug Evaluation and Research, Food and Drug Administration (USA), 2005.

Evaluation of Analgesic activity

Hot plate test

Rats were divided in three groups (n=6) i.e. Vehicle control, Standard (Morphine), Combination of HMAF. The HMAF were given orally for 7 days. Morphine was used as standard drug at dose of 7 mg/kg bw. On 7^{th} day, 1 hr after administration of HMAF and Morphine in respective groups, hot-plate test was performed as previously described by Gupta YK et al., 2004 [13]. Briefly, rats were placed on a hot plate maintained at 56 ± 0.2 ° C and the reaction time (paw licking latency) was observed. Paw licking latency is the interval from the instant animal reached the hot plate until the moment animal licked its feet or jumped out. A cut off time of 45 s was followed to avoid any thermal injury to the paws. The paw licking latency was recorded at 0 (baseline), 15, 30, 60, 90, and 120 min after treatment.

Radiant heat tail-flick test

Rats were divided in three groups (n=6) i.e. Vehicle control, Standard (Morphine), Combination of HMAF. HMAF were given orally for 7 days. Morphine was used as standard drug at dose of 7 mg/kg bw. On 7^{th} day, 1 hr after administration of HMAF and Morphine in respective groups, radiant heat tail-flick test was performed as previously described by Katyal J & Gupta YK, 2012 [14]. The analgesic activity was studied by measuring the sensitivity (tail flick latency) of the rats to radiant heat. The intensity of the light beam has been experimentally defined such that naive animals will withdraw their tails within 2-3 s, to heat stress applied to their tails by using analgesiometer. The strength of the current passing through the naked Ni-chrome wire was kept constant at 5 ampere. The application site of the heat on the tail was maintained within 2 cm, measured from the root of the tail. Cut-off time

was 15 s to avoid any tissue injury during the process. Tail-flick latency i.e. time taken by rats to withdraw (flick) the tail was measured at 0, 15, 30, 60, 90 and 120 min time interval after treatment.

Evaluation of Anti-inflammatory activity

Carrageenan induced paw edema test

Rats were divided in three groups (n=6) i.e. Carrageenan control, Standard (Indomethacin), Combination of HMAF. The HMAF were given orally for 7 days, orally. Indomethacin was used the sensitivity of the system and as standard drug at dose of 3 mg/kg bw. On day 7^{th}, 1 hr after administration of HMAF/ indomethacin, intraplantar injection of 100 µl of 1% carrageenan was given to induce paw edema [15]. Paw volume was determined using a plethysmometer (Ugo Basil, Italy) at 0, 1, 2, 3, 4 and 5 h interval after administration of carrageenan. The percent paw edema was calculated as

$$\% \text{ paw edema} = \frac{(\text{Vol. of swollen paw} - \text{Vol. of paw before injection}) * 100}{\text{Vol. of paw before injection}}$$

Evaluation of Anti-depressant activity

Forced swim test

Rats were divided in three groups (n=6) i.e. Vehicle control, Standard (Fluoxetine), Combination of HMAF. The HMAF were administered orally for 7 days. On 7^{th} day, 1 hr after treatment, rats were placed individually in plexi glass cylinder of 25 cm height and15 cm internal diameter, filled with water (25° C) up to a depth of 10 cm. This depth was sufficient to keep adult rats from supporting themselves by placing their paws or tails on the base of the cylinder. After an initial struggle (climbing and swimming), the rat becomes immobile wherein it floats by making only movements necessary to keep its head above the water. There were two sessions; the first was "15 min pre-test swim" and 24 hours later a

second "5 min swim test". The standard drug fluoxetine was given thrice i.e. following the initial 15 m pre-test (23.5 hr prior to test swim), 5 h and 1 h prior to 5 min swim test.

After 24 hr, during 5 min swim test session, time-sampling technique was used wherein the predominant behaviour (Immobility/ Swimming/ Climbing) over each 5-s period was observed. [17].

Tail suspension test

Rats were divided in two groups (n=6) i.e. Vehicle control and Combination of HMAF. The HMAF were administered for 7 days. On 7th day, after 1 hr of last dose, tail suspension test was performed. Rats were suspended 20 cm above the floor in a visually isolated area by adhesive tape placed 3 cm from the tip of the tail. Immobility duration was recorded for last 4 min during 10 min test session. Rats were considered immobile only when they hung passively without movements [18].

