Original article

Evaluation of analgesic, antiamnesic and antidiarrheal potentials of *Medicago denticulata* extract using animal model

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A B S T R A C T

The analgesic, antidiarrheal, and neuro-pharmacological potentials of *Medicago denticulata* leaves extract were screened in animal models. Potential analgesic response was noted ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) in formalin, acetic acid and heat-induced pain models in a dose-dependent manner. Maximum activity by means of writhing inhibition was documented for *Medicago denticulata* at 300 mg/kg that was found to be 71.79% (17.43 ± 1.31). In first phase, the *Medicago denticulata* at a dose of 150 and 300 mg/kg showed analgesic activity and reduced the pain by 54.18% (18.39 ± 1.67) and 62.90% (14.89 ± 1.56), respectively. In second phase, the *Medicago denticulata* at a dose of 150 and 300 mg/kg showed analgesic activity and reduced the pain by 69.48% (19.78 ± 1.44) and 70.89% (18.86 ± 1.58), respectively. In hot plate method, the *Medicago denticulata* at a dose of 150 and 300 mg/kg showed the maximum response of 61.16% (8. 47 ± 1.23) and 67.39% (10.09 ± 1.04), respectively at 60 min. Scopolamine significantly reduces spontaneous alteration in Y-maze model for antiamnesic activity. *Medicago denticulata* significantly increased the discrimination index in a dose-dependent manner using novel object recognition test (NORT) model. Exploration time in sec for the novel object was increased significantly (P < 0.001) by donepezil decreased for familiar one with a discrimination index (DI) of 62.18%. *Medicago denticulata* significantly increased the discrimination index by 60.86% and 57.24% at 300 and 150 mg/kg b.w, respectively. The lowest DI of 53.80% at 75 mg/kg was observed in comparison to the amnesic group. The *Medicago denticulata* significant decreased the elevated levels of acetylcholinesterase (AChE) and malondialdehyde (MDA and enhancing level of acetylcholine (ACh), superoxide dismutase (SOD) and catalase (CAT) acting as an antioxidant agent. *Medicago denticulata* reduced the total number of diarrheal feces to lesser extent at dose-dependent manner. From the study results, it is suggested that the *Medicago denticulata* extract possess good analgesic and antiamnesic activity however the antidiarrheal effects of plant were negligible. In the current study, the traditional use of the plant as a source of medicine has been validated.

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1. Introduction

The plant species having medicinal importance are widespread used due to low price, lesser side effect and easily accessibility (Alamgeer et al., 2018). Approximately 80% of the world population is dependent on indigenous herbal drugs (THD) as per statement of World Health Organization (WHO). Internationally, out of 422,000 flowering species (Ahmad et al., 2014), 35,000 to 50,000 species of

plants are approved medicinally and in Pakistan, 400 to 600 among 6000 plant species are medicinally important with recommendations from hakims and local healers (Hussain et al., 2019). The Northern parts of Pakistan is rich in economic and medicinally important varieties of plant species for indigenous communities (Malik et al., 2019). Plants and their phytochemicals is used as natural medicines to treat various diseases since ancient times and reported to possess antioxidant, anti-depressant, antispasmodic, anti-inflammatory, analgesic, immunomodulatory, antipyretic and cardioprotective potentials (Yuan et al., 2016).

The Fabaceae or Leguminosae, known by family of legume, pea, or bean is a large flowering plant family have economic importance. The family has a range from trees to shrubs with a variety of annual and perennial herbaceous plants which can be recognized easily through their leguminous fruits. Due to its wide distribution, the family has third-largest number of species on land, behind only the Orchidaceae and Asteraceae, having approximately 751 known genera and round 19,000 species (Rahman and Parvin, 2014). The *Medicago* variety (Fabaceae family) comprises of 83 distinguished species of herbs or bush fundamentally disseminated around the Mediterranean basin yet in addition adjusted to a range of environmental conditions, while producing some specific metabolites, for example, coumarins, flavonoids, naphthoquinones, alkaloids and some saponins (D'Addabbo et al., 2020). Traditionally and scientifically, the species of *Medicago* have aptitudes in the treatment of diabetes, enhancing memory, curing kidney pain, relief from coughing and having anti-inflammatory, antioxidant, hypolipidemic, neuroprotective, anti-asthmatic, antimicrobial, and against leishmania potentials (Bora and Sharma, 2011, Abbasi et al., 2013). Based upon the supported literature of *Medicago* genus, the study was designed to explore the possible use of *Medicago denticulata* for the management of pain, diarrhea and amnesia.

