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Exploring the Analgesic Potential of Homeopathic Formulation of Tincture Apis

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Abstract

Apis is a homeopathic formulation developed from the venom of *Apis mellifera*. *Apis* is extensively used to treat various skin disorders, pleurisy, and peritonitis. The Global Burden of Disease Study 2016 firmly stated the high prominence of pain and pain-related diseases are the leading cause of disability and disease burden globally. The current investigation was aimed to explore the analgesic effect of homeopathic formulation tincture *Apis* using formalin induced flinching, acetic acid induced writhing and Eddy's hot plate test. Tincture *Apis* pretreatment significantly reduced the number of flinching's in both neurogenic and inflammatory phase in formalin test. Moreover, in Acetic acid and Eddy's hot plate test, tincture *Apis* pre-treatment significantly reduced the acetic acid induced writhing's and increased the paw licking latency, respectively. The effect of *Apis* was prominent to that observed with standard diclofenac in formalin and acetic acid test. Amino acid analysis revealed that tincture *Apis* contains 14 amino acids including seven essential amino acids. Furthermore, morphological studies revealed that administration of 10 times concentrated tincture *Apis* had no visible changes in acute toxicity screening such as change in skin color, salivation, convulsions, tremors, diarrhea, and paralysis or mortality in 14 day study. However, abnormal movement of body and eye were observed in the first 4 h. Overall outcome of present study revealed that tincture *Apis* has an excellent analgesic potential, which might be linked to the presence of essential amino acids including leucine, histidine, and GABA in tincture *Apis*.

Keywords: Acetic acid, Analgesic, *Apis*, Formalin, Pain

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Introduction

Pain is an enormous problem globally. Pain is the first symptom of a variety of ailments including traumatic injury and chronic organic disorders.[1] Pain is considered to be warning sign to institute medical treatment so as to prevent further deleterious changes in the body.[2] Sensory perception of pain is transmitted through nociceptors.[3] Nociception involves a complicated medley of inflammatory mediator such as cytokines, bradykinin, neutrophils, chemokines, and eicosanoids ion channels.[4] The Global Burden of Disease Study 2016 firmly stated the high prominence of pain and pain-related diseases are the leading cause of disability and disease burden globally.[5] The current therapeutic management of pain relies mainly on the non-steroidal anti-inflammatory (NSAIDS) agents and opioids.[6] The NSAIDS is mostly used for acute pain and inflammatory condition such as surface injuries and mild surgeries including dental extraction. However, the NSAID therapy is limited by the various side effects of these agents; chief of which is gastric ulceration.[7] Opioids, on the other hand, are prescribed for more severe referred pain arising from organic tissue damage such as severe referred pain in congestive heart failure.[8] Although the opioids are very effective in pain management, their tendency to precipitate tolerance and addiction has been of major concern.[3,9] Due the limitations of the classical analgesics, many efforts are being made to explore the alternative systems of managing pain. Homeopathy is an alternative therapy that works on the principle that "like heals like," that is, a disease can be cured by an agent that precipitates same symptoms as the inflammogen.[10] Homeopathy follows the law of "Infinitesimals," that is, lower the dose of the curative, the higher is the efficacy of the curative. The system of homeopathic practice is documented to be as old as 18th century.[10]

Tincture *Apis* is a homeopathic formulation developed from the venom of *Apis mellifera*. *Apis is* prepared from the stingers of the female honey bee. The active constituents of *Apis* include mellitin, apamin, and MCD peptide 401.[11] *Apis* also contains hyaluronic acid, apamin, adolpin, secapin, minimine, phospholipase A_2, histamine, etc.[12,13] Venom preparation of *Apis* (honey bee) has been reported to inhibit the mast cell degranulation induced by compound 48/80 treatment in BALB-c mice.[14] Apidaecin, a peptide found in bee venom, is known to modulate the human mast cells and monocytes, both of which play a central role in inflammatory process.[15] Studies have also revealed that bee venom ameliorates the inflammation induced neuronal injury and death by preventing mitochondrial disruption in transgenic mouse model of amyotrophic lateral sclerosis.[16]

Formalin-induced nociception and acetic acid-induced writhings are well accepted animal models for studying analgesic activity. These tests are important screening tools for assessing antinociceptive and anti-inflammatory agents.[17] Writhing is an overt body reaction to the enormous pain actuated by acetic acid through nociceptors described by episodes of withdrawal of midsection and extension of hind limbs. The signals passed to CNS in retaliation to pain caused by any irritant, results release of various mediators, that is, prostaglandins, which adds to the

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expanded aversion to nociceptors.[18] The present investigation was designed to explore analgesic potential of tincture Apis using formalin induced flinching's, acetic acid-induced writhing's, and Eddy's hot plate test.

