Annals of Phytomedicine 6(1): 107-113, 2017
DOI: 10.21276/ap.2017.6.1.16 ; Print ISSN : 2278-9839 and Online ISSN : 2393-9885
Ann. Phytomed., 6(1):107-113 (2017)

Original article

Scientific validation of traditional wisdom on analgesic effect of selected plant sources

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Received January 3, 2017: Revised January 28, 2017: Accepted February 5, 2017: Published online June 30, 2017

Abstract

The World Health Organization (WHO) estimated that 80% of the population of developing countries rely on traditional medicines, mostly plant based drugs for their primary healthcare needs. Indian System of Medicine (ISM) mainly gives plant based remedies for most of the diseases. It offers most appropriate cure against many diseases like jaundice, asthma, arthritis, diabetes, *etc*. where there is no complete cure in allopathy. In the Indian System of Medicines, plants occupy a predominant place in the therapeutic field. Traditional knowledge is unique in any society which is fabricated on their sound belief, norms and the culture of the society to which they belong. Along with this, there is an urgent need to safe guard and reaffirm traditional wisdom about home remedies. The present study was undertaken on animals to find out the analgesic effect of selected plants, *i.e*., Tulsi (*Ocimum sanctum* L.), Guarpatha (*Aloe vera* L.), Ajwayan (*Hyoscymus niger* L.) and Sonth (*Zingiber officinale* L.) and their effective dose to relieve the pain threshold. The analgesic effect was statistically significant by dose concentration and type of plant. The maximum dose of 0.9 ml was most effective in comparison to other doses, indicating higher the concentration of extract, better is the effect. The results confirm the scientific base of using selected plant as home remedies to relieve pain threshold.

Key words: Analgesic effect, medicinal plants, threshold, traditional wisdom, inflammation

1. Introduction

India has 15 agroclimatic zones and 17000-18000 species of flowering plants of which 6000-7000 are estimated to have medicinal usage in folk and documented systems of medicine, like Ayurveda, Siddha, Unani and Homoeopathy. Medicinal plants are not only a major resource base for the traditional medicine and herbal industry but also provide livelihood and health security to a large segment of Indian population. The World Health Organization (WHO) estimated that 80% of the population of developing countries rely on traditional medicines, mostly plant based drugs, for their primary healthcare needs. Indian System of Medicine (ISM) mainly gives plant based remedies for most of the diseases. It offers most appropriate cure against many diseases like jaundice, asthma, arthritis, diabetes, *etc*., where there is no complete cure in allopathy (Biradar, 2014). The exploration of the effectiveness of plant-based drugs used in the traditional medicine has been given great considerations because they are cheap and have little side effects (Kakoti *et al*., 2013). Despite rapid strides and remarkable progress in scientific technology, the contribution of Indian indigenous drugs is still valuable. Therefore, there is a compelling need for detailed scientific validation of all traditional medicinal plant drugs to establish their efficacy and safety in light of modern science. Now, it is increasingly recognized that combination therapy is better to treat complex diseases involving several drug targets. With the traditional knowledge in the preparation of polyherbal formulations to treat various diseases, new rational polyherbal formulations have to be developed using extracts, active fractions and for active principles (Subramoniam, 2016).

Traditional knowledge is unique in any society which is fabricated on their sound belief, norms and the culture of the society to which they belong. It is also termed as traditional wisdom and defined as the knowledge possessed by the rural persons, passed from their ancestors and practiced by them. Current trends in research and therapy are indicative of bright future prospects of indigenous drugs. In order to overcome the adverse effects of the conventional NSAIDs, there has been a search for analgesic activity in indigenous drugs for years together. Various medicinal plants have been selected for scientific testing and screening of analgesic and anti-inflammatory action.

