Evaluation of the analgesic activity of standardized aqueous extract of *Withania somnifera* in healthy human volunteers using Hot Air Pain Model.

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Abstract

Pain is the most prevalent health care problem. Most analgesics available today have good analgesic activity but are associated with lot of side effects. So analgesics with minimal side effects are required which can be evaluated by hot air pain model in healthy volunteers. *Withani somnifera* with various properties due to its active constituents-withanolides is widely used in Ayurvedic medicine. This study was done to evaluate the analgesic effect and tolerability of single oral dose (1000mg) of standardized aqueous extract of *Withania somnifera* using Hot Air Pain model in healthy human volunteers as per ICH GCP Guidelines after taking written informed consent to protocol approved by IEC. Subjects were randomised to receive either single oral dose of 1000mg standardized aqueous extract of *Withania somnifera* or identical placebo in a double blind manner. Mean Pain Threshold Time at baseline and 3hrs after drug administration were noted. Washout period of 10-14 days was given for cross-over between the two treatments. Safety assessments were conducted before and at end of study Total twelve subjects were enrolled. Mean Pain Threshold Time with *Withania somnifera* increased from 43.99±6.79 to 49.89±7.07 sec (p<0.05). No significant change was observed with placebo. Both drugs were well tolerated.

Key words: *Withania somnifera*, Hot Air Analgesiometer, Pain Threshold Time.

1. Introduction

Pain is the most prevalent and most distressing health care problem with which the patient approaches a health care professional. The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (Bonica, 1979). Early treatment of pain is important as it relieves patient discomfort (Wolf, 2010). Analgesics are the drugs that provide pain relief. The most common analgesic drugs available today are narcotic analgesics, (also termed opioids) and nonsteroidal anti-inflammatory drugs (NSAIDs); and acetaminophen. These drugs have very good analgesic activity but they are associated with a lot of side effects ranging from drowsiness, constipation, nausea, vomiting, dry mouth, itching, dependence potential and respiratory depression seen with opioid analgesics (Andrea et al, 2008) and ulcers, anemia, gastrointestinal hemorrhage, perforation, hypertension, renal damage etc seen with NSAIDs even when used in their therapeutic doses (Brennan, 1984).

To overcome this drawback intense research is going out for the past two decades to develop analgesics with minimal side effects in allopathic as well as complementary and alternative systems of medicine (Bone et al, 1996; Mellar, 2010).Now drugs can first be evaluated in experimentally induced pain models involving healthy volunteers. Experimentally-induced pain, with its greater precision and the ability to use healthy subjects in a controlled environment, is a better model in early clinical investigation of analgesics (Staahl et al ,2012).Many experimentally induced pain models like thermal pain(radiant heat ,hot air, cold pressor), electrical, chemical and mechanical etc are available today (Naidu et al, 2008) Hot air analgesiometer is one of the standardized thermal pain models for testing the analgesic activity of newer drugs. It delivers variable, quantifiable and reproducible heat stimulus to induce thermal pain on the volar surface of subjects' forearm. This model used in the present was developed and standardized in the department and was found to be safe, and effective. (Khambam et al, 2011)

Ayurvedic medicine is a Hindu system of traditional medicine native to India and is a form of alternative medicine. Extracts from various part (root,stem,leaves,bark,seeds) of plants like *Withania somnifera ,Terminalia arjuna, Calotropis procera, Emblica officinals* have been used in ayurveda to treat various conditions including pain since vedic times. *Withania somnifera* (Ashwagandha) is widely used in Ayurvedic medicine (Mishra et al, 2000). It is an active ingredient in many formulations prescribed for a variety of musculoskeletal conditions like osteoarthritis, rheumatism etc.(Singh et al, 2011).Several animal and human studies with this plant indicated that it possesses anti-inflammatory,(Agarwal et al, 1999) antitumor(Jai Prakash et al, 2001), anti-stress, antioxidant, immunomodulatory, hematopoietic and rejuvenating properties besides positively influencing the endocrine, cardiopulmonary and central nervous systems(Raut et al, 2012).The present study was thus designed to evaluate the analgesic efficacy of standardized aqueous extract of *Withania somnifera* in experimentally induced Hot Air Pain model.

Material and Methods

The present study was conducted in Department of Clinical Pharmacology and Therapeutics at Nizam's Institute of Medical Sciences. This was a randomized, double-blind, placebo-controlled, cross over study. Written informed consent was taken from the participants to the protocol approved by the Institutional Ethics Committee. The participants were then screened for eligibility into the study by taking medical history conducting physical and clinical examination. Safety laboratory tests to assess hematological, hepatic and renal parameters were also conducted. The subjects were trained on two separate occasions on the study procedure prior to participation.