Neurobehavioral parameters

Rats were divided in two groups (n=6) i.e. Vehicle control and Combination of HMAF. The HMAF were administered in combination at equivalent rat dose, p.o, for 28 days. The neurobehavioral parameters were assessed at 0, 7, 14, 21 and 28 day after treatment.

Elevated Plus maze test: The acquisition (Initial latency) and retention (Retention latency) of memory processes was assessed using elevated plus maze according to the method of Sharma and Gupta, 2001 [19].

Passive Avoidance test: The memory retention deficit was evaluated by a passive avoidance (step through) apparatus according to the method described by Reeta *et al*, 2009 [20].

Actophotometer test: Locomotor activity (index of wakefulness /alertness of mental activity) was evaluated by Actophotometer. It is expressed in in term of total photobeam counts per five minutes per rat [21].

Rota Rod test: Motor coordination was evaluated using Rotarod Apparatus. Fall off time due to loss of muscle grip is an indication of motor cordination.The difference in the fall off time from the rotating rod between the control & treated rats is taken as index of muscle cordination [21].

Muscle Grip Strength Test: The grip test was performed according to methods of Sinha K *et al*., 1995 [22]. The apparatus with a 50 cm string pulled between 2 vertical supports elevated 40 cm above from the flat surface. The rat was placed on the string at a point midway between the supports and is evaluated by the scale 0: Fall off from the string, 1: Hangs onto string with two forepaws, 2: Hangs onto the string with two forepaws, but also attempt to climb on the string, 3: Hangs onto the string with two forepaws, plus one or both hind paws, 4: Hangs onto the string with all paws, plus the tail wrapped around the string, 5: Escape.

Anti-seizures activity

Experimental protocol of pentylenetetrazole (PTZ) - induced seizures model

Rats were divided in three groups (n=6) i.e. PTZ - control, Valproate and Combination of HMAF. The HMAF were administered in combination at equivalent rat dose, p.o, for 28 days. PTZ was administered at a dose of (60 mg/kg, i.p), 30min after the administration of the Valproate/ HMAF. The dose of PTZ has been standardized earlier in our laboratory as 100% convulsing dose with minimal mortality in rats [23]. Valproate was used as standard drug at 300 mg/kg bw. The latency to myoclonic jerks and incidence of generalized tonic clonic seizures (GTCS) with loss of righting reflex were observed.

Experimental protocol of maximal electroshock (MES)-induced seizures model

Rats were divided in three groups (n=6) i.e. MES - control, Phenytoin and Combination of HMAF. The combination of HMAF was administered in orally for 28 days. Phenytoin was used as standard drug (40 mg/kg,i.p.). Electroconvulsions were produced by a supra-threshold fixed current sinus wave stimulus (current intensity: 70mA, duration: 0.2s)

delivered via ear clip electrodes using ECT unit (Ugo Basile, Italy) [24]. The rats were observed for occurrence of tonic hind limb extension (THLE), i.e., the hind limb of animals outstretched 180º to the plane of the body axis and duration of THLE.

Statistical analysis

All data are expressed as mean ± SEM. Drugs treated groups were compared to normal control and positive control group using one way ANOVA with posthoc Bonferroni test. The p value < 0.05 was considered statistically significant. All the statistical analyses were performed using software (SPSS, version 16).

Results:

Analgesic activity

Hot plate test:

The standard drug morphine (7 mg/kg, i.p), showed analgesic effect which was indicated by significant ($p<0.001$) increase in latency in comparison to control. The combination of HMAF (Narikel Lavan, Rason Vati, Godanti Mishran and Numax) did not cause any significant increase paw licking latency as compared to control group (Figure 1A).

Radiant heat tail flick test:

Morphine (7 mg/kg, i.p) used as a standard drug, showed analgesic effect which was indicated by significant ($p< 0.001$) increase in tail flick latency in comparison to control. The combination of HMAF (Narikel Lavan, Rason Vati, Godanti Mishran and Numax) did not cause any significant change in tail flick licking latency as compared to control (Figure 1B).