2. Material and methods

2.1. Chemicals and animals

Tween-80, scopolamine was purchased from Sigma Aldrich Chemical Company, Germany. Diclofenac sodium, tramadol, naloxone, castor oil, magnesium sulphate and indomethacin were purchased locally. Solvents and chemicals like methanol, acetic acid, donepezil used were of analytical grade extra pure purchased from E. Merck. Male Balb/C mice weighing 20–25 gm procured from National Institute of Health (NIH) Islamabad Pakistan were used in the study and kept in animal house with free access to food and water ad libitum. The animals were kept at room temperature around 22–25 °C with light and dark cycle of about 12 h each (light at 6:00 am) and a relative humidity of 50–55%. A study was conducted as per approval from the Departmental Ethical Committee vide notification Pharm/ECC/22–2020 in compliance with the Animal Bye-Laws.

2.2. Plant collection and extraction

The fresh plant having flowers and fruits was collected from the Mountain of village Shamozai, District Swat and identified and authenticated. The plant leaves were cut and rinsed with tap water and dried under shade. The shade dried leaves were ground in an electric chopper machine to obtained fine powder forms. The grinded material (powder form) of about 8 kg was soaked in 15L of methanol. The soaking process was completed in three days with occasional shaking. Then the soaked materials were filtered out initially in a clean sterilized beaker using a muslin cloth, and the final filtration was done through a filter paper, especially

designed for plant extract formation. The rotary evaporator (*HeidolphLaborta* 400 efficient) was used to concentrate the extract. After some hours a thick past of crude extract of red–black color was received, weighing of approximately 200 g.

2.3. Pharmacological activities

2.3.1. Acute toxicity

For acute toxicity in mice, the activity was carried out in two phases at different dose concentration in various groups (n = 6). The mice in each group were observed for any untoward effects or mortality for 24 h followed by its observation for 14 days with free access of food and water. Animals were observed daily for two weeks to observe signs of convulsions, tremor, diarrhoea, salivation, lethargy, and sleeping. The body weight was also measures as per weekly observation (Lorke, 1983).

2.3.2. Writhing test by acetic acid

The extract *Medicago denticulata* was given orally (i.p.) to mice in experimental groups (*n* = 6) at different dose concentrations of 75 mg, 150 mg and 300 mg/kg b.w 30 min prior to administration of acetic acid (0.6%, 10 mL/kg, i.p.). The nociception intensity was noted in response to number of writhes produced within 30 min after acetic acid administration (Koster, 1959).

2.3.3. Licking response by formalin

The mice were treated in experimental groups (*n* = 6) with crude extract *Medicago denticulata* (i.p.) at different dose concentrations 1 h before the treatment of animals with formalin (1%,50 µL) in respective groups of animals in the right hind paw. The treated paw of mice was experiential for 30 min in an plexiglass box and the paw licking time in seconds was recorded in two phases, 0–5 min (neurogenic pain), and 15–30 min inflammatory pain (Hunskaar and Hole, 1987, Shoaib et al., 2019).

2.3.4. Hot plate test and involvement of the opioid system

Following the previously reported protocol, the test was carried out to determine reaction time of mice as licking, shaking of the paw or jumping off from the hot surface maintain at 55 ± 2 °C. The data was recorded 30 min after administration of with crude extract *Medicago denticulata* or vehicle (10 mL/kg, i.p.). Tramadol (20 mg/kg) was given 30 min before the hot plate test. Naloxone (2 mg/kg) was used to assess the involvement of opioid system (Eidi et al., 2016).

3. Antiamnesic study of *Medicago denticulata* on memory

3.1. Experimental design

The *in-vivo* activity was carried out by using male Balb/C mice weighing 19–23 g by divided into different groups (n = 6). Normal saline was given to Group I, while Group II and Group III was treated with scopolamine and scopolamine plus donepezil, respectively. Group IV-VI received SA at the dose of 75, 150 and 300 mg/kg plus scopolamine. This test was utilized to evaluate the impact of *Medicago denticulata* regulated for 10 continuous days against memory debilitation initiated by 1 mg/kg i.p scopolamine single injection. The behavioral tasks were designed to assess the impact of the treatment in novel object recognition test and Y maze model.

