ANTINOCICEPTIVE EFFECTS OF SAUSSUREA LAPPA ROOTS ETHANOL EXTRACT IN EXPERIMENTAL ANIMALS

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Abstract

The current study was designed to investigate both central and peripheral analgesic activities of crude extract of roots of Saussurea lappa (S. lappa) plant at 100% ethanol concentration. These activities were processed and evaluated through Eddy's hot plate and acetic acid induced-writhing methods, respectively, on laboratory Swiss albino mice. Reference positive control used was paracetamol, and negative control was distilled water, and statistical analysis done by SPSS version 25 to test one-way ANOVA for group mean differences, followed by Tukey and Dunnett two-sided post-hoc tests. Before the mentioned assessment, an acute oral toxicity test by a fixed-dose procedure method (FDP) was taken to check the safety of the plant. This plant was safe under experimentation, and it exerted significant ($P < 0.01$) analgesic effects at a dose of 500mg/kg. The crude extract revealed a noticeable increase of latency time to thermal pain stimuli when compared to paracetamol positive control at 100mg/kg concentration by 36.42%, and it reduced the number of writhing contractions by 21.73% when compared even to the positive control. It is presumed that S. lappa contains particular phytoconstituents that are responsible for presenting these effects. In both applied tests, S. lappa proved that it could be used as a safe, powerful analgesic treatment.

Key words: *Central Analgesic effect, Crude extract, Peripheral Analgesic effect Saussurea lappa, Soxhlet extraction.*

Introduction

Pain is an odious sensation and protective mechanism associated with tissue damage due to burns, wounds, irritants, and ischemia (1). Pain itself is not a disease, but rather a medical symptom. It can range from mild localized discomfort to agony feelings to present as acute or chronic pain that can be debilitating. Thus, by identifying the source/cause of pain, it will help to prevent or control it. Sometimes ache source cannot be identified, and pain control needs the intervention of another agent (drug). Thus, analgesics are added in the management of patient's prerequisites. Pain analgesics are considered as one of the most important therapies in the medical field; analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) are the utmost popularly used agents these days as pain killers, which relief nonspecifically for both acute and chronic pain (2).

Aniline analgesics, NSAIDs, opioids, and analgesic adjuvants are the main pharmacological therapies available for pain management in modern medicine. Prolonged usage of these drugs leads to undesirable side effects such as gastrointestinal bleeding, peptic ulcer, renal toxicity, respiratory depression, and dependence and addiction. These effective pain-relieving medicines have limited usage due to their adverse effects. Thus, research has been conducted to find potent alternative analgesics with fewer side effects (3).

Herbal medicine refers to the use of medicinal plants as a treatment for various health conditions (4). It shows a rapid and substantial growth in use due to its effectiveness that historically recorded without noticed side effects. Medicinal plants are rich in secondary metabolites that can be extracted, purified, and used as natural chemical treatments. Many plants were recognized by ancient people to be used as pain killers, such as chamomile and cannabis. Saussurea lappa (S. lappa) is one of the useful medicinal plants used in Asia a long time ago and shows many beneficial medical activities.

Anciently, S. lappa used by Korean physicians to treat many conditions, such as peptic ulcer, asthma, cough, and pharyngitis. Even it is used as an anti-inflammatory, anti-septic, and anti-tumor agent (5). This plant considered as one of the most widely used ethno-pharmacological plants in the Shopian forest of Kashmir (6). S. lappa family is widely spread in Pakistan and India. It is useful in the treatment of many medical conditions according to Ayurveda clinicians. Additionally, from the Indian literature, there are many drugs described by Ayurveda classics used in the treatment of Tudikeri (a disease that has the same signs and symptoms of tonsillitis) (7).

S. lappa follows the family of Asteraceae, which includes nearly one thousand genera and thirty thousand species that spread globally but concentrated mainly in the Indian subcontinent (8). This plant is known by many names, viz. Kur (Bengali), Postkhai (Kashmiri), Sepuddy (Malayalam), and Kuth (Hindi) (9). Long time ago, S. lappa had great importance; its composition of sesquiterpene lactones has a role in initiating the release of pro-inflammatory mediators, which share its anti-asthmatic, anti-inflammatory and anti-tumor properties during many experiments (10).