Anti-inflammatory activity

Carrageenan induced paw edema

Intraplantar injection of carrageenan causes time dependent increase in paw volume, with maximum % paw edema (66.0 ± 6.9) at 3 hr. Indomethacin has significantly ($p< 0.001$) decreased % paw edema as compared to carrageenan/control group at all the time intervals.

The combination of HMAF did not cause significant reduction in per cent paw edema as compared to carrageenan only group at any time interval (Figure 2).

Anti-depressant activity

Forced swim test

The standard drug fluoxetine, has significantly reduced immobility time ($p<0.001$) and significantly increased climbing and swimming time ($p<0.001$), as compared to vehicle control group indicating anti-depressant effect. However, combination of HMAF (Narikel Lavan, Rason Vati, Godanti Mishran and Numax) at calculated equivalent rat dose, for 7 days, did not cause any significant change in immobility, climbing and swimming time as compared to control (Figure 3A).

Tail suspension test

The combination of HMAF (Narikel Lavan, Rason Vati, Godanti Mishran and Numax) at calculated equivalent rat dose, for 7 days, did not cause any significant change in mobility as compared to control (Figure 3B)

Effect on neurobehavioral parameters

Elevated plus maze test: The combination of HMAF; Narikel Lavan, Rason Vati, Godanti Mishran and Numax up to 28^{th} day of treatment did not cause any significant change in the retention transfer latency as compared to control group (Figure 4A)

One-trial Passive Avoidance task: The retention escape latencies of treatment group (combination of HMAF) and control group was not found significantly different up to 28^{th} day of treatment (Figure 4B).

Actophotometer: There was no significant difference the movement counts of treatment group (combination of HMAF) and control group, up to 28^{th} day of treatment. (Figure 4C)

Rota Rod: No significant change was observed in the fall off time in group treated with combination of HMAF as compared to control group up to 28th day of drug treatment. (Figure 4D).

Muscle Grip Strength Test

The combination of HMAF did not cause significant change in the muscle grip strength as compared to control group up to 28th day of treatment (Figure 4E).

Anti-seizures activity

PTZ induced seizures model:

The combination of HMAF (Narikel Lavan, Rason Vati, Godanti Mishran and Numax), at calculated rat dose for 28 days, did not show any protection against Generalised tonic clonic seizures (GTCS) in PTZ induced seizures model in rats.(Table 1).

MES- induced seizures model:

The combination of HMAF (Narikel Lavan, Rason Vati, Godanti Mishran and Numax) at calculated rat dose for 28 days also did not show any protection against tonic clonic hind limb extension (THLE) in MES- induced seizures model. However significant ($p<0.001$) reduction in the duration of THLE in combination group was observed indicating reduction in severity of seizures (Table 2).

Discussion

Migraine is the most frequent neurological disease and is a major health problem causing ictal disability [25, 26]. Increased attack frequency leads to chronic migraine which becomes less responsive to symptomatic as well as prophylactic migraine medications. The prolonged use of modern medication such as beta blockers, calcium channel blockers, tricyclic antidepressants, anti-convulsants, triptans, NSAIDs etc. are also associated with moderate to severe intolerable side effects and drug interactions. Failure of adequate trial with appropriate dose & duration results in situation called refractory chronic migraine (RCM).

Though no epidemiological data available, but it is estimated that nearly 10% of the patient have refractory chronic migraine. Because of such limitations of modern medicine in management of migraine, the other therapies are being tried and explored for prevention, symptomatic relief and cure of the migraine including Ayurvedic practices. The efficacies of few Ayurvedic approaches in management of migraine have also been reported [27-29]. However, these studies have limitations in terms of study design, statistical appropriation and mechanistic explanation of the efficacy.

A clinic runs by an established Ayurvedic practitioner, reported efficacy of an Ayurvedic therapy in prophylaxis of migraine with aura. The Ayurvedic treatment protocol AYTp consisted of four herbomineral Ayurvedic formulations (HMAF) namely Godanti mishran, Narikel lavan, Numax and Rason Vati along with regulated diet and life-style modification. In the uncontrolled, open label study, out of 406 migraine patients, 204 completed the 90 days of treatment and complete disappearance of headache was seen in 72 and in rest the improvement of symptoms varying degree was observed [9].