Group I received only normal saline (10 mL/kg) standing as control group. Group II (amnesic group, scopolamine): Received normal saline (10 mL/kg). Group III (Donepezil group): Received donepezil at the dose of 2 mg/kg. Groups IV–VI (tests groups): Received one of the three doses of *Medicago denticulata* (75, 150

and 300 mg/kg). Each group was subjected to the related treatment in a sequence of 10 successive days. On 10th day after 30 min of treatment, scopolamine was injected all the groups (1 mg/kg, i. p.) aside from distal water feed group. The behavioral tests were conducted after 30 min of scopolamine injection.

3.2. Y-maze test

Y-maze test was aimed for assessing the mice short-term memory by recording unconstrained variation during single session on 10th day. Y-maze had three arms, each 35 cm long, 15 cm wide, and 8 cm in height) and a symmetrical three-sided focal territory. One hour after the last treatment and 30 min after scopolamine injection (aside from group treated with distilled water), each mouse, beforehand acclimatized to the maze, was put toward the one arm and were permitted to move openly through the maze for total of 08 min. The percent of spontaneous alternation was calculated as; (number of alternations/total entries − 2) X 100 (Kouémou et al., 2017).

3.3. Novel object recognition (NOR) test

The NOR test is aimed at memory recognition of mice. For the test, an apparatus with open field was used made up of plywood having square geometry (40 cm × 40 cm × 25 cm). On the preceding day of the test (8th day after treatment with drug), each mouse was acquainted for 5 min with the apparatus after 01 h of drug treatment. On the first day of testing (9th day after treatment with drug) the animals were exposed to two indistinguishable items 01 h after treating with drugs for a total period of 5 min observation. An animal investigates an item when it contacts the object or it coordinates its nose a good way off under 2 cm to the object. On the test 2nd day (10th day after treatment with drug), 30 min after injecting scopolamine (aside the group treated with distilled water), another article supplanted one of the objects introduced in the first day. The time taken by animal for investigating the new object (tB) and the acquainted (tA) objects was noted for 5 min. The index of discrimination (DI) was calculated as; DI= (tB/tB + tA). Whereas tB new object and tA familiar object (Rajendran et al., 2014).

3.4. Assessment of biochemical parameters and biomarker study

The animals after in-vivo study were executed after anesthetizing with ether and the brain from each animal was excised to extract brain in phosphate buffer saline that is ice cold (chilled). The portions were subjected to analysis of biochemical parameters that includes acetylcholinesterase (AChE), acetylcholine (ACh), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) contents (Mushtaq et al., 2018).

3.5. Antidiarrheal activity of Medicago denticulata

3.5.1. Castor oil-induced diarrhea

For studying antidiarrheal activity, diarrhea was induced in mice by treating with castor oil as indicated by the technique depicted by Shoba and Thomas (2001). The mice were screened for the trial by inducing diarrhea after feeding each mouse with 0.5 mL castor oil (p.o.). The overnight fasted mice were treated orally (p.o) with extract, vehicle, or standard drug. Then each mouse was orally treated with castor oil (0.5 mL dose) after 01 h. At that point, every mouse was set in a different enclosure with a smudging paper-lined floor. The observation was carried out for 04 h to record the trademark diarrheal droppings. Using the following formula, percent (%) defecation inhibition was calculated as:

% Inhibition of defecation = [(A − B) / A] × 100; where, A = number of castor oil induced defecation. B = number of drug/ extract induced defecation (Shoba and Thomas, 2001, Imam et al., 2012).

3.5.2. Magnesium Sulfate-Induced diarrhea

Following the method of Doherty (1981), the test was performed by inducing diarrhea by feeding with magnesium sulphate dose (02gm/kg) orally (Doherty, 1981). The animals were grouped after screening. An oral dose of magnesium sulfate (02gm/ kg) was given 30 min after administering the drug/extract and vehicles. In the wake of watching the diarrheal droppings for the following 04 h, percent (%) defecation inhibition was calculated (Imam et al., 2012).

3.6. Statistical analysis

The data is presented as mean ± SEM. Graph Pad Prism 5 software version 5.01 (GraphPad Software Inc., San Diego, CA, USA) was used to analyze variance and Dunnett's test was calculated. P-values ≤ of 0.05 were considered significant.

4. Results

4.1. Acute toxicity

It is important to study and evaluate the pharmacological biopotentials of the plant extracts. Studying the acute toxicity of plant extract is significant in light of the fact that there are numerous agents that have potential remedial impacts yet because of their harmfulness can't be utilized as a therapeutic source. During the current study, Medicago denticulata has a safety profile up to 2000 mg/kg dose without any behavioral changes and mortality noted.