Despite potent bioactivities of folk plant medicines in facing many diseases, the total profile of active plant ingredients and their exact effects have not been clearly mentioned because of complicated chemical composition. However, part of the chemical form that is responsible for inducing medical properties have been suggested and reported by many researchers; these compounds include alkaloids, flavonoids, ligans, peptides, polyphenols, polysaccharides, and saponins (11). Therefore, this study was initiated to evaluate the analgesic effects of S. lappa roots extract for prospective medical interest.

Methodology

The experiments and testing procedures were approved and endorsed by the research and management center (RMC) of Management and Science University (MSU), Malaysia, and ethics form registered by the number: MSU-RMC-02/FR01/05/L3/014.

Plant Gathering and Identification

S. lappa dry roots were bought from G-M herbal shop, Shorkot city, Punjab, Pakistan. According to the seller, it was cultivated from Jammu and Kashmir area after the rainy season, enclosed well, and transported in the contained jar and preserved naturally at room temperature. It is known domestically as Kuth.

Specimen of the plant was sent to the Forest Research Institute of Malaysia (FRIM) to be identified and authenticated for plant taxonomy under report number FRIM (S).600-5/6/1 Klt. 2 (40). The institute confirmed and authenticated the sample of the plant (HSID 006/19) through high-performance thin-layer chromatography (HP-TLC) as reported and referenced in the 17th edition of Japanese Pharmacopoeia.

Plant Extract Preparation

The extraction process was assessed through Soxhlet method as it is highly preferable for highly phytoconstituents yield (12). The roots were clean and dried thoroughly. Firstly, they smashed into small pieces as they are adamant, then crushed and ground into fine powder by using a blender to ensure the best treating with the solvent. 50gm of roots powder poured in the cellulose thimble inside Soxhlet apparatus, and the flask filled up by 300ml of 100% ethanol solvent as it is highly effective solvent for extraction (13). After the fixation of the apparatus, we started running the heating adjusted at 70°C as the boiling point of ethanol is 78°C for approximately twenty hours. The extract inside the flask was filtered with No. 1 Whatman filter paper, then placed into the rotary evaporator to get rid of the used solvent. The rotary bath heating was adjusted on 45°C, and the pressure settled on 670 hpa for few hours until the dark brown sticky material was precipitated on the walls of the rotary flask. The precipitated material was taken by spatula, weighed and preserved in dark container at 2-8°C refrigerator.

Experimental Animals

Thirty-six randomly selected albino mice of Swiss strain, which were obtained from KRK SERI ENTERPRISE under MSU lab supervision, weighing between 26-30gm. The animals lived at room temperature of 25 ± 2°C with a relative humidity of 75 ± 5% under 12 hours dark and 12 hours light cycle. The mice were divided for each experiment into three groups of six mice in each group, in separate sterilized polyethylene cages (MSU standardized cages) having the dimensions of 408 mm × 280 mm × 150 mm. The animals were maintained under standard free access to rich pellet diet and water, cages bedded with Chipsi classic woody bedding, and they were allowed to accommodate to the environment for seven days before experimentation. Animals fasted on a diet only overnight before the experiments.

Testing Methodology

Fixed-Dose Procedure (FDP)

Acute toxicity study processed according to the Organisation for Economic Cooperation and Development (OECD) guidelines 420 (Fixed single-Dose Procedure-FDP). These guidelines comprise four important steps, half of the lethal dose (LD50) estimation, body weight measurement and observation, monitoring physical signs of toxicity, and evaluation of gross pathological changes (14). These experiments not depending on the death of the animal as an endpoint for evaluation as had performed previously in the old conventional method; instead, it works by increasing of tested doses gradually by using fixed-dose levels. This approach avoids the death of animals as it depends mainly on life condition, weight monitoring, and observation of signs of toxicity (15).