The migraine is a complex condition with multiple etiopathology associated symptoms e.g. pain, inflammation, depression, anxiety, oxidative stress, behavioural and somatic changes. The herbal components of HMAF have been described in literatures to possess analgesic, anti-inflammatory, anti-depressant, anti-oxidant and also neuroprotective activity (Table 3) in addition to several other properties.

In the present study, it was therefore considered worthwhile to investigate the efficacy of these HMAF on experimental models of pain, inflammation, depression in rats. The HMAF were given as combined formulation at 7.3 gm per day dose in Ayurvedic practice. However, for logistic purpose of dosing, we administered these formulations at an interval of 15 min at equivalent rat dose. Interestingly in the doses, up to 300 mg/kg, 40 mg/kg, 500 mg/kg and 150 mg/kg for Narikel Lavan, Godanti Mishran, Rason Vati and Numax respectively, these

HMAFs did not showed significant analgesic effect in the tail flick and hot plate model. The other experimental model of pain and higher doses of AYFs were not tried.

The combination of HMAF (Narikel Lavan, Rason Vati, Godanti Mishran and Numax) at calculated equivalent rat dose, for 7 days, did not cause any significant change in forced swim and tail suspension test. These HMAF also failed to show significant anti-inflammatory effect in carrageenan induced paw edema model in rats.

Chronic treatment (28 days) with HMAF has no effect on seizure threshold in both MES and PTZ induced seizures models. However severity of seizures was reduced as indicated by significant ($p < 0.001$) reduction in the duration of THLE in MES-induced seizures model. There was no significant effect on neurobehavioral parameters for learning and memory, anxiety, loco-motor activity.

In the absence of significant effect on the above parameters, yet the demonstrated efficacy in the documented Ayurvedic practice, we are tempted to consider the hitherto unexplored and un-established prepositions of involvement of gastro-intestinal (G.I.) system in migraine. Tesot in 1973 [57], reported for the first time distinguished migraine from common headache by linking the former with the reflexes from stomach and gall bladder. Later Vaidya PB et al., also suggested a correlation between symptoms of migraine with those of *Amla-pitta* (state of acid-alkali imbalance in the body) causing symptoms such as: *brahma* (confusion), *moorcha* (fainting), *aruchi* (anorexia), *aalasya* (fatigue), *chardi* (vomiting), *prasek* (nausea), *mukhmadhurya* (sweetness in the mouth) and *shiroruja* (headache).

As the plant components of these HMAF have also been traditionally used for G.I. related symptoms as evidenced in various studies, we consider that the efficacy of HMAF could be due to combined mechanism at central (brain) and peripheral (G.I.) level (Table 3). However, at present, it is largely conjectural and needs study with graded doses of HMAF on expanded models with simultaneous assessment of CNS and GI parameters. A randomized controlled,

clinical trial with adequate power, on migraine patients having associated G.I. symptoms especially gall bladder is also important to scientifically validate the reported findings of the efficacy of the aforementioned HMAF.