4.2. Analgesic activities

4.2.1. Acetic acid-induced writhing method

The analgesic action of Medicago denticulata was evaluated at a dose of 75, 150, and 300 mg/kg by inducing writhing using the acetic acid method. Writhing is a characteristic action of extending the abdomen and hind limb stretching when acetic acid is injected into mice shown in Table 1.

The writhing frequency was counted in which the Medicago denticulata expressed a significant reduction in total writhes at all doses. At dose 75 mg/kg and 150 mg/kg the Medicago denticulata has shown 61.78% (23.61 ± 1.67, P < 0.01, n = 6) and 67.13% (20. 31 ± 1.72, P < 0.001, n = 6), respective inhibitory activity upon comparing with control group (61.05 ± 1.21, n = 6). Maximum activity by means of writhing inhibition was documented for Medicago denticulata at 300 mg/kg that was found to be 71.79% (17.43 ± 1. 31, P < 0.001, n = 6) as stated in Table 1.

4.2.2. Formalin test

Formalin induced pain method was used for analgesic effect evaluation of Medicago denticulata at a dose of 75, 150, and

Table 1
Acetic acid-induced analgesic activity of Medicago denticulata.

Treatment/Dose		Writhes	% inhibition
Control (2% Tween 80)		61.79 ± 1.65	—
Medicago denticulata	75 mg	23.61 ± 1.67**	61.78
	150 mg	20.31 ± 1.72***	67.13
	300 mg	17.43 ± 1.31***	71.79
Diclofenac sodium	10 mg	9.11 ± 1.14***	85.25

Mean ± SEM (n = 6). **P < 0.01, ***P < 0.001 vs control group.

300 mg/kg. The *Medicago denticulata* at a dose of 75 mg/kg showed analgesic activity and reduced the pain by 51.29% (19.55 ± 1.71, P < 0.01, n = 6) and 60.06% (25.88 ± 1.67, P < 0.05, n = 6) in a biphasic manner (first phase, second phase), respectively when compared to control group (40.14 ± 1.24, n = 6 and 64.81 ± 1.91, n = 6) as shown in Table 2.

Similarly, in first phase, the *Medicago denticulata* at a dose of 150 and 300 mg/kg showed analgesic activity and reduced the pain by 54.18% (18.39 ± 1.67, P < 0.001, n = 6) and 62.90% (14.89 ± 1.56, P < 0.001, n = 6), respectively upon comparing with the control group (40.14 ± 1.24, n = 6). In second phase, the *Medicago denticulata* at a dose of 150 and 300 mg/kg showed analgesic activity and reduced the pain by 69.48% (19.78 ± 1.44, P < 0.001, n = 6) and 70.89% (18.86 ± 1.58, P < 0.001, n = 6), respectively by comparing with the control group (64.81 ± 1.91, n = 6).

Indomethacin pretreated animals (10 mg/kg) produced a substantial drop in the total time of paw-licking up to 76.10% (9.47 ± 1.37, P < 0.001, n = 6) during 2nd phase test while the mild reduction was observed in the time of paw-licking as 22.30% in the first phase when compared to their respective control groups.

The current study shows that doses of *Medicago denticulata* (75, 150, and 300 mg/kg) abridged the number of paws licking remarkably in inflammatory as well as neurogenic pain response P < 0.05, P < 0.01, and P < 0.001, n = 6).

4.2.3. Hot plate test and involvement of the opioid system

Table 3 expresses the hot plat test result of the analgesic response of *Medicago denticulata* shown at a dose of 75 mg, 150 mg, and 300 mg/kg. Maximum analgesic effect of *Medicago denticulata* was observed at 60 min latency increased.

The *Medicago denticulata* at a dose of 75 mg/kg showed 56.19% (7.51 ± 1.18, P < 0.01, n = 6) at 60 min that was comparable to control group 3.29 ± 1.31, n = 6.

Similarly, the *Medicago denticulata* at a dose of 150 and 300 mg/kg showed the maximum response of 61.16% (8.47 ± 1.23, P < 0.01, n = 6) and 67.39% (10.09 ± 1.04, P < 0.001, n = 6), respectively at 60 min when compared to control group 3.29 ± 1.31, n = 6 as shown in Table 3.