Materials and Methods

Drugs and Chemicals

Tincure *Apis* was purchased from Jain Pharmacy, Amritsar. Diclofenac sodium was procured from Novartis India Ltd. Acetic acid and formalin were also procured from Sisco Research Laboratory Pvt. Ltd. (SRL).

Experimental Animals

Swiss albino mice of either sex (30–40 g) were procured from Indian Institute of Integrative Medicine, Jammu. Mice were housed in the animal house facility of GNDU. The room was maintained at a temperature of 25 ± 5°C with a 12:12 h of light-dark cycle. Animals had a free access to food and water during housing. All animals used in this study were fully approved by Institutional Animal Ethics Committee, constituted under CPCSEA (Protocol No. (226/CPCSEA 2015/09). Schematic representation of detailed experimental design used in formalin test is shown in Figure 1.

Pharmacological Studies

Formalin induced pain

About 2% freshly prepared solution of formalin in a volume of 50 µL was injected on to the plantar surface of the right hind paw of mice, subcutaneously. The mice were placed individually in an observation chamber and lickings and biting of the injected paw (flinchings) were monitored. Analgesic effect was determined in two phases. The early neurogenic phase (first phase) was recorded during the first 5 min, while the late, inflammatory phase (second phase) was recorded during the 25–30 min.[17,19]

Acetic acid-induced writhing test

Chemicals may also produce painful reactions in animals. Intraperitoneal injection of 1%, 10 ml/kg body weight of mice produces pain reactions which are called as writhing response.[14,16] The number of abdominal contractions (writhing's) was taken as measure of pain in this test. This behavior of animals was recorded for 30 min.[20]

Eddy's hot plate test

Temperature of hot plate (55 ± 1°C) does not cause any harm to skin but paws of mice are very sensitive to that temperature. Animals were individually placed on hot plate and time at which animal make hind paw lickings was recorded as an observation (paw licking latency). A total of 15 sec was kept as a cut off time to avoid any thermal injury to mice.[20] The latency of response was taken after 30 min of *Apis* injection**.**

Amino acid analysis of tincture Apis

The amino-acid fingerprinting of *Apis* tincture was obtained with the help of amino acid analyzer using amino acid digestion method. 0.5 ml of sample was mixed with 0.5 ml of 6% sulphosalicylic acid and kept in refrigerator for 24 h. From this, 0.2 ml of sample was taken and 0.8 ml of 0.1 N HCl was added to it. Thereafter, the mixture was filtered from 0.2-micron filter paper. The filtered aliquot was loaded in amino acid analyzer.

Acute Toxicity Studies

Acute toxicity study was carried out in accordance with OECD guidelines with a few modifications (OECD, 2021). Mice were divided into two groups each group contains three animals. In first group, the animals were administered normal saline. Whereas, in second group, the animals were administered tincture of *Apis* in 10 times concentrated form. The behavioral changes were recorded continuously for a period of 4 h followed by daily monitoring for 14 days after tincture *Apis* administration.

Statistical Analysis

All results were articulated as mean ± SEM. One-way analysis of variance followed by Tukey's multiple comparison test was used to establish statistical significance using the Graph pad prism software.

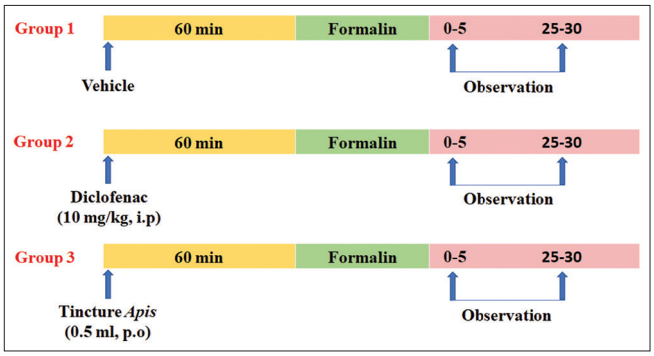

Figure 1: Schematic representation of detailed experimental design used in formalin test