Eddy's Hot Plate Method

Eddy & Leimbach employed this method to analyze the central analgesic activity of particular material. It is used to measure latency time for developed analgesia against induced thermal pain through the ability of the animal to sense and react to mild acute thermal pain via measuring animal's voluntary hiding (moving) away from its body part from the heated floor (16). The neurogenic mechanism assessed by the hot plate method resulted from the complex process within the brain, which results in a behavioral reflex of moving and licking of paws after exposure to heat since this method can measure central pain, therefore, non-opioids (non-narcotics) such as aspirin, diclofenac sodium, and ibuprofen drugs (with the exception of paracetamol) can't relieve such type of somatic pain (17). It is still unclear the exact mechanism of action of paracetamol, despite its shares some central and peripheral activities. Some studies mentioned that it might affects the endogenous cannabinoid system in the brain. This can appear through its AM404 metabolite, which has ability to inhibit cannabinoid/vanilloid anandamide reuptake, hence avoiding pain sensation. At the same time, the weak anti-inflammatory action of paracetamol can translate its peripheral action through reducing COX, which means decreasing formation of prostaglandins (PGs) and bradykinin (BK), even at less extent (18).

Mice were selected for normal reaction time one day before experimentation, in which normal hind paw licking time is 4-6 seconds, and normal jumping response time is 2-3 seconds. Then they were divided into three groups; every group contained six mice. The first group (1), which marked as the negative control group, was fed on distilled water. The second group (2) was administrated orally by 500mg/kg of plant extract. The third group (3), which marked a positive control group, was orally administrated

by paracetamol 100mg/kg. A study undertaken for pharmaceutical evaluation of four market available commercial paracetamol, it revealed that there was no significant differences upon assessment; therefore, paracetamol was obtained from MSU labs (19). The surface of the hot plate apparatus was sterilized well and kept clean all the time, and the device was operated and maintained at 55 ± 0.5°C. The testing started twenty minutes after absorption of orally given treatments, and the test time-controlled not to exceed 25 seconds to prevent injury of animals to heat. Each animal placed individually and watched for jumping, licking of paws and vocalizing response, and the time recorded at 20, 60, and 90 minutes.

Finally, percentage protection against thermal pain stimulus = (Control mean - Test mean / Control mean) * 100. All results processed by one-way ANOVA for analysis of mean differences between tested groups, then followed by Tukey and Dunnett post hoc tests to specify the exact mean differences magnitude and to show the comparison between them (20).

Acetic Acid Induced-Writhing Method

This method even called acetic acid abdominal constriction method (21). It is a chemical method that depends on inducing pain of peripheral origin (nociceptors) in mice using the injection of chemical irritants such as acetic acid, and this is why it is used to estimate peripheral pain. The signals transmitted from nociceptors to central nervous system in response to this pain-causing release of mediators like prostaglandins which in turn induce writhings (as an arching of back of animal, extension of hind limbs and contraction of abdominal muscles), hence this type of visceral pain can be treated by NSAIDs (non-narcotic analgesics) such as ibuprofen. Those writhings (contractions), which are the manifestation of such reaction remain for a long time, and with supplying analgesic compounds to test animals, this will help in the decrease of mummer of writhings. This method processed by intraperitoneal injection of 0.1 to 0.2 ml of 1% acetic acid, this injection will induce muscular contractions which counted five minutes after acetic acid injection, and it is continued overtime period of 20 minutes.

Eighteen Swiss albino mice randomly selected and divided into three groups and marked well. The first group (1) fed by distilled water orally and considered as a negative control group. The second group (2) fed by 500 mg/kg of plant extract through an 18G nasogastric tube. The third group (3), which considered a positive control group, administrated paracetamol 100mg/kg orally. All mice kept thirty minutes before the intraperitoneal injection of 0.1 ml of 1% acetic acid. All mice were observed individually on the observation table for the start, number, course, and end of writhings. The results were recorded. A reduction in the number of writhes is an indication of analgesic property. The number of writhes for each group compared to the control group and the percent reduction in writhes count and the percentage of inhibition calculated as follow: Ncontrol – Ntest/ Ncontrol * 100, where N=mean number of writhes in each group accordingly. All findings statistically checked by one way ANOVA, followed by Tukey and Dunnett post hoc tests, as mentioned previously (20).