Whereas Tramadol a centrally acting opioid analgesic agent, revealed significant post-treatment activity which was found to be 71.80%, 11.66 ± 1.41, P < 0.001, n = 6 when compared with the control group 3.29 ± 1.31, n = 6.

Animals treated with naloxone and morphine produced a significant reduction in the analgesic activity standard used. However, naloxone pretreatment causes partial reversion of *Medicago denticulata* analgesic effect at dose-dependent manner in mice in the hot plate model indicating some other possible mechanisms apart from opioid receptors involvement.

4.2.4. Y-maze spontaneous alteration

Table 4 expresses significant reductions in spontaneous alteration by scopolamine 79.83% to 43.08% (P < 0.001, n = 6) in the Y-maze test. Upon using donepezil normalizes the spontaneous alteration by increasing the % alteration to 77.08 ± 2.64. *Medicago*

denticulata at a dose of 75, 150, and 300 mg/kg showed also increased percent alteration. *Medicago denticulata* showed a major increase in spontaneous alternation (P < 0.01, P < 0.001) at 150 and 300 mg/kg after comparison with the amnesic group that was found to be 73.91 (P < 0.001, n = 6) and 68.75 (P < 0.01, n = 6).

4.2.5. Novel object recognition test

Results from antiamnesic activity of *Medicago denticulata* on NOR test model for short term memory are shown in Table 5. In the sample phase, no change was observed significantly in exploration time for the objects for all samples tested groups. In the test phase, exploration time was significantly higher for a novel object than that of the identical objects in groups treated with *Medicago denticulata* at 75, 150, 300 mg/kg and standard donepezil (2 mg/kg).

Exploration time in sec for the novel object was increased significantly (P < 0.001, n = 6) by donepezil decreased for familiar one with a discrimination index of 62.18% (P < 0 001, n = 6). *Medicago denticulata* significantly increased the discrimination index by 60.86% (P < 0 001, n = 6), 57.24% (P < 0 001, n = 6) at 300 and 150 mg/kg b.w, respectively. The lowest DI (53.80%, P < 0.01, n = 6) and 75 mg/kg were observed in comparison to the amnesic group.

Results from antiamnesic activity of *Medicago denticulata* on NOR test model for long term memory are shown in Table 6. In the sample phase, no change was observed significantly in exploration time for the objects for all samples tested groups. In the test phase, exploration time was significantly higher for a novel object than that of the identical objects in groups treated with SACrd at 75, 150, 300 mg/kg and standard donepezil (2 mg/kg).

Exploration time in sec for long term memory in the novel object was increased significantly (P < 0.001, n = 6) by donepezil decreased for familiar one with discrimination index of 64.08% (P < 0 001, n = 6). *Medicago denticulata* significantly increased the discrimination index by 61.29% (P < 0 001, n = 6), 60.70% (P < 0 001, n = 6) at 300 and 150 mg/kg b.w, respectively. The lowest DI (52.69%, P < 0.01, n = 6) and 75 mg/kg were observed in comparison to the amnesic group.

4.2.6. Biochemical parameters and biomarker study

Scopolamine in this study significantly substantially elevated the AChE and MDA level, decreased the content of ACh, boosted oxidative stress in mice as confirmed by decreases in the SOD and CAT level in the brain. The *Medicago denticulata* showed significant effects on these changes by decreasing the content of AChE and MDA. Similarly, it also enhances the level of ACh, SOD, and CAT content indicating the possible role of *Medicago denticulata* on oxidative stress as an antioxidant (Table 7).

4.2.7. Castor oil-induced diarrheal test

In the model of castor oil-induced diarrheal test, all doses of *Medicago denticulata* (75, 150, and 300 mg/kg b.w.) abridged diarrheal feces total number to a lesser extent in a dose-dependent manner. The inhibition of diarrhea in percentage at a dose of 75,

Table 2
Paw-licking response induced by formalin.

Treatment/Dose		Licking time (Sec)		Inhibition (%)	
		1st Phase	2nd Phase	1st Phase	2nd Phase
Control (2% Tween 80)		40.14 ± 1.24	64.81 ± 1.91	——	——
Medicago denticulata	75 mg	19.55 ± 1.71**	25.88 ± 1.67*	51.29%	60.06%
	150 mg	18.39 ± 1.67***	19.78 ± 1.44***	54.18%	69.48%
	300 mg	14.89 ± 1.56***	18.86 ± 1.58***	62.90%	70.89%
Indomethacin	10 mg	32.41 ± 1.77	18.35 ± 1.22***	19.25%	71.68%

Mean ± SEM,n = 6. *P < 0.05, **P < 0.01 and ***P < 0.001 *vs* control

Table 3

Analgesic response using the hot plate method.