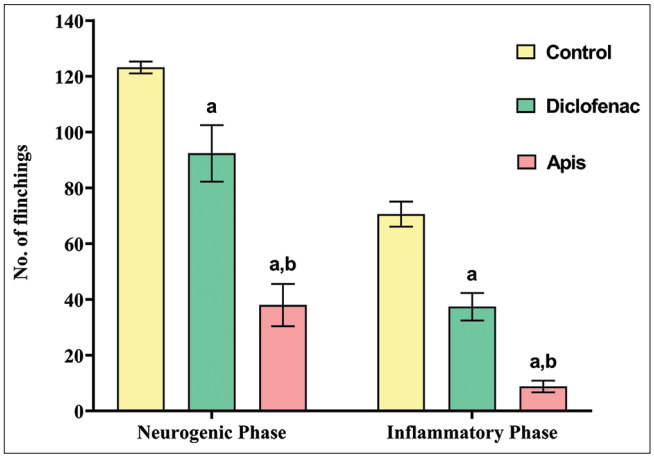

Figure 2: Effect of *Apis* on neurogenic and inflammatory phase in formalin-induced hyperalgesia. All values are expressed as mean ± SEM. Data were analyzed using a two-way analysis of variance followed by Tukey's test ($^{a}P < 0.05$ vs. formalin control group, $^{b}P < 0.05$ vs. diclofenac)

Results

Effect of *Apis* on Formalin Induced Nociception

Formalin is known to produce a biphasic pain response quantified by the number of flinching's an animal exhibits over a period of 60 min. The first phase is the neurogenic phase and occurs as early as 5–10 min of formalin injection whereas the second phase is the inflammatory phase which occurs between 30 and 45 min after formalin injection. Formalin treatment induced the flinching response (a measure of nociception) in mice. Standard diclofenac (10 mg/kg, i.p) pre-treatment significantly reduced the number of flinching's in both neurogenic and inflammatory phase as compared to control group. Similarly, *Apis* (0.5 ml, p.o) pre-treatment also reversed the effect of formalin in both neurogenic and inflammatory phase. A significant reduction in number of flinching was observed in *Apis* pre-treated group as compared to the formalin control group [Figure 2]. Area under curve data of total 60 min showed a significant decrease in AUC (flinchings 0–60 min) in diclofenac and *Apis* pretreated group as compared to formalin treated controls [Figure 3].

Effect of *Apis* on Acetic Acid Induced Writhings

Intraperitoneal administration of acetic acid (1%) was found to induce writhing response in mice. Standard diclofenac (10 mg/kg, i.p) pre-treatment was found to reduce the number of writhing's as compared to the control group. *Apis* (0.5 ml, p.o) pretreatment also reversed the effect of acetic acid. A significant reduction in number of writhing's was observed in *Apis* pretreated group as compared to the acetic acid control group. The effect of *Apis* was found to be more prominent than diclofenac [Figure 4a].

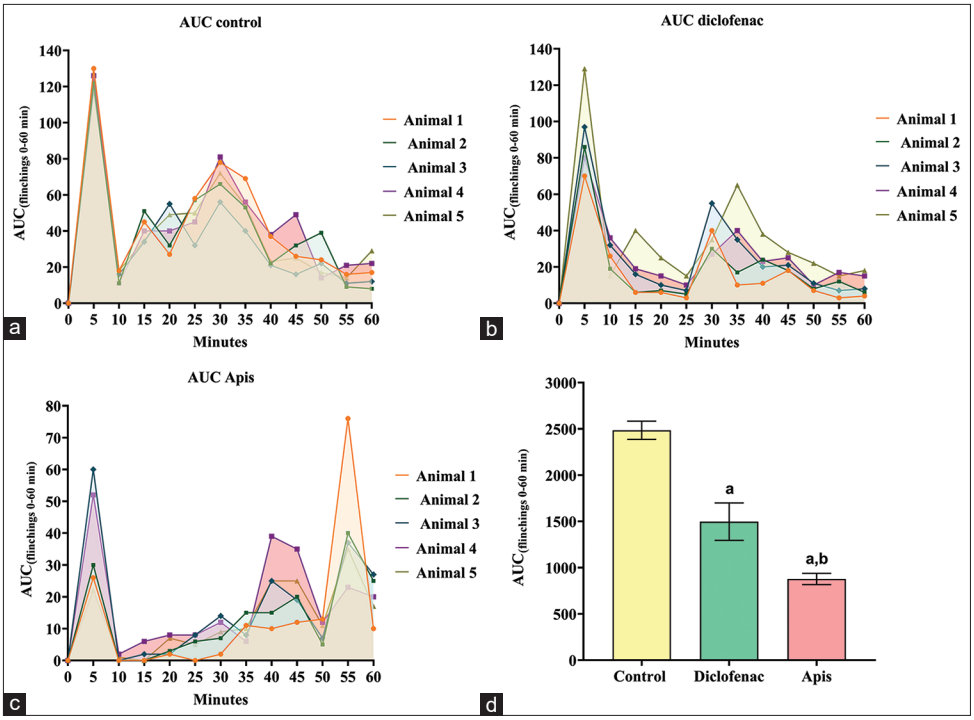

Figure 3: (a-d) Effect of various intervention on area under curve in formalin induced hyperalgesia test