RESULTS

Acute Toxicity Experiments Results

According to the Commission of the European Communities, Council Directive 83/467/EEC, the classification of chemical lethality is as follows: very toxic when LD50 of test material is less than 25 mg/kg body weight, toxic when LD50 is 25-200 mg/kg, harmful substances when LD50 is between 200 to 2000 mg/kg, and with an LD50 is greater than 2000 mg/kg body weight are termed 'unclassified' (22). S. lappa roots ethanol extract is considered a safe plant as it falls in category five on the globally harmonized system (GHS) for the classification of chemicals which cause acute toxicity. The limit dose was found to be safe for more than 2000 mg/kg upon being given orally to mice. Therefore, the conclusion of the testing dose will be 2000 mg/kg < LD50 < 5000 mg/kg. Moreover, the plant extract did not affect the daily life of tested mice, did not affects their weights, did not exhibit any toxic signs and all animal reactions, and vital signs were normal and did not cause any abnormalities upon gross necropsy. Thus, this plant considered to be safe for consumption.

Eddy's Hot Plate Method

Outcomes of testing of 100% ethanol extract of S. lappa roots for its analgesic effects as the central analgesic agent by hot plate test are presented in table 1.

According to recorded results, S. lappa ethanol extract of its roots exhibited potent central analgesic effect, and there was a prominent increase in registered latency time when compared to paracetamol as a control drug. This was eminent throughout the entire course of the experiment after the oral administration of plant extract. It was clearly seen that S. lappa has elongated the latency time when compared to the negative control (dH2O) by 82.37 % after twenty minutes, then by 6.87 % after one hour and finally by 43.47 % after ninety minutes. The same was happening for positive control (paracetamol) group, which seen to increase the latency time by 48.3 %, 22.97 %, and 22.67 % after 20, 60, and 90 minutes respectively, when matched to the negative control group. The last percentages indicate that the latency time was more extended with the plant group by 82.11% than that of the paracetamol group (45.69%) with a difference of 36.42%.

Table 1. The mean effects of 100% ethanol extract of Sansevera lappa roots on reaction time for central analgesic effects.

TREATMENT tested	DOSE (mg/kg/ bw)	TIME OF PAW LICKING OR JUMPING					
		AFTER 20 MINUTES		AFTER 60 MINUTES		AFTER 90 MINUTES	
Distilled water (-ve) control	1ml/100 gm	10.43 ± 0.70	% of inhibition → 76.77%	10.80 ± 0.71	% of inhibition → 3.54%	9.06 ± 0.32	% of inhibition → -16.11%
Plant extract	500mg/ kg	10.76 ± 0.91	3.16% → 82.37%	11.50 ± 0.87	6.48% → 6.87%	16.50 ± 0.45	**82.11%** → 43.47%
Paracetamol (+ve) control	100mg/ kg	8.75 ± 0.48	-16.10% → 48.3%	10.76 ± 0.69	-0.37% → 22.97%	13.20 ± 0.93	**45.69%** → 22.67%

Inspecting the table for the significance (Sig.) value of 0.004 indicates it is significant value, as it is considerably lower than our significance threshold of P < 0.01. Therefore, it is assumed to reject the null hypothesis and to accept the alternative hypothesis. Hence, after the administration of plant extract to mice, there was a significant difference in elongation of latency time between experimental groups (F (2, 51) = 6.034, P < 0.01). Statistically, ANOVA test showed that there were significant differences between groups only, and it does not give an idea about where the difference is located and its direction, therefore we need to run post hoc tests.