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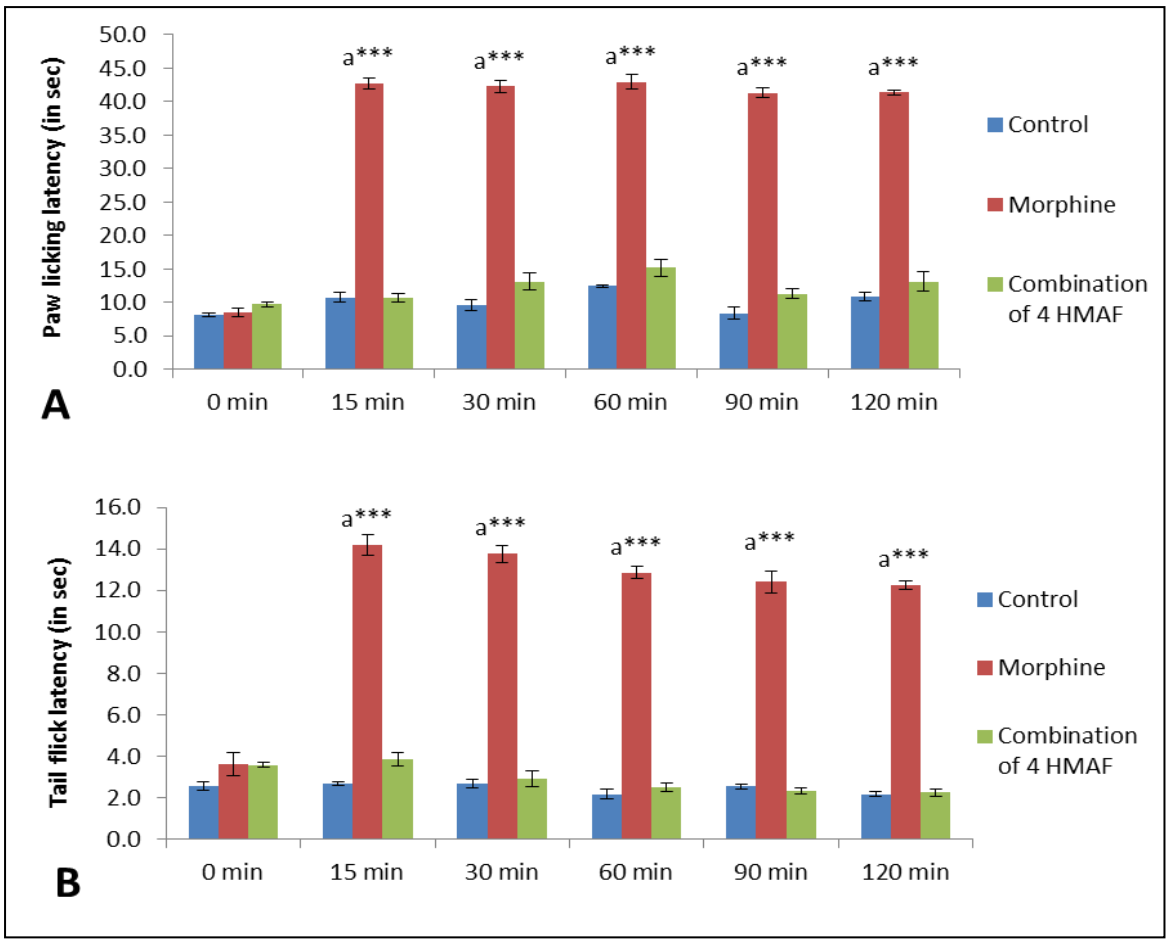

Figure1: Effect of Ayurvedic formulations on; (A) Paw licking latency of rats, at different time intervals in hot plate test, (B) Tail flick latency of rats at different time intervals in tail flick test. Data represented as Mean ± S.E.M (n = 6), *** p< 0.001, a: compared to control group, HMAF: Herbomineral Ayurvedic Formulations.