O. sanctum natively known as Tulsi, is mentioned in the Charaka Samhita, an ancient Ayurvedic text by Charaka for its medicinal importance. Its extracts are used in Ayurvedic remedies for common colds, headaches, stomach disorders, heart disease, inflammatory and allergic disorders (Kalabharti *et al.*, 2011). *A. vera* is also known as Guarpatha, a succulent plant having fleshy, thick, serrated, lanceolate shaped green to grayish leaves and is strongly considered a beneficial agent for wound healing, protecting mucous membrane, treating ulcer, preventing diabetes and being effective in skin care

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(Ni and Tizard, 2004; Ni *et al.*, 2004; Eshun and He, 2004; Boudreau and Beland, 2006). *Z. officinale* (Sonth) has been used in traditional medicine to aid digestion and treat stomach upset, diarrhea, nausea and arthritis for centuries. In addition to this, sonth is used as support in inflammatory conditions such as arthritis and may even be used in heart disease or cancer and androgenic property (Morakinyo and Adeniyi, 2008). *H. niger* (Ajwain) has been commonly used in traditional medicine systems for a variety of medicinal and pharmacological aspects (Lateef *et al.*, 2006). Also, it was used for the reduction of undesired effects related to the opioid withdrawal. It was also introduced as a potent analgesic and anti-inflammatory agent. Therefore, it was applied on the affected area solely or in combination with egg white or honey. Persian practitioners used ajwain in chronic fevers and gripes (Tonekaboni and Tohfat, 2007).

Analgesic drugs are one of the most products that are used in numerous diseases for alleviating the pain. Most analgesic drugs, accessible in the market, exhibit an extensive range of adverse effects including gastrointestinal disorders, kidney problems and other unwanted effects. Due to potential side effects and low efficacy of synthetic and chemical drugs, consumption of other complementary drugs especially herbal remedies to control pain is increasing (Nasri and Shirzad, 2013). Medicinal plants have been suggested to presence reliable remedies for prevention and treatment of pain related conditions. Keeping India's rich biodiversity in mind, the present research work has been undertaken on animals to find out the analgesic effect of selected plants.

2. Materials and Methods

The study was conducted in R.N.T. Medical College, Udaipur, Rajasthan, India. The experimental protocol of the study was approved by the Institutional Animal Ethical Committee (IAEC) of the University. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (916|ac|05-CPCSEA). The animals were procured from Chaudhary Charan Singh Haryana Agricultural University. (CCSHAU), Hissar, Haryana, India.

2.1 Selection of plants

The plants having analgesic properties and are being used as a remedy at home level were identified on the basis of available literature. These four plants, *i.e.* Tulsi (*O. sanctum*), Guarpatha (*A. vera*), Ajwaian (*H. niger*), and Sonth *(Z. officinale)* were selected. The voucher specimen of plants were authenticated by Dr. Anand Singh Jodha, Horticulturist, K.V.K, Udaipur, India. Herbarium Voucher specimen (Ajwain-01-98; Guarpatha-02-98; Sonth-03-98; Tulsi-04-98) were prepared and deposited in the Department of Horticulture, R.C.A., Udaipur, India for future reference.

2.2 Selection of animals

A total of 50 healthy albino rats were selected for the study on the basis of their age and weight. The albino rats were procured from C.C.S., Haryana Agriculture University, Hissar, India. The rats aged more than 9 weeks were taken for the purpose. The weights of these animals ranged between 85 to 205 g. The experimental group consisted of rats of both sexes equally. All the animals were physically examined for not having any disease or injury before experiment. For first 6 days, all the 50 animals were fed on standard laboratory diet and acclimatized to the conditions of the animal house. Rats were divided into 8 groups, consisted of six rats in each of the group. Two rats were kept aside for any replacement.

2.3 Preparation of doses

The preparation of doses was carried out with each plant extracts separately. No combinations were used. Fresh leaves of tulsi were taken, cleaned and crushed in a mortar to prepare a paste. Fresh guarpatha leaves were taken and peeled to take out its pulp. The seeds of ajwaian and stem of sonth in dried form were selected to make the extract from these plants and each was used separately for the purpose. The extracts from plants were prepared using a standardized technique as suggested by Khandal (1992). For preparation of doses: one part of plant material, eight parts of water was added and boiled till it reduced to one-fourth of its content. The boiled mixture was then strained through a linen cloth to get the extract which was cooled at room temperature. From this extract, three doses, *i.e.* mild, moderate and maximum were prepared for each plant. The doses are as follows:

- Control dose
  0.9 ml of distilled water as placebo
- Dose (I) : Mild dose
  0.3 ml extract + 0.6 ml distilled water
- Dose (II) : Moderate dose
  0.6 ml extract + 0.3 ml distilled water
- Dose (III) : Maximum dose
  0.9 ml extract + 0.0 ml distilled water

Name of the plant	No. of animals with each dose			
	Control dose	Mild dose	Moderate dose	Maximum dose
Ajwain	6	6	6	6
Guarpatha	6	6	6	6
Sonth	6	6	6	6
Tulsi	6	6	6	6

Six animals were grouped for each dose of each plant.