Reviewing of multiple comparisons of post hoc tests results disclose that there is a significant difference between means of groups (1, 2), and less significant (at p < 0.05) between groups (3, 2). By treating test group by S. lappa roots extract, there was a significant difference in the elongation of latency time mainly between two groups (1 and 2) and partially between groups (3 and 2) of (F (2, 51) = 6.034, P < 0.01), specifically, the negative control (10.1 ± 1.594), plant extract (12.9 ± 3.172), and positive control (10.9 ± 2.511) groups were significant differences from each other at (P<0.01).

Tukey HSD post hoc test reveals that mean differences between groups 1 and 2 are more significant than differences between groups 1 and 3 and mean differences between groups 2 and 1 are more significant than differences between groups 2 and 3. To assure these findings, Dunnett-t (2-sided) post hoc test showed significant differences between means of plant extract group 2 to negative control group 1 at a p-value of 0.003, and there was no significant (p = 0.531) mean differences between groups 1 and 3. This means that S. lappa extract 500 mg/kg is more potent in action than paracetamol at a dose of 100 mg/kg as seen on Figure 1.

Comparing clustered bar mean of three groups indicates the manifest change in the second group (plant extract test group), in which the first negative control group showed no effects on latency time until the first hour after administration of treatment, then latency time decreased with time which indicates to the absence of treatment upon this group. From figure 1, both group 2 and 3 (plant extract and paracetamol) groups revealed their potent action to expand latency time (time of pain-sensing) which denotes to their active effects from twenty to thirty minutes from administration of treatments accordingly, that was more apparent with plant extract by percentage of 82.11 % when compared to paracetamol percentage of 45.69 %.

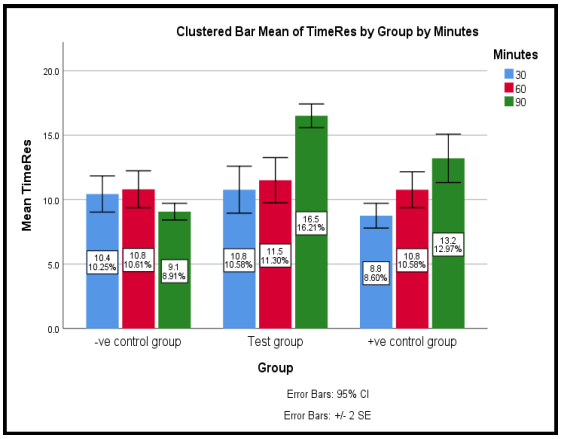

Figure 1. Clustered bar means of time response effect (latency time) by plant extract compared to both negative and positive control groups recorded three times.

Even more, both groups, two and three have strong affectivity to propagate latency time after the first hour from the administration of treatments by percentages of 43.47% and 22.67% respectively when compared to their effect of percentages of 6.87% and 22.97% respectively after thirty minutes of administration of treatment.

Acetic Acid Induced-Writhing Method

Results of testing of 100% ethanol extract of S. lappa roots for its analgesic effects as the peripheral acting agent by acetic acid induced-writhing method were presented in table 2.

As seen from the table, both plant extract and paracetamol have a considerable amount of effects. Both of them had elongated the onset and end of writhings (characteristic contractions), and both of them had reduced number of writhings for the same test time. It is apparently seen that S. lappa extract was more potent than paracetamol, in which plant extract has diminished writhings in the count by 46.42% more than that of positive control, which has a minimized number of contractions by 24.69%. Both of them have the same effect upon leverage on the duration of trunk contractions.

Table 2. The mean effects of 100% ethanol extract of S. lappa roots on the number of writhing in the evaluation of peripheral pain analgesia.

TREATMENT tested	DOSE mg/kg	WRITHING RESPONSE				
		ONSET per minutes	RECOVERY per minutes	NUMBER OF WRITHINGS	DURATION	% OF INHIBITION
Distilled water (-ve) control	1ml/ 100gm	4.05	16.50	56 ± 2.75	2.25	-
Plant extract	500mg/kg	7.05	16.45	30 ± 1.59	1.6	**46.42%**
Paracetamol (+ve) control	100mg/kg	4.42	17.76	42.17 ± 3.34	1.6	**24.69%**

Values are expressed in means ± SEM, n=6, group differences determined by one-way ANOVA at p-value <0.01, followed by post hoc Tukey and Dunnett tests.