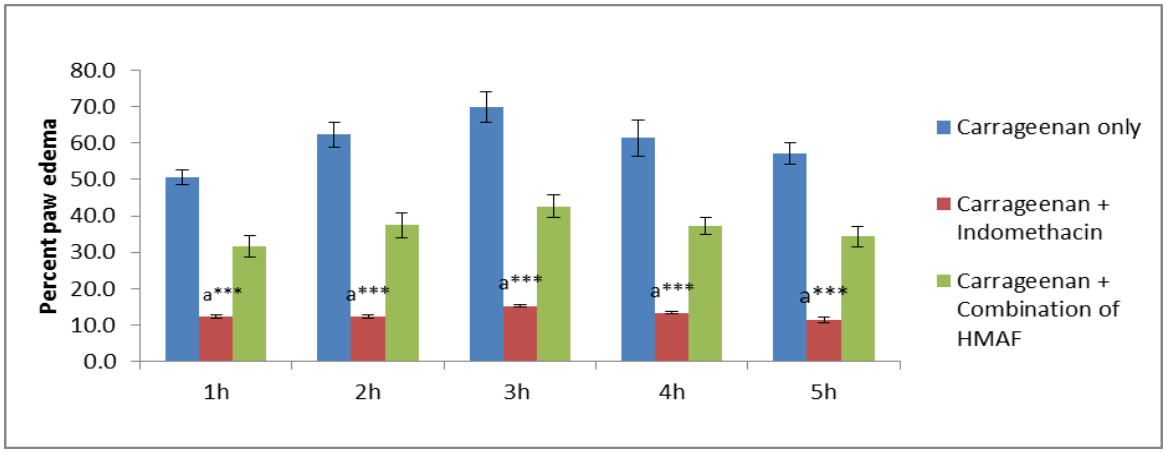

Figure 2: Per cent paw edema of rats in different groups at 1,2,3,4 & 5 h after treatment. Data represented as Mean ± S.E.M (n = 6), ***p< 0.001, a- compared to Carrageenan group. HMAF: Herbomineral Ayurvedic Formulations.

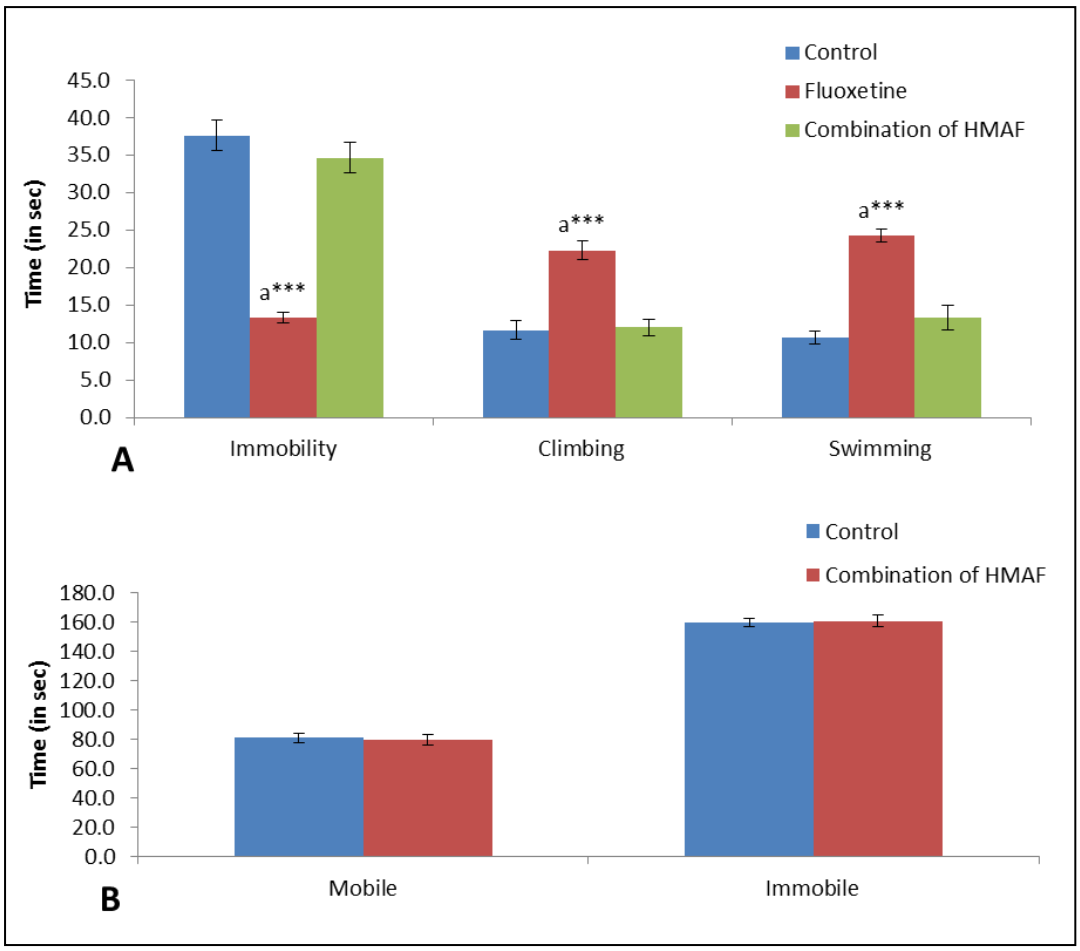

Figure 3: Effect of Ayurvedic formulations in combination; (A) on immobility, climbing and swimming counts of rats in forced swim test, (B) on mobility counts of rats in tail suspension test. Data represented as Mean ± S.E.M (n = 6), *** p< 0.001, a: compared to vehicle control group.