2.4 Experiment

Artificial pain was induced using 'rat tail method' (Davies *et al.*, 1946). In this method, an apparatus 'technoanalgesiometer' was used. The test dose was given to rats intraperitonially in form of aqueous suspension through tuberculin syringe to find out the analgesic effect. The experiment was conducted in two parts: once with two plants, *i.e.*, tulsi and guarpatha in a day on a group of 24+24 rats. Then a gap of 15 days was maintained before conducting the second part of experiment with remaining two plants, *i.e.*, sonth and ajwaian. With each plant material, a different group of 6 rats were used as a control group to avoid variations in readings of control group at each stage of experiment and to have conformity in results. During this gap, a standard laboratory diet was fed to these rats and they have been kept comfortably in a same manner as prior to the experiment. Analgesia is reflected in a prolongation of the reaction time, the increase over normal in the mean reaction time of the treated animals was taken as a measure of analgesic effect. The

reaction time before and after the drug treatment was noted up to two hours with an interval of 15 minutes up to 1 hour and 30 minutes in next one hour.

2.5 Statistical analysis

The data were statistically analyzed, using one-way analysis of variance (ANOVA) for mean reaction time by different plants and doses at different time intervals, *t*-test for analgesic effect of different plants and regression analysis for functional relationship between various doses of selected plants at different time intervals. All values are expressed as mean ± SE.

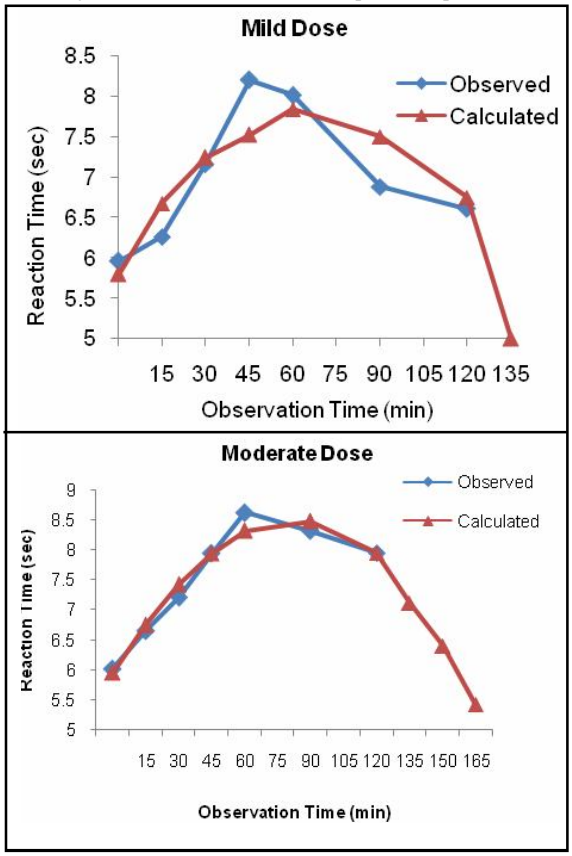
The study was conducted to evaluate the analgesic effect of selected plants, used as home remedies on the albino rats in the laboratory.

3. Results and Discussion

To assess the traditional wisdom used in India, four plants namely; tulsi, guarpatha, sonth and ajwaian were evaluated for their analgesic effects. Results revealed that the analgesic activity of four plants was statistically significant with all the three doses ($p \leq 0.01$).

3.1 Ajwaian (*Hyoscyamus niger* L.)

The mild dose of ajwaian (Figure 1) showed a significant increase in reaction time at 30 and 45 min. Peak effect was observed at 60 min which was 24.13% from initial value. The moderate dose showed an increase of 43.36% and maximum dose of 68.70% at 60 min from its initial observation. The regression analysis showed that the mild dose would be effective upto 2 h and 15 min; moderate and maximum dose up to 2 h and 45 min. Petramfar *et al*. (2013) conducted a randomized controlled placebo control clinical trial, the herb essential oil was assayed for the analgesic effect in neuropathic feet burn. Results revealed that ajwain essential oil significantly reduced the feet burn compared to placebo.