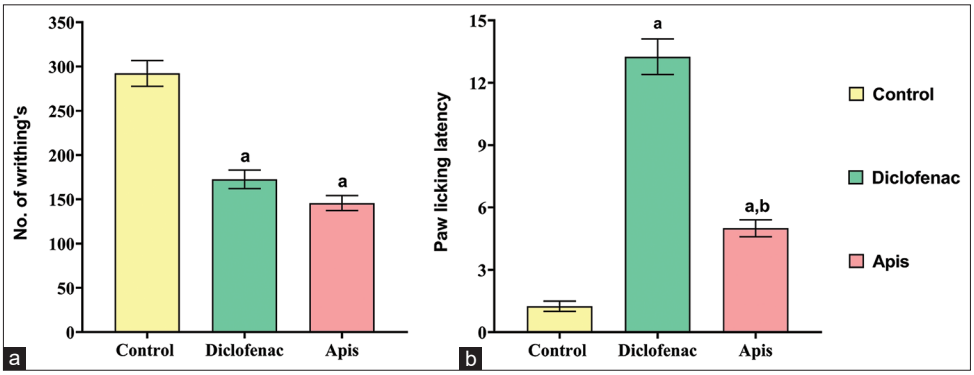

Figure 4: Effect of *Apis* on (a) acetic acid induced writhing and (b) paw licking latency in Eddy's hot plate test. All values are expressed as mean ± SEM. Data were analyzed using a one-way analysis of variance followed by Tukey's test ($^{a}P < 0.05$ vs. acetic acid control group, $^{b}P < 0.05$ vs. diclofenac)

Effect of *Apis* on Thermal Hyperalgesia in Eddy's Hot Plate

The normal control animals were found to show pain response i.e., a paw licking response in Eddy's hot plate at a temperature of 55 ± 1°C. Standard diclofenac (10 mg/kg, i.p) pre-treatment significantly increased the paw licking latency as compared to the normal control group. *Apis* (0.5 ml, p.o) pre-treatment also increased the paw licking latency as compared to the normal control group [Figure 4b].

Amino Acid Analysis of Tincture *Apis*

Amino acid analysis carried out in amino acid analyzer revealed that tincture *Apis* contain 14 amino acids; namely, aspartic acid, glutamic acid, asparagine, serine, histidine, GABA, arginine, cystine, valine, methionine, isoleucine, leucine, lysine, ornithine. The concentration of ornithine, methionine, histidine, serine, and aspartic acid was relatively higher [Figure 5 and Table 1].

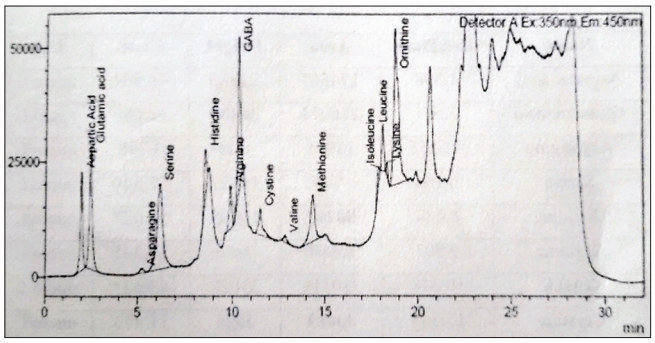

Figure 5: Amino acid analysis of tincture *Apis*

Table 1: Studied parameters in amino analysis of tincture *Apis*

Peak	*Name of Amino Acid*	*Ret. Time*	*Area*	*Height*	*Concentration*
1	Aspartic acid	1.998	174567	21385	48.564
2	Glutamic acid	2.501	276074	26469	42.686
3	Asparagine	5.5251	13985	900	3.798
4	Serine	6.194	397157	18421	57.849
5	Histidine	8.583	98292	10205	58.627
6	Arginine	9.907	85901	9276	11.335
7	GABA	10.378	310118	33027	43.627
8	Cystine	11.505	80683	5522	11.473
9	Valine	12.848	18100	1289	15.504
10	Methionine	14.360	185457	9990	66.819
11	Isoleucine	17.942	32771	3322	7.090
12	Leucine	18.162	81478	9696	16.107
13	Lysine	18.387	16428	3053	0.000
14	Ornithine	18.841	582960	34183	128.131
Total			2353970	186738	

Effect of Tincture *Apis* on Morphological Parameters

Acute toxicity studies revealed that 10 times concentrated tincture *Apis* administration had no sign of change in skin color, salivation, convulsions, tremors, diarrhea, paralysis, and paw licking. However, abnormal movement of body was observed with for the first 4 h after administration. Thereafter, no abnormal movement of body was observed. Besides, this abnormal eye movement was also observed with 10 times concentrated tincture *Apis* [Tables 2 and 3].