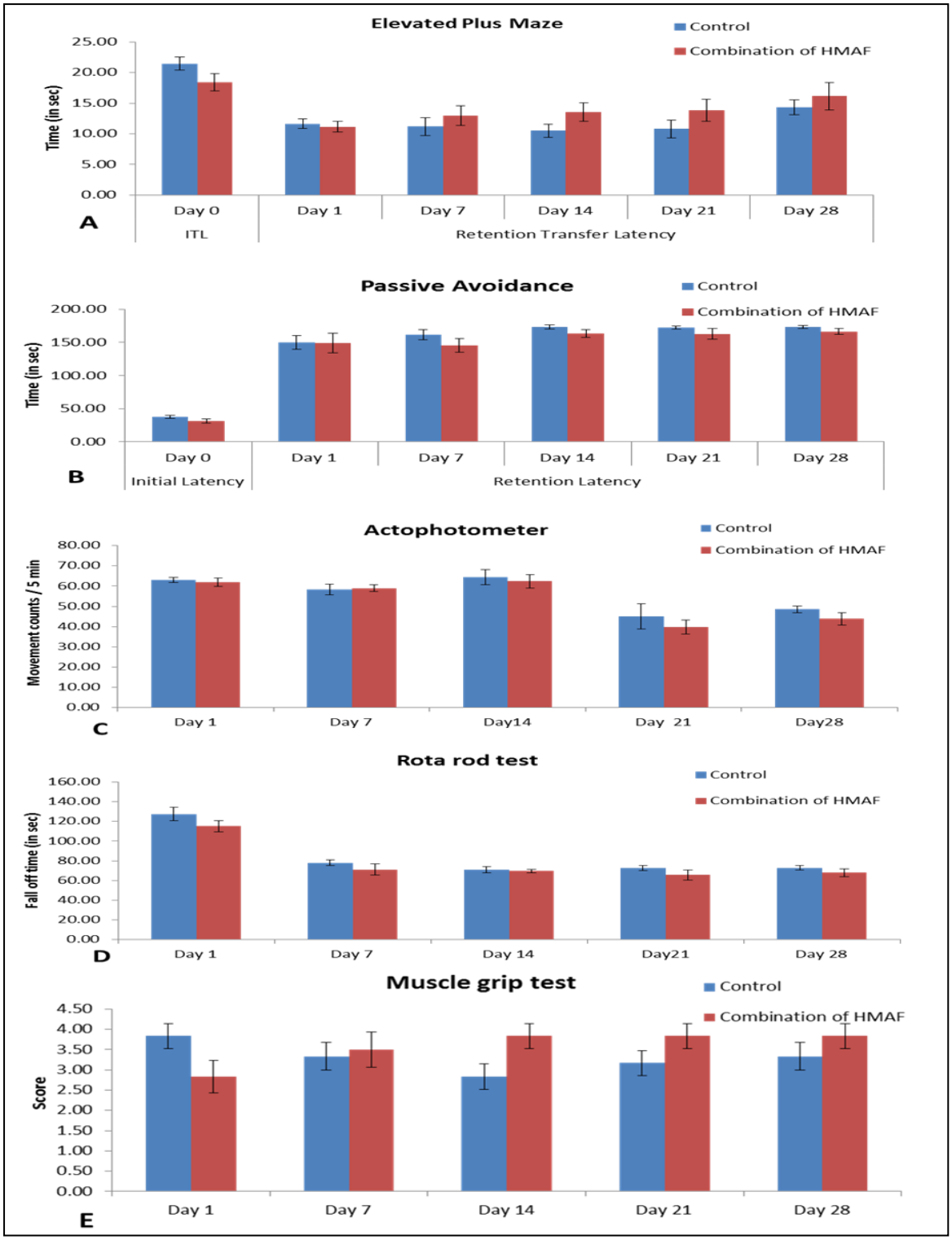

Figure 4: Effect of combination of Ayurvedic formulations on Day 1, 14, 21, & 28 after treatment on; (A) Retention Transfer Latency in Elevated Plus Maze Test, (B) Retention Escape Latency in Passive Avoidance Test, (C) Movement counts in Actophotometer (D) Fall off Time in Rota rod Test, (E) Score in Muscle Grip Strength Test. Data represented as Mean ± S.E.M (n = 6), HMAF: Herbomineral Ayurvedic Formulations.