ANOVA table states that there is a significant difference between groups' mean of 0.000 value (p-value < 0.01), subsequently, it is supposed to reject the null hypothesis and to accept the alternative hypothesis to say that after administration of plant extracts to mice, there was a significant difference in reduction of writhings number between experimental groups (F (2, 15) = 23.842, P < 0.01). Tukey HSD post hoc test reveals there are significant differences in the mean of all groups between each other, mostly apparent between first and second groups.

Thus it can be stated that administration of S. lappa extract to test group gave a significant difference in decreasing number of writhings in all test groups of value (F (2, 15) = 23.842, P < 0.01), specifically, the negative control (56.00 ± 6.753), plant extract (30.00 ± 3.899), and positive control (42.17 ± 8.183) groups were significant differences from each other at (P < 0.01). Tukey HSD post hoc test disclosed mean differences between groups 1 and 2 are more significant than mean differences between groups 1 and 3 and mean differences between groups 2 and 1 are more significant than differences between groups 2 and 3. To emphasize the last findings, Dunnett-t (2-sided toward negative control) post hoc test indicated that there is a significant differences between means of plant extract group 2 (sig. 0.000) and paracetamol positive control group 3 (sig. 0.004) as compared to negative control group, with a little bit more potency towards the second group. To depict discussed differences between the plant test group and paracetamol positive control group, both compared to the distilled water group as the negative control (Figure 2).

The graph uncovers the potent efficiency of S. lappa root extracts to decrease the frequency of body contractions when compared to paracetamol as well as to decrease the time of each contraction. Both of plant extract and paracetamol disclosed the same effect towards contraction time (1.6 minutes) with the advantage of minimizing writhings for plant extract over paracetamol. From the same figure, S. lappa lessened the number of contractions by a percentage of 46.42% when compared to paracetamol treatment of percentage 24.69%, which means that there is an amount of 21.73% difference between two treatments in favor of plant extract.

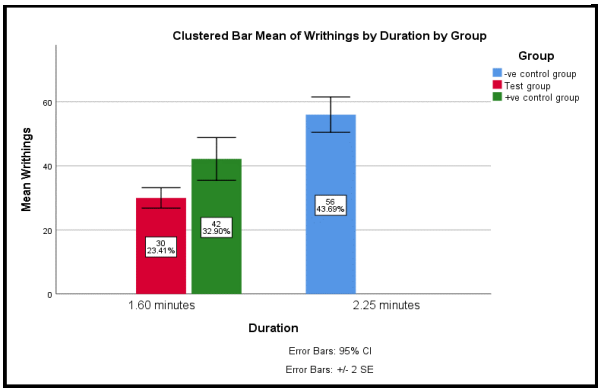

Figure 2. Clustered bar means of animal's contractions (writhings) response effect of three test groups with their effects on the duration of contractions.

Discussion

Many models can process evaluation of nociception for lab pre-clinical studies. The hot plate method is a substantial model for testing high-intensity stimuli for both acute and persistent pain. It can evaluate the supraspinal level pathway on pain sensation, then the pain is interpreted and perceived in the brain (20). Central pain testing model marked by significant raise in pain via the central pain pathway, which initiated by stimuli (heating), then complex processes control the pain centrally like endogenous opioids, opioidergic neurotransmission, opioid receptors, mechanoreceptor fibers and nociceptors fibers. Analgesics mainly opiates affect this pathway to reduce pain. Opioids are substances that act on opioid receptors to produce morphine-like effects. These drugs modulate the incoming pain information in the spinal and central sites, as well as relieve pain temporarily (23).