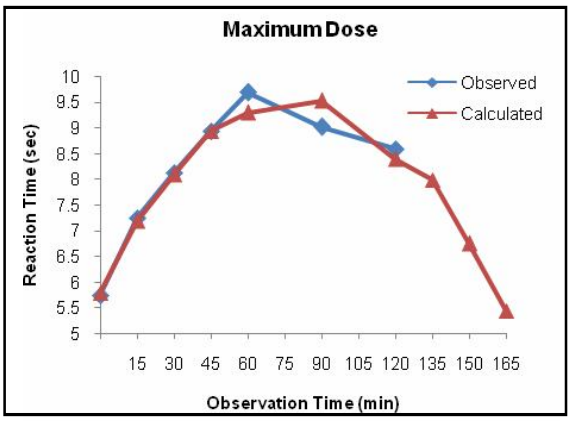
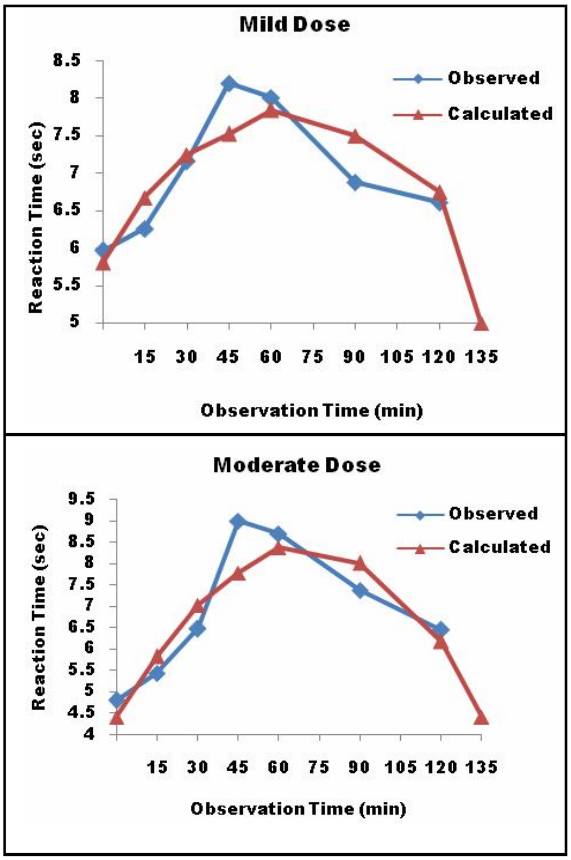


Figure 1: Regression line of analgesic effect of ajwaian (*H. niger*).

3.2 Guarpatha (*Aloe vera* L.)

The peak effect of guarpatha was observed at 45 min with all the three doses. The percentage increase in mean reaction time observed was 37.58% with the mild doses, 87.5% with moderate dose and 138.10% with the maximum dose from the initial values. The regression line showed that the effect of guarpatha would be minimized up to 2 h and become negligible at further 15 min irrespective of dose concentration (Figure 2). Zhang *et al*. (2006) found that aqueous extract of *A. vera* exhibited antinociceptive activity, which indicates both central and peripherally mediated antinociceptive properties. The analgesic activity of the plant is attributed to the presence of the enzymes, carboxypeptidases and bradykinase that tend to relieve pain.