A hot air analgesiometer (**Figure1**) was used to deliver the thermal pain stimulus for the study that has been developed, validated and described in detail elsewhere. (Sunil Kumar Reddy Khambam et al, 2012)
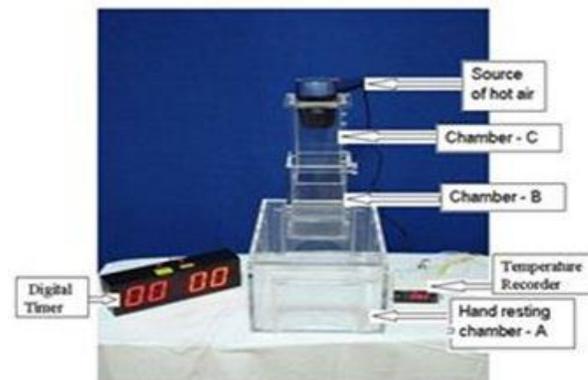

Figure1: Hot Air Analgesiometer

2.1 Study Participants

Healthy male participants aged 18 – 40yrs with BMI 19.5 – 25.9 kg/m^2 who were willing to give written informed consent and able to perform the test procedure as per protocol and not using other analgesics, anti-inflammatory, anti-pyretic drugs known to alter pain sensation either topically or systemically in the past 2 weeks were included the study. Subjects were excluded if there was any evidence of physical illness, drug abuse or abnormal laboratory test parameters, using other anti oxidants and over-the counter medications over the past 2 weeks or any other investigational drugs, within 3 months prior to the enrollment into present the study during screening.

2.2 Study medication

In the present study we used two capsules of 500 mg each (total dose 1000 mg) of SENSORIL® and 2 Identical Placebo capsules. All the study medication were supplied by Natreon, Inc.USA. SENSORIL® contains patented standardised aqueous extract of *Withania somnifera* with an average of 15.7% withanolide glycosides,40.2% oligosaccharides,0.24% withaferin-A. The SENSORIL® product was selected for this study because withanolide glycosides not the aglycones are known to be the active ingredients with oligosaccharides as bio-carrires and SENSORIL® contains very high levels of withania glycosides.

2.3 Study Procedure

On the day of the study after a good overnight sleep the participants reported to the department at 7:00 am in the morning. After having light breakfast they were

seated in a quiet room with ambient temperature of 22ºC ± 2 ºC and humidity for half an hour before the initiation of test procedure. After blindfolding they were asked to keep their non-dominant hand exposing the volar aspect of the forearm into the lower chamber -A as shown in the **Figure 2** and the source of heat was turned on.
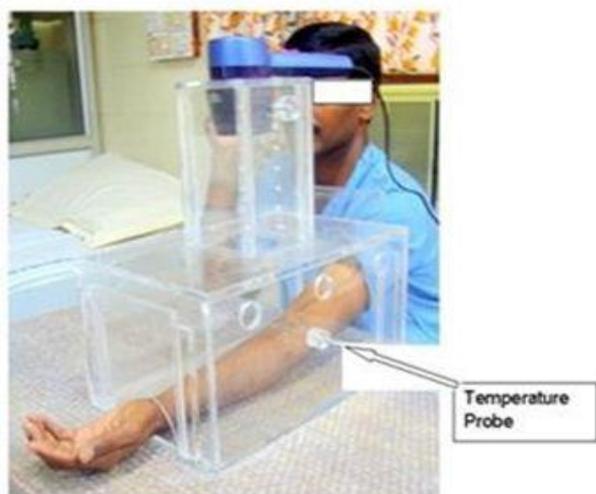

Figure 2: Participant placing his hand in Hot Air Analgesiometer

The subjects were instructed to indicate as soon as they perceived the heat sensation as painful (**Pain Threshold)**, by raising their index finger of the other hand following which the hair drier was turned off immediately and the experimental hand was removed from the chamber. The time elapsing between the turning on of the heat source and indication of heat sensation as painful, was measured in seconds, by a digital clock. The test procedure was repeated for three times with inter-stimulus interval of five minutes. The mean of the three measurements was taken as baseline and was used for analysis.

The participants were then given either two capsules of 500 mg(1000 mg) standardised aqueous extract of *Withania somnifera* or identical looking placebo capsules with 240ml of water as per prior randomization schedule at 8:00 am in a double blind fashion. After taking the drug the participant was asked to sit upright for atleast 2 hrs in a chair. The test procedure was repeated after 3 hrs of taking the drug and the pain threshold time was noted and used for analysis. Participants were asked to report any side effects during the study. Safety laboratory parameters were evaluated at the end of the study. After two week wash out period the second drug was given according to the randomization sequence and same procedure was repeated.

2.4 Statistical Analysis

The data on pain threshold time was recorded in seconds and presented as mean ± SD. ANOVA and Paired Student t-test were used to compare the difference within the group, at 80% power and $p<0.05$ was used to test the significance. All statistical analysis were performed using the Graph pad PRISM software 4 (Graph pad software Inc., USA).

3. Results and Discussion:

Total of 14 participants were screened and 12 enrolled for the study. Those two participants were excluded because their liver function tests were> three times the upper limit of normal levels. The demographic characters are depicted in **Table 1**.

Table 1: Demographic characteristics of the study group

Age in years (mean±SD)	35.28±3.31
BMI in kg/m2 (mean±SD)	21.33±1.012

The results of outcome measures are shown in **Table 2**.