Generally, the results of this study revealed that 100% ethanol extract of S. lappa roots displayed different degrees of antinociceptive activity in a hot-plate model of pain, less non-significant at first stage and significant at a late stage. Presumed analgesic outcomes regarded as an indicator of significant phytoconstituents that participate this action, mainly flavonoids, tannins, and terpenoids, which have been reported for the responsibility of analgesic effect in many studies (24). Furthermore, the plant extract showed a notable increase of latency time to thermal pain when compared to the positive control (by 36.42%) at the dose of 500 mg/kg, which may be attributed to the inhibition of histamine or kinins pathway

to reduce the pain (22). The plant roots contain a lot of important phytoconstituents, particularly the bioflavonoid glycoside (solanoflavone), steroid alkaloids and glycoalkaloids, hence the presence of alkaloids is responsible for exerting significant analgesic effect (25 and 26).

Acetic acid at the scarce dose used as a nociceptive agent to induce pain of peripheral origin marked by abdominal muscle contractions, this model used to evaluate peripherally acting analgesics. This pain caused by the liberation of inflammatory mediators; these chemicals detected at local peritoneal receptors include serotonin, histamine, PGs (mainly PG-E2 and PG-F2α), bradykinins, and substance-P as well as lipooxygenase products. The cyclooxygenase enzyme inhibited by NSAIDs is the prominent mechanism that leads to inhibition of both COX1 and COX2, then leads to inhibition of prostaglandins, leukotrienes, and other endogenous substances (27).

This experiment revealed that 100% ethanol extract of S. lappa roots showed valuable peripheral analgesic activity in the acetic-acid induced-writhing method. The plant roots as many plants contain an important phytoconstituents like flavonoids, alkaloids, and tannins, particularly the bioflavonoid glycoside (solanoflavone), steroid alkaloids and glycoalkaloids which have a role in pain decreasing effect, hence their presence is responsible for exerting significant analgesic effect (28).

Ethanol extract of S. lappa roots exhibited a significant ($P < 0.01$) analgesia at dose of 500 mg/kg to reduce peripheral pain in terms of followings, it extended onset of writhings by 74.07%, it helped in minimizing time of recovery from writhing contractions by 0.3%, it reduced number of writhings by 46.42%, and it helped in lowering of duration of writhings by 28.88% of inhibition when compared to paracetamol 100 mg/kg as a positive control.

The first phase reflects neurogenic pain, which manifested by direct chemical stimulation of pain afferent C-fibers nociceptors. The second phase of pain which known as inflammatory pain initiated by release of inflammatory mediators such as histamine, PGs, BK, and serotonin in the peripheral tissues. Morphine and other opiates can prevent the first type of pain, while NSAIDs can block the second type. For example, aspirin exerts its action to reduce peripheral pain through inhibition of the formation of pain chemicals in tissues, while PGs and BK are responsible for sharing properties in inducing pain (29). The present study indicates that S. lappa roots extract shown its peripheral pain analgesia in acetic acid-induced writhing method, as it acts by antagonizing effects of mentioned mediators or by suppressing their action. The study showed that the dose of S. lappa, which exhibits potential analgesia is 500 mg/kg.

The outcomes of the experiment enhance the theory of inhibition of prostaglandins formation since the nociceptive mechanism requires the emission of pain metabolites through cyclooxygenase (COX) pathway and prostaglandin biosynthesis. Furthermore, and as discussed before, flavonoids participate in inhibiting PGs synthase enzyme and formation of them. Thus, these phytoconstituents have an important role in this type of analgesia (30). Therefore, this experiment suggested that S. lappa has a potent effect on peripheral analgesia.

Conclusion

Medicinal plants are copious by beneficial phytoconstituents, which share a lot of therapeutic effects. The S. lappa crude extract produced significant central and peripheral antinociceptive activities. The plant extract at a dose of 500 mg/kg disclosed eminent increase of latency time to thermal pain when compared to paracetamol positive control by a percentage of 36.42%, and it reduced the number of writhings by 46.42% when compared to paracetamol 100 mg/kg. It is supposed that S. lappa contains many chemical compounds that participated in these effects, mainly alkaloids, tannins and flavonoids. Therefore, this plant can be used as a potent analgesic agent to reduce pain. Furthermore, S. lappa roots extract was safe upon testing of its acute toxic effects on experimental animals. It is recommended to seriously taking this plant for further studies to check its mechanism of action.

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