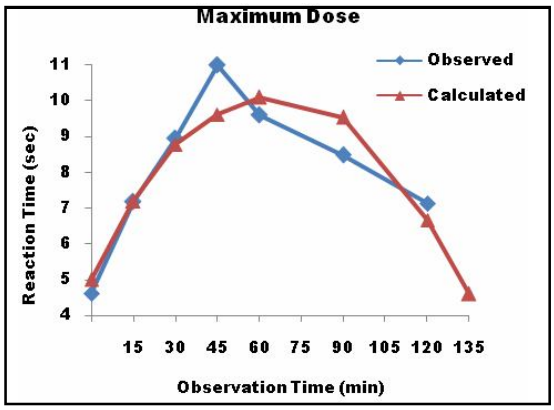
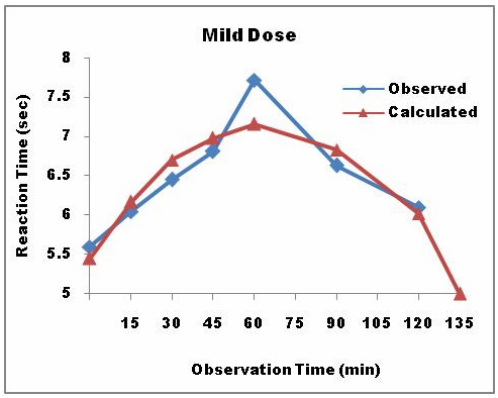
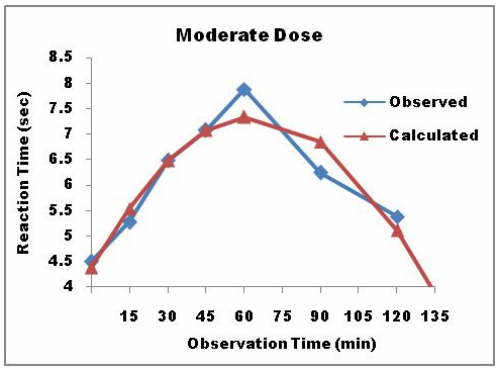


Figure 2: Regression line of analgesic effect of guarpatha (*A. vera*).

3.3 Sonth (*Zingiber officinale* L.)

The mild, moderate and maximum doses of sonth showed a peak effect at 60 min and the increase in reaction time was of 26.56%, 75.11%, and 51.09%, respectively from their initial values. The regression analysis indicated that sonth would be effective up to 2 h and 15 min (Figure 3). The anti-inflammatory activities of compounds obtained from ginger essential oil (GEO) have been reported by various investigations using ginger extract. These anti-inflammatory actions could be owing to the inhibition of prostaglandin release and hence, ginger may act in a way similar to other non-steroidal anti-inflammatory drugs which interfere with prostaglandin biosynthesis. GEO was found to contain monoterpenes and sesquiterpenes as principal compounds, suggesting that the anti-inflammatory and analgesic effects could be correlated to these essential oil constituents (Vendruscolo and Takaki, 2006).





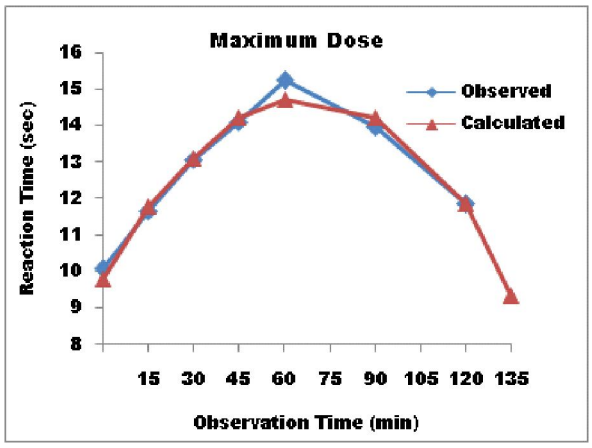
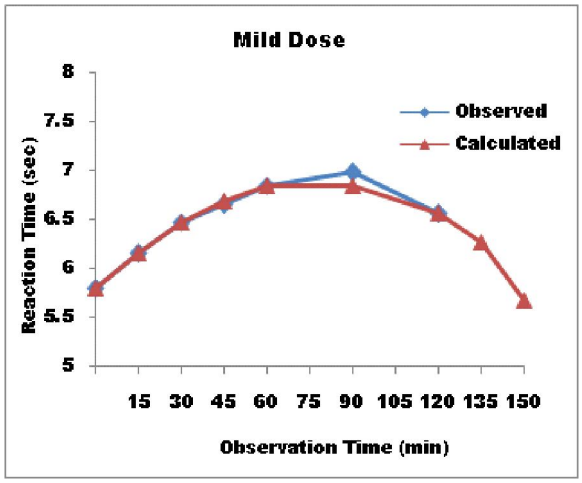
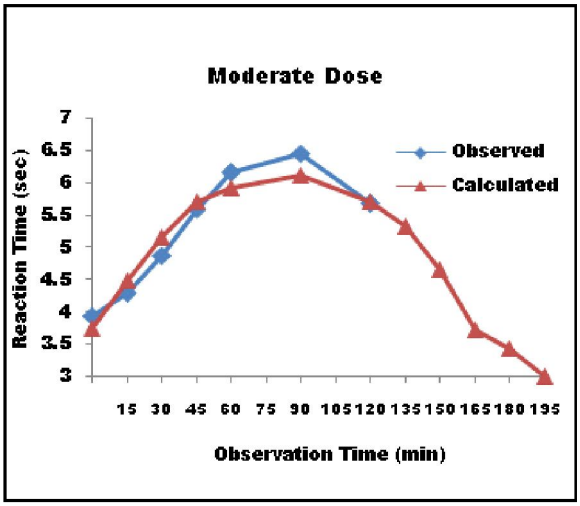