Treatment/Dose (mg)		0 min	30 min	60 min	90 min	120 min	%response at 60 min
Control (2% Tween 80)		3.31 ± 1.23	3.26 ± 1.32	3.29 ± 1.31	3.36 ± 1.21	3.41 ± 0.97	–
Medicago denticulata	75	3.34 ± 1.31	5.39 ± 1.41	7.51 ± 1.18**	6.98 ± 1.34	6.79 ± 1.42	56.19%
	150	3.43 ± 1.78	7.81 ± 1.11	8.47 ± 1.23**	8.11 ± 1.41	7.31 ± 1.06	61.16%
	300	3.31 ± 1.34	8.11 ± 1.23	10.09 ± 1.04***	9.66 ± 1.26	7.22 ± 1.11	67.39%
Tramadol	20	3.25 ± 0.97	8.32 ± 1.17	11.66 ± 1.41***	10.22 ± 1.0	10.01 ± 1.3	71.80%
M.denticulata + Naloxone	75 + 2	3.21 ± 0.89	4.41 ± 0.98	4.89 ± 0.96	4.73 ± 1.61	4.71 ± 1.67	–
	150 + 2	3.36 ± 1.41	5.21 ± 0.91	5.40 ± 1.11	5.10 ± 1.23	4.91 ± 1.11	–
	300 + 2	3.31 ± 1.52	5.09 ± 1.23	5.87 ± 1.21	5.11 ± 1.01	4.90 ± 1.14	–
Tramadol + Naloxone	20 + 2	3.29 ± 1.22	3.19 ± 1.19	3.44 ± 1.14	3.37 ± 1.02	3.29 ± 1.21	–

Mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* control.

Table 4

Spontaneous alteration for memory.

Treatment/Dose		Spontaneous alternation performance
Control		79.83 ± 2.61
Scopolamine	1 mg	43.08 ± 2.01###
Medicago denticulata	75 mg	51.66 ± 1.41*
	150 mg	73.91 ± 1.71***
	300 mg	68.75 ± 1.29**
Scop + DNZ	1 + 2 mg	77.08 ± 1.41***

Mean ± SEM (n = 6). $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* scopolamine group. $^{###}P < 0.001$ *vs* control.

150, and 300 mg/kg of the *Medicago denticulata* was found to be 19.17% (11.8 ± 0.81, n = 6), 31.16% (10.1 ± 1.05, P < 0.05, n = 6) and 23.97% (11.1 ± 1.01, P < 0.05, n = 6), respectively when compared to control group (14.6 ± 0.68, n = 6). Loperamide HCL at a dose of 5 mg/kg significantly reduced diarrhea and was found to be 84.24% (2.3 ± 0.84, P < 0.001, n = 6) as displayed in Table 8.

4.2.8. MgSO₄ induced diarrheal test

In the MgSO₄ induced model of the diarrheal test, all doses of *Medicago denticulata* (75, 150, and 300 mg/kg b.w.) reduced the total number of diarrheal feces to a lesser extent in a dose-dependent manner. The inhibition of diarrhea in percentage by the 75, 150, and 300 mg/kg of the *Medicago denticulata* was found to be 11.36% (11.7 ± 1.03, n = 6), 26.51% (9.7 ± 1.21, P < 0.05, n = 6) and 28.03% (9.5 ± 0.87, P < 0.05, n = 6), respectively when compared to control group (13.2 ± 1.18, n = 6). Loperamide HCL at a dose of 5 mg/kg significantly reduced diarrhea and was found to be 80.30% (2.6 ± 0.91, P < 0.001, n = 6) as displayed in Table 9.