Table 2: Pain Threshold time in seconds: Mean ± SD

Pain threshold time in seconds	Aqueous extract of *Withania somnifera.*	Placebo
Baseline (Mean ± SD)	43.99±6.79	43.79±6.19
Post-drug (Mean ± SD)	49.89±7.07	44.28±9.19

There were no significant differences in baseline characteristics between the two groups. As seen from the **Table 2**, standardised aqueous extract of *Withania somnifera* significantly increased Pain Threshold Time compared to that of baseline and placebo. As shown in Table 2 compared to baseline Pain threshold time increased from 43.99±6.79 to 49.89±7.07 sec with standardised aqueous extract of *Withania somnifera*, whereas with placebo, it increased from 43.79±6.19 to 44.28±9.19 sec only. The mean percentage change in Pain Threshold Time was 12.85% compared to 0.4% with placebo as shown in **Figure3**.
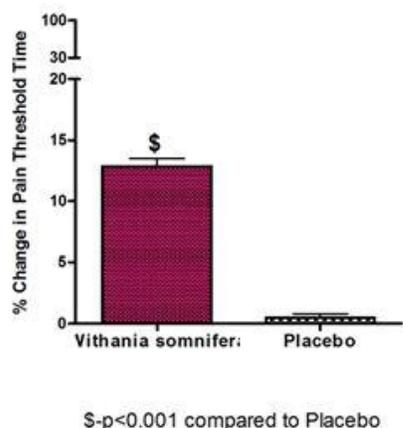
$-p<0.001 compared to Placebo

Figure3. Percentage change in Pain Threshold Time with *Withania somnifera* and placebo

Compliance to study medication was excellent as it was supervised administration. Both the medications were well tolerated, no subject discontinued the study because of adverse events. All safety laboratory parameters, ECG were repeated after the test procedure with placebo and standardised aqueous extract of *Withania somnifera* and were found to be within normal limits.

The present study was conducted to evaluate the analgesic activity of *Withania somnifera* (SENSORIL®) in healthy human volunteers using hot air pain model. Widely regarded as the oldest form of healthcare in the world, Ayurveda is an intricate medical system that originated in India thousands of years ago. *Withania somnifera*, also known as ashwagandha, is a plant in the Solanaceae and is used extensively as a herb in Ayurvedic medicine. It is used for the treatment of various ailments owing to its myriad properties (Mir 2012). In the present study we found that standardised aqueous extract of *Withania somnifera* improved the Pain Threshold Time significantly following single oral dose (1000mg) compared to placebo using Hot Air Analgesiometer. This experimental pain model was found to be sensitive in humans.

Withania somnifera was reported to show potent analgesic and antipyretic effect with the absence of gastric damage at different dose levels in experimental models in animals , with withanolide glycosides being the active ingredients(Budhiraja et al, 1989)Though there is not much data on the effects of *Withania somnifera* on pain threshold time in healthy human participants, research done by Anbalagan et al, as early as in 1984; and Al-Hindawi et al, in 1992 on the extracts of *Withania somnifera* in a variety of rheumatological conditions, reported that *Withania somnifera*

significantly improved the symptoms including pain and disability associated with these conditions. In the present study there was significant increase in the mean percentage change in pain threshold time(12.85%)compared to placebo. The mechanism by which *Withania somnifera* exerted the beneficial effects is presently not clear, however previous animal studies done by Pradeep.S et al for the Preclinical evaluation of anti-nociceptive effect of *Withania somnifera* (ashwagandha) in diabetic peripheral neuropathic rat models found to be mediated both by the central and peripheral mechanisms. (Sahni Y. P. 1995, Pradeep.S 2000)Sabina et al in 2009 tested *Withania somnifera* containing withanolides(withaferinA) to evaluate the analgesic, anti-pyretic and ulcerogenic effect in mice compared to indomethacin. The analgesic activity was tested using acetic acid induced abdominal constriction and hot plate tests the results of this study has clearly shown that the active constituents of withania –the withanolides produced profound analgesia without causing any gastric damage demonstrating the effectiveness of *Withania somnifera* as an analgesic.

Clinical trials have shown that *Withania somnifera* can alleviate pain in chronic painful conditions like rheumatoid arthritis, osteoarthritis without having any distressful side effects (Kulkarni et al, 1999) .These studies have shown that besides the symptoms, inflammatory markers like ESR, RA factor have decreased significantly in the *Withania somnifera* group compared to control group (Thamaraiselvi et al, 2012).These results are in agreement with our data where we could demonstrate the beneficial effects of standardised aqueous extract of *Withania somnifera* on pain threshold in healthy subjects.(Nalini et al, 2012)

4. Conclusion

Relief of pain is the most important aspect of treating patients suffering from various disorders. In the present study treatment with standardised aqueous extract of *Withania somnifera* produced significant increase in Pain Threshold time compared baseline and placebo. Further studies are needed to confirm the results of the present study in patients suffering from chronic pain so that this drug can be effectively used as a good therapeutic alternative in patients like osteoarthritis, rheumatoid arthritis and other pain conditions requiring long term analgesics as it is devoid of their distressing side effects.

Acknowledgemets

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