Table 1: Effect of combination of herbomineral Ayurvedic Formulations on latency of myoclonic jerks and GTCS in PTZ-induced seizures.

Groups	Myoclonic jerk latency (sec)	GTCS latency (sec)
PTZ-Control	51.57± 3.76	151.7 ± 16.07
Valproate	0.0 ± 0.0	0.0 ± 0.0
Combination of HMAF	106.17 ± 26.07	154.2 ± 29.02

Data represented as Mean ± SEM, (n = 6)

Table 2: Effect of herbomineral Ayurvedic formulations, on onset and duration of THLE in MES-induced seizures.

Groups	Onset of THLE (sec)	Duration of THLE(sec)
MES Control	13.48 ± 2.13	45.2 ± 5.82
Valproate	0 ±0	0 ± 0
Combination of HMAF	12.48 ±1.31	22.1 ± 2.47a**

Data represented as Mean ± SEM, (n = 6), **p<0.01, a- compared to MES control group

Table 3: Pharmacological activities of plant components of HMAF

Plant	Activity	Reference
Alium sativum	Analgesic activity	Jayanthi MK and Jyoti MB, 2012 [30]
Bambusa arumdinaceae	Anti-inflammatory activity Neuro-protective activity	Muniappan M and Sundararaj T, 2003 [31] Hong EJ et al, 2010 [32]
Eclipta alba	Analgesic activity	Pandey PS et al., 1997[33] Leal LKAM et al., 2000 [34] Sawant M et al., 2007 [35]
	Anti-inflammatory activity	Kumar SS et al., 2005 [36]
	Neuro-pharmacological Activities	Thakur VD and Mengi SA, 2005 [37] Banji O et al., 2007 [38] Lobo OJF et al., 2008 [39] Bhaskar M and Chintamaneni M, 2014 [40]
	Antioxidant activity	Kaur G et al., 2009 [41] Uddin N et al., 2010 [42] Baldi R et al., 2011 [43] Mansoorali KP at al., 2012 [44]
Ellettaria cardamomum	Analgesic activity	Amrit PS and Samir M, 2008 [45]
	Anti-inflammatory activity	Amrit PS and Samir M, 2008 [46] Mehjabeen et al., 2015 [47]
Cinnmomum zeylanica,	Analgesic activity	Atta AH and Alkofahi A, 1997 [48]
	Anti-inflammatory activity	Atta AH and Alkofahi A, 1997 [49]
Ferula northrax	Analgesic activity	Bashir S et al., 2013 [50]
Piper longum	Analgesic activity	Vedhanayaki Get al., 2003 [51]
	Anti-inflammatory activity	Choudhary GP, 2006 [52]
	Anti-depressant activity	Lee SA et al., 2008 [53]
Piper nigrum	Analgesic activity	Bukhari IA et al., 2013 [54]
	Anti-inflammatory activity	Bang JS et al., 2009 [55]
	Anti-depressant activity	Mao QQ., 2014 [56]

Table 4: Plant components of herbomineral ayurvedic formulations (HMAF) reported to have activities on gastrointestinal symptoms.

Plant component	References
Alium sativum,	Kagyung R et al., 2010 [58]
Cinnmomum zeylanica,	Ranasinghe P et al., 2013 [59]
Ellettaria cardamomum,	Pandey M.M et al., 2013 [60]
Piper longum,	Samy R.P et al., 2008 [61]
Piper nigrum,	
Zingiber officinalis,	
Cinnamomum cassia,	Vangalapati M et al., 2012 [62]
Ferula northrax	Bashir S et al., 2013 [63]