5. Discussion

The study was designed to the reveal the analgesic activity of *Medicago denticulata* L extract to elucidate the pain-relieving effects in different testing methodologies such as acetic acid, formalin, tail immersion, and hot plate test. Furthermore, the exploration of possible mechanisms of these effects highlighted it's

pharmacological activities. The crude extract significantly (P < 0.01 and P < 0.001) delayed the onset as well as inhibited writhing episodes (Table 1) in the writhing test. The test is a steadfast technique for the assessment of the peripheral and central analgesic effect of new agents (Le Bars et al., 2001). Administration of formalin in the sub plantar region of paw educes the biphasic nociceptive pain. During the first phase, the neurogenic pain while in the second phase, inflammatory pain is produced (Lopes et al., 2019). The crude extract significantly (P < 0.05, P < 0.01 and P < 0.001) reduced the nociceptive response by formalin of both phases and the effect was more prominent in the inflammatory phase suggesting the involvement of supraspinal systems in the analgesic response. These findings signified the central analgesic as well as anti-inflammatory potentials.

The tail immersion and hot plate methods are used for the assessment of centrally acting drugs and can be differentiated based on their nociception pathways. Nociception produced by tail immersion in hot water is spinally mediated whereas hot plate is selectively used for the supra-spinally mediated nociception (Shoaib et al., 2016, Gunn et al., 2011). Opioid agents involve spinal and supraspinal receptors for their analgesic action (Pathan and Williams, 2012). Therefore, the results (Table 3) signified that inhibition of nociception by plant extract could be linked with supraspinal opioid receptors operate via the central nervous system. This was confirmed by naloxone antagonism of the analgesic effect in the hot plate model (Table 3). Naloxone is a nonselective opioid receptor antagonist (Li et al., 2019). In the current study, we can see that the activity of Tramadol (standard opioid analgesic) and those of the test samples go parallel and are almost comparable. In the same manner, once again the analgesic potential of test samples and Tramadol was considerably affected by the prior administration of Naloxone. It clearly shows the involvement of opioid receptors, which have been blocked by the Naloxone, and the test samples and Tramadol were unable to exhibit analgesic response via the opioid receptors.

Scopolamine is commonly injected i.p to test animals for studying cognitive impairments. It has structural similarities with ACh neurotransmitter by exerting its function through blockage of muscarinic Ach receptors resulting in cholinergic dysfunction and

Table 5

NORT for short term memory.

Treatment/Dose		Sample Phase		Test Phase		DI (%)
		Identical Object A1	Identical Object A2	Novel Object A1	Familiar Object A2	
Control		19.05 ± 1.41	18.21 ± 1.63	1 8.83 ± 1.33	17.31 ± 1.29	52.10
Scop		9.05 ± 1.65	7.83 ± 1.23	6. 71 ± 1.67###	13.33 ± 1.33	33.48
Medicago denticulata	75 mg	14.86 ± 1.76	15.10 ± 1.39	14.86 ± 1.41**	12.76 ± 1.49	53.80
	150 mg	15.36 ± 1.67	15.43 ± 1.51	14.86 ± 1.71**	11.10 ± 1.57	57.24
	300 mg	14.48 ± 1.39	16.81 ± 1.41	14.48 ± 1.81*	9.31 ± 1.61	60.86
Scop + DNZ	1 + 2 mg	13.86 ± 1.45	14.26 ± 1.66	13.85 ± 1.23***	8.43 ± 1.34	62.18

Mean ± SEM (n = 6). $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* scopolamine group. $^{###}P < 0.001$ *vs* control.

Table 6
NORT for long term memory.

Treatment/Dose		Sample Phase		Test Phase		DI(%)
		Identical Object A1	Identical Object A2	Novel Object A1	Familiar Object A2	
Control		20.30 ± 1.24	21.80 ± 1.39	21. 65 ± 1.44	11.78 ± 1.51	64.74
Scop		13.08 ± 1.31	11.41 ± 1.49	6. 26 ± 1.56$^{!!!}$	12.28 ± 1.31	33.76
Medicago denticulata	75 mg	17.73 ± 1.22	16.40 ± 1.67	12.03 ± 1.67*	10.80 ± 1.45	52.69
	150 mg	18.40 ± 1.44	16.73 ± 1.45	17.46 ± 1.22**	11.30 ± 1.56	60.70
	300 mg	17.23 ± 1.55	19.06 ± 1.67	19.21 ± 1.31***	12.13 ± 1.67	61.29
Scop + DNZ	1 + 2 mg	16.45 ± 1.39	17.95 ± 1.41	21.61 ± 1.37***	12.11 ± 1.41	64.08

Mean ± SEM (n = 6). *P < 0.05, **P < 0.01 and ***P < 0.001 *vs* scopolamine group. $^{!!!}$P < 0.001 *vs* control.