Figure 3: Regression line of analgesic effect of sonth (*Z. officinale*).

3.4 Tulsi (*Ocimum sanctum* L.)

Tulsi showed an increase of 20.34% with mild dose, 43.80% with moderate dose and of 51.47% with maximum dose at 90 min after injection. The regression line indicated the analgesic effect remain up to 3 h irrespective of dose concentration (Figure 4).





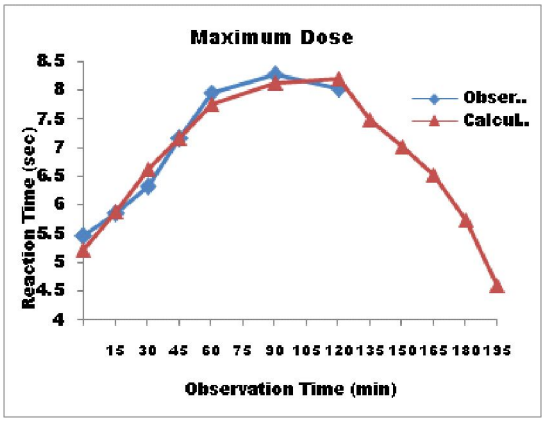


Figure 4: Regression line of analgesic effect of tulsi (*O. sanctum*).

A study conducted by Tiwari *et al.* (2016) showed that the leaf extracts of *Murraya koenigii, Agele marmelos, Azadirachta indica* and *Ocimum sanctum* showed significant scavenging activity against free radicals which may be correlated with the presence of phenolic compounds in extensive amount in these extracts. Other studies also reported that the presence of phenolic compounds including flavonoids is responsible for DPPH scavenging activity in plant extract (Dehpour *et al.*, 2009; Uddin *et al.*, 2015; Pongjanta *et al.*, 2016). *O. sanctum* leaf was also used by folk medicinal practitioners (FMP) as an analgesic to treat rheumatic pain. The analgesic and anti-inflammatory effects of leaves of the plant have been demonstrated (Hannan *et al.*, 2011). Comparison of analgesic effect of selected plants showed that at 15 and 30 min, the difference in mean reaction time was not significant between plants. But guarpatha showed a significant higher ($p \geq 0.05$) mean reaction time at 45 min than tulsi and ajwaian, indicating a better analgesic effect than other plants. At 60 min of observation, guarpatha and sonth showed a significantly better analgesic effect than tulsi. Further at 90 and 120 min. the difference in mean reaction time was not found statistically significant among four plants. The analgesic effect was statistically significant by dose concentration and type of plant (Figure 5 and Tables 1a b c). Guarpatha showed an early and best effect (6.38 sec) amongst four plants reaching to its peak at 45 min, followed by sonth (5.05 sec) and ajwaian (3.95 sec) which showed peak effect at 60 min. Tulsi showed its peak effect (2.80 sec) at 90 min, which is slow and lowest among selected plants. The maximum dose of 0.9 ml was most effective in comparison to other doses, indicating higher the concentration of extract, better is the effect.