Discussion

Management of different types of pain is a very big challenge. Some of the existing treatments have addiction potentials, whereas NSAIDS is associated serious side effects.[21] The present study explored the analgesic potential of tincture *Apis*. The outcome of this study revealed that homeopathic formulation of *A. mellifera*, that is, tincture *Apis* significantly abrogated the formalin- and acetic acid-induced nociception. Formalin-induced nociception and acetic acid-induced writhings are well accepted animal models to study the analgesic potential of different pharmacological interventions.[17] In humans, formalin injection on index finger has been reported to produce a sharp and burning pain. After an interval of 5 min, it has been replaced by a steady, and throbbing ache, which get disappeared after a period of 30–60 min.[22] In this study, formalin and acetic acid treatment induced the flinching's and writhing's response in mice, respectively. Tincture *Apis* significantly attenuated chemical hyperalgesia as evidenced by a significant reduction in the number of flinching's in formalin test, and writhing's in acetic acid test. Moreover, it also abrogated the thermal hyperalgesia as evidenced by an increase in paw licking latency (response time) in the Eddy's hot plate test.

Depletion of neurotransmitters due to pain syndrome and treatments might add to an unfortunate treatment result. Deficiency of neurotransmitters might be linked to enhanced turnover rate or insufficient intake of essential and conditional/semi essential amino acids.[21] Shell *et al*. documented that amino acid precursor's treatment results a reduction in chronic back pain and inflammation.[21] Furthermore, Miller amd Butler studied potential of redox active plants in combination with L-Leucine against osteoarthritis. A significant decrease in pain score was

Table 2 : Effect of tincture *Apis* on the morphological parameters in acute toxicity studies (0–120 min)

Observations	*30 min*				*60 min*				*90 min*				*120 min*			
	C	*A1*	*A2*	*A3*	*C*	*A1*	*A2*	*A3*	*C*	*A1*	*A2*	*A3*	*C*	*A1*	*A2*	*A3*
Skin and fur color	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal Eye movement	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+
Salivation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lethargy	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+
Mild jerks	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+
Convulsions	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal movement	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+
Paralysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Paw licking	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*C indicates mean of control animals (*n*=3); A, A2, A3 indicate 3 tincture *Apis* treated animals

Table 3: Effect of tincture *Apis* on the morphological parameters in acute toxicity studies (from 120 to 240 min)

Observations	*150 min*				*180 min*				*210 min*				*240 min*			
	C	*A1*	*A2*	*A3*	*C*	*A1*	*A2*	*A3*	*C*	*A1*	*A2*	*A3*	*C*	*A1*	*A2*	*A3*
Skin and fur color	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal Eye movement	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lethargy	-	-	-	-	-	+	+	+	-	-	+	+	-	+	+	+
Mild jerks	-	+	+	+	-	-	+	+	-	+	+	+	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal movement	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+
Paralysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Paw licking	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*C indicates mean of control group (*n*=3); A1, A2, A3 indicate tincture *Apis* treated animals

observed with this mixture.[23] Moreover, systemic treatment of L-arginine exerts an anti-nociceptive effect and also upregulates the brain kyotorphin, an analgesic peptide.[24] In formalin test, isovaline has been found to reduce formalin-induced nociception. It has been reported that analgesic potential of isovaline is devoid of toxicity.[24,25] Amino acid analysis investigation showed that tincture *Apis* contain 14 amino acids including seven essential amino acids, that is, Arginine, leucine, isoleucine, lysine, histidine, valine, and methionine. Conclusively, the outcomes of current investigation delineate that analgesic potential of tincture *Apis* may be due to the presence of inhibitory amino acids such as GABA and arginine. Further exploration studies on its active constituent might give a good lead molecule to manage pain.

Conclusion

Overall outcomes of current investigation delineate that the analgesic potential of tincture Apis might be attributed to the presence of inhibitory amino acids such as GABA and arginine.

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