Table 7
Effect of *Medicago denticulata* on AChE, ACh, SOD, CAT and MDA level in the brain.

Sample Test	AChE (μmoles) of substrate hydrolyzed / min / g tissue)	Ach (mmol/min/mg protein)	SOD	CAT	MDA
Control	12.09 ± 1.14	16.01 ± 1.49	15.39 ± 1.25	60.09 ± 1.28	0.39 ± 0.71
Scopolamine	27.21 ± 1.32§§§	6.03 ± 1.56§§§	5.29 ± 2.11###	16.11 ± 1.56###	1.19 ± 0.67###
Donepezil	11.31 ± 1.76***	14.40 ± 1.26***	16.09 ± 2.09***	62.89 ± 1.78***	0.41 ± 0.51***
Medicago denticulata 300 mg	14.22 ± 1.42***	11.51 ± 1.19**	11.61 ± 2.03**	54.81 ± 1.37***	0.51 ± 0.63***

Mean ± SEM (*n* = 6). §§§P < 0 001 and ###P < 0 001 *vs* control. *P < 0.05, **P < 0.01, ***P < 0.001 *vs* amnesic.

Table 8
Effect of *Medicago denticulata* on castor oil-induced diarrhea.

Treatment/Dose		Diarrheal feces	% inhibition
Control		14.6 ± 0.68	—
Medicago denticulata	75 mg	11.8 ± 0.81	19.17
	150 mg	10.1 ± 1.05*	31.16
	300 mg	11.1 ± 1.01*	23.97
Loperamide	5 mg	2.3 ± 0.84***	84.24

Mean ± SEM (n = 6). *P < 0.05, ***P < 0.001 *vs* control group.

Table 9
Effect of *Medicago denticulata* in MgSO$_4$ induced diarrhea.

Treatment/Dose		Diarrheal feces	% inhibition
Control		13.2 ± 1.18	—
Medicago denticulata	75 mg	11.7 ± 1.03	11.36
	150 mg	9.7 ± 1.21*	26.51
	300 mg	9.5 ± 0.87*	28.03
Loperamide	5 mg	2.6 ± 0.91***	80.30

Mean ± SEM (n = 6). *P < 0.05, ***P < 0.001 *vs* control group.

cognition impairment (Shabani and Mirshekar, 2018). ACh is a neurotransmitter with a key role in the central cholinergic system for function regulation. There is an elevated level of acetylcholinesterase (AChE) perceived in the brain of Alzheimer's patients responsible for acetylcholine (ACh) breakdown (Muramatsu et al., 2018).

Lately, study results showed that scopolamine actuated memory impairment in animal models was connected with expanded oxidative stress inside the brain because of a modification of antioxidant enzymes in the brain (Niedzielska et al., 2016).

In the treatment of neurodegenerative, especially AD, the phytoconstituents of therapeutic plants assume a significant job. Accordingly, it is worthwhile to investigate the convenience of therapeutic plants for the treatment of different cognitive diseases. The animal models of scopolamine-induced dementia and oxidative stress serve as the main screening test for the determination of the anti-Alzheimer effect of unknown plants or drugs in this study. The crude extract significantly increased (P < 0.01,

P < 0.001) in spontaneous alternation in Y-maze. The discrimination index DI was increased significantly (P < 0.05, P < 0.01, P < 0.001) in short and long-term memory using the Novel Object Recognition test.

Therapeutic plants are storage facilities of phytochemicals for the treatment of innumerable major and minor diseases. Various species of the *Medicago* genus contain many important substances including saponins, alkaloids, sterols, triterpenes, coumarins, flavonoids, phenolics, vitamins, proteins, minerals, and other nutrients (Ghani and Ahmad, 2019). The constituents found in the species of *Medicago* genus are suggested to have a variety of biological and pharmacological applications including analgesic, antiamnesic, and antidiarrheal based on their reported literature. Due to the chemical structural diversity and various analgesic mechanisms of these secondary metabolites in several studies, secondary metabolites of the plant could be promising candidates for new natural analgesic drugs.

Taken all together the current study attested to the role of *Medicago denticulata* as an analgesic and memory enhancer. This plant may serve as promising candidates and warrant consideration in further research and development for the management of pain.

6. Conclusion

The current study of *Medicago denticulata* L extract warrants its traditional use as an analgesic and antiamnesic agents while the role to subside diarrhea was mild. Further work is required to isolate the active compounds from the plant responsible for the management of pain and amnesia.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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