Table 1(a): Mean ± S.E. values of tail withdrawal reaction time (sec) observed at various time intervals using selected plants

S. No.	Plants	Time dose	Before injecting the drug	15 min	30 min	45 min	60 min	90 min	120 min
1	Ajwain	Control	5.53 ± 0.38	5.63 ± 0.56	5.47 ± 0.37	5.65 ± 0.37	5.58 ± 0.35	5.68 ± 0.35	5.58 ± 0.36
		Mild	5.76 ± 0.20	6.10 ± 0.22	6.46 ± 0.24	7.11 ± 0.21	7.15 ± 0.18	6.96 ± 0.19	6.66 ± 0.23
		Moderate	6.02 ± 0.13	6.65 ± 0.21	7.21 ± 0.19	7.95 ± 0.18	8.63 ± 0.21	8.33 ± 0.11	7.96 ± 0.22
		Maximum	5.75 ± 0.24	7.25 ± 0.31	8.13 ± 0.36	8.95 ± 0.31	9.70 ± 0.27	9.03 ± 0.46	8.60 ± 0.39
2	Guarpatha	Control	6.68 ± 0.43	6.63 ± 0.37	6.73 ± 0.47	6.55 ± 0.43	6.53 ± 0.43	6.70 ± 0.50	6.31 ± 0.43
		Mild	5.96 ± 0.22	6.26 ± 0.23	7.16 ± 0.19	8.20 ± 0.24	8.01 ± 0.22	6.88 ± 0.34	6.61 ± 0.25
		Moderate	4.80 ± 0.24	5.43 ± 0.30	6.48 ± 0.26	9.00 ± 0.25	8.70 ± 0.28	7.38 ± 0.30	6.45 ± 0.41
		Maximum	4.62 ± 0.50	7.18 ± 0.44	8.95 ± 0.32	11.00 ± 0.51	9.60 ± 0.47	8.48 ± 0.56	7.13 ± 0.50
3	Sonth	Control	6.43± 0.61	6.70 ± 0.80	6.50 ± 0.88	6.68 ± 0.94	6.73 ± 0.78	6.66 ± 0.85	6.80 ± 0.76
		Mild	5.60± 0.91	6.05 ± 0.90	6.45 ± 0.83	6.81 ± 0.83	7.72 ± 0.93	6.63 ± 0.74	6.10 ± 0.79
		Moderate	4.50± 0.50	5.28 ± 0.36	6.48 ± 0.60	7.08 ± 0.68	7.88 ± 0.72	6.25 ± 0.58	5.38 ± 0.31
		Maximum	10.08± 0.36	11.67 ± 0.46	13.08 ± 0.67	14.10± 0.47	15.23 ± 0.48	13.95 ± 0.37	11.85 ± 0.48
4	Tulsi	Control	6.23± 0.56	6.35± 0.57	6.16 ± 0.47	6.40 ± 0.61	6.36 ± 0.67	6.38 ± 0.47	6.18 ± 0.63
		Mild	5.80± 0.60	6.16 ± 0.61	6.48 ± 0.61	6.66 ± 0.63	6.85 ± 0.62	6.98 ± 0.66	6.56 ± 0.51
		Moderate	3.95± 0.29	4.30 ± 0.25	4.88 ± 0.38	5.60 ± 0.38	6.16 ± 0.38	6.46 ± 0.38	5.68 ± 0.32
		Maximum	5.46±0.75	5.86 ± 0.74	6.32 ± 0.74	7.17 ± 0.65	7.96 ± 0.59	8.27 ± 0.58	8.03 ± 0.61
	Level of significance			**	**	**	**	**	**

** $p \geq 0.01$

Table 1(b): Analysis of variance of difference in mean increase in reaction time by four selected plants

Time Intervals	Ajwain	Guarpatha	Sonth	Tulsi	CD at 5%	L.S.
15 min	0.642	0.863	0.679	0.313	0.7255	NS
30 min	1.050	1.817	1.367	0.579	1.030	NS
45 min	1.692	3.154	1.929	1.083	1.4075	**
60 min	2.000	2.696	2.717	1.433	0.8163	**
90 min	1.740	1.842	1.692	1.638	0.5642	NS
120 min	1.438	1.113	0.850	1.287	0.5681	NS

NS = Non significant
** Significant at 5% and 1% levels of significance ($p \geq 0.01$)

Table 1(c): Analysis of variance of difference in mean reaction time by dose concentration

Time Intervals	Control	Mild	Moderate	Maximum	CD at 5%	L.S.
15 min	0.071	0.363	0.604	1.458	0.7255	*
30 min	-0.021	0.858	1.442	2.533	1.030	**
45 min	0.087	1.417	2.617	3.737	1.4075	**
60 min	0.046	1.650	3.029	4.121	0.8163	**
90 min	0.112	1.083	2.295	3.421	0.5642	**
120 min	-0.008	0.704	1.596	2.396	0.5681	**

* Significant at 5% level of significance $p \geq 0.05$

** Significant at 5% and 1% level of significance ($p \geq 0.05$, $p \geq 0.01$)

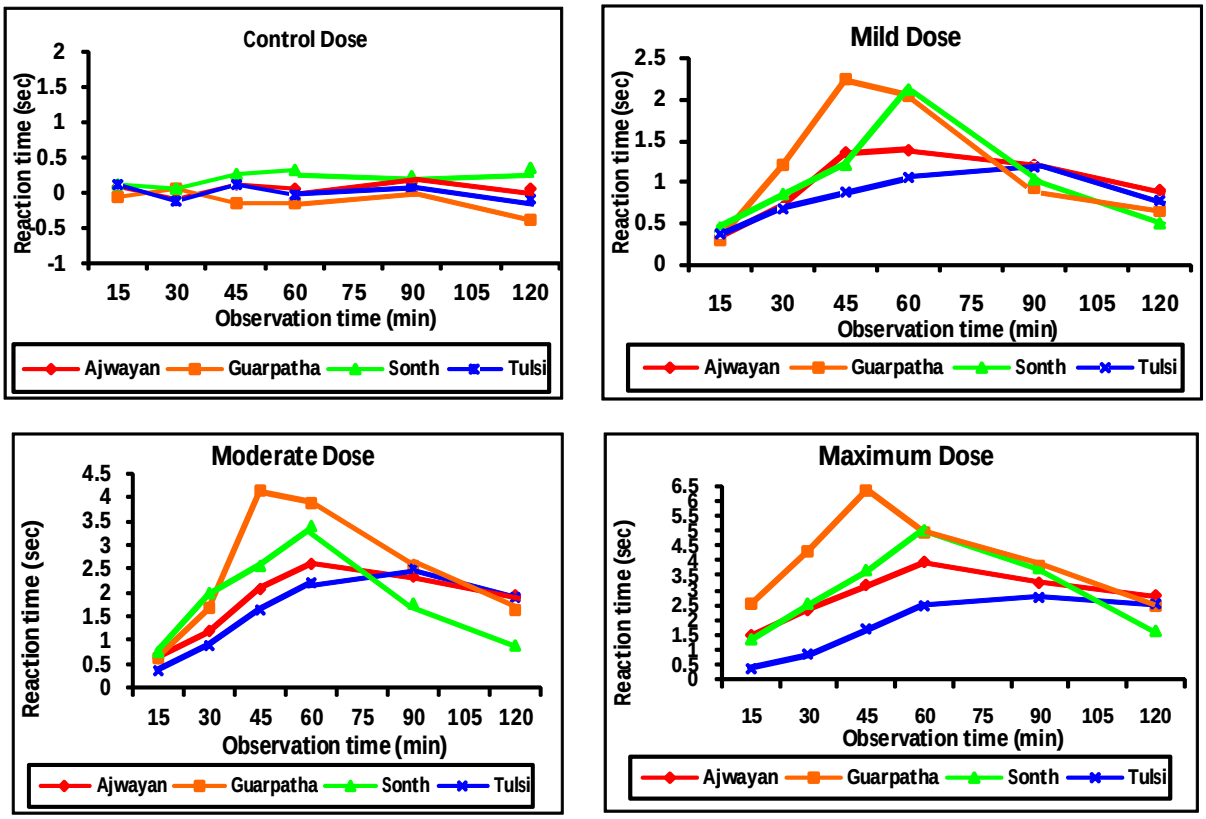


Figure 5: Comparison of analgesic effect of different doses of selected plants at different time intervals.

4. Conclusion

Guarpatha showed an early and best effect amongst four plants reaching to its peak, followed by sonth and ajwain. Tulsi showed slow and lowest peak pain relieving effect among selected plants. The maximum dose was most effective in comparison to other doses, indicating higher the concentration of extract, better is the effect. The result confirms that all the four selected plants showed analgesic activity to relieve pain threshold.

Conflict of interest

We declare that we have no conflict of interest.

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