BMC Complementary and
Alternative Medicine

RESEARCH ARTICLE

Open Access

CrossMark

Scientific investigation of crude alkaloids from medicinal plants for the management of pain

Mohammad Shoaib[1*], Syed Wadood Ali Shah[1], Niaz Ali[2], Ismail Shah[1], Shafi Ullah[1], Mehreen Ghias[1], Muhammad Nawaz Tahir[3], Farah Gul[4], Sohail Akhtar[5], Abd Ullah[1], Wajid Akbar[1] and Asad Ullah[1]

Abstract

Background: Tissue damage is associated with pain, which is an alarming sign. Aspirin and morphine have been widely used in recent decades for management of pain. Medicinal herbs have been in use for treatment of different diseases for centuries. Many of these herbs possess analgesic activity with relatively less incidences of adverse effects. The strong positive correlation of alkaloids in medicinal plants for analgesic activity persuades an intention to determine possible analgesic activity of total alkaloids extracted from the selected medicinal plants using animal models to answer its possible mechanisms.

Methods: Crude alkaloids from selected medicinal plants (*Woodfordia fruticosa, Adhatoda vasica, Chenopodium ambrosioides, Vitex negundo, Peganum harmala* and *Broussonetia papyrifera*) were extracted as per reported literature. The test crude alkaloids were screened for acute toxicity study. Writhings induced by acetic acid, tail immersion method and formalin-induced nociception assay procedures were used for possible analgesic effects of the crude alkaloids.

Results: Crude alkaloids were safe up to dose of 1250 mg/kg body weight in mice. The alkaloids significantly reduced the abdominal constrictions, and increased the time for paw licking response in both phases with a significant raise in latency time in nociception models ($P \leq 0.05$). Moreover, the antinociceptive response was significantly attenuated by pretreatment with naloxone suggesting involvement of the opioid receptors for possible antinociceptive action.

Conclusions: Crude alkaloids of *Woodfordia fruticosa* and *Peganum harmala* showed prominent analgesic potentials through inhibition of peripheral as well as central nervous system mechanisms. Further work is required for isolation of the pharmacologically active constituents.

Keywords: Crude alkaloids, *Woodfordia fruticosa*, *Peganum harmala*, Traditional uses, Pain, Naloxone, Opioid

Background

International Association for the Study of Pain (IASP) defines pain as "a disagreeable sensory and arousing experience coupled by real or potential tissue damage" [1]. Usually pain is thought to be an alarming sign of actual or apparent tissue damage that warns the subject for possible unwanted outcome. Therefore, the subject seeks averting reaction for possible defense [2]. Aspirin and morphine are in use for analgesic purposes in recent

decades. Opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) have been reported to relieve pain sometimes by 50 % in 30 % of recipients [3]. The uses of these analgesics are associated with incidences of adverse effects like opiates cause physical dependence, tolerance and addiction. NSAIDs are frequently associated with gastrointestinal disorders like gastric or duodenal ulceration [4].

This necessitates for discovery of relatively safe alternatives for treatment of pain. Medicinal herbs have been used for therapeutic purposes for centuries. Many of these herbs had been used for pain management without any evident adverse effects [5]. Ethno-pharmacologically

* Correspondence: mohammadshoaib13@yahoo.com
[1]Department of Pharmacy, University of Malakand, Malakand, Khyber Pakhtunkhwa, Pakistan
Full list of author information is available at the end of the article

Shoaib *et al. BMC Complementary and Alternative Medicine* (2016) 16:178

Page 2 of 8

guided research has brought considerable contributions to new drug development [6, 7]. There has been an increasing interest to find new and safe anti-inflammatory and analgesic drugs from natural sources including medicinal plants [8]. Medicinal plants have been very useful source for lead structure for subsequent synthetic modification and optimization of bioactivity.

Alkaloids are naturally occurring active diverse group of secondary metabolites in plants that has been used in medicine for hundreds years [9].

Plants like *Woodfordia fruticosa, Adhatoda vasica, Chenopodium ambrosioides, Viburnum cotinifolium, Vitex negundo, Peganum harmala* and *Broussonetia papyrifera* have been investigated scientifically for presence of alkaloids with reference to their ethno pharmacological profile in pain management [10, 11–16].

Analgesic activity of alkaloids isolated from plants is reported with different mechanistic approaches [17–20]. The strong positive correlation of alkaloids in medicinal plants for analgesic activity persuades an intention to determine possible analgesic activity of total alkaloids extracted from the mentioned medicinal plants using animals' model.

Methods
Plant materials
The collected plant species namely *Woodfordia fruticosa* (voucher specimen Wf-01-2015, *Lythraceae,* Swat, *aerial parts*), *Adhatoda vasica* syn. *Justicia adhatoda* (voucher specimen Av-02-2015, Acanthaceae, Kohat, leaves), *Chenopodium ambrosioides* (voucher specimen Ca-03-2015, *Chenopodiaceae, Peshawar, leaves*), *Vitex negundo* (voucher specimen Vn-04-2015, Verbenaceae, Swat, *aerial parts*), *Peganum harmala* (voucher specimen Ph-05-2015, *Zygophyllaceae,* Peshawar, *aerial parts*) and *Broussonetia papyrifera* syn. *Morus papyrifera* (voucher specimen Bp-06-2015, Moraceae, Dir Lower, *aerial parts*) were identified by Dr. Jehandar Shah, ex Vice Chancellor, University of Malakand. The materials were dried under shade. Respective voucher specimens were submitted to the herbarium of Department of Botany, University of Malakand, Dir Lower, Khyber Pakhtunkhwa.

Extraction of crude alkaloids
The crude alkaloidal extracts from the plants were obtained according to the method of Harborne with slight modifications. Briefly, dried powdered plants materials (100 g) were defatted using Soxhlet apparatus (Quickfit, UK) followed by an extraction process with 10 % acetic acid (200 ml) in ethanol for 24 h in Soxhlet apparatus. The extract was concentrated using rotary evaporator till a concentrated mass. The pH was adjusted to 9 by the addition of concentrated ammonium hydroxide solution. It was then extracted with chloroform (50 ml) three

times. During extraction, the contents were subjected to vigorous shaking. The chloroform layer was separated using separating funnel. The chloroform portion was concentrated using rotary evaporator. Presence of alkaloids was confirmed with Dragendorff's, Mayer's or picric acid reagent. The alkaloids were stored in a clean amber glass vial at 4 °C. Similar procedure was used to extract crude alkaloids from the aforementioned specimens. The alkaloids were successively labeled as *Woodfordia fruticosa* crude alkaloid (Wf Cr.A), *Adhatoda vasica* crude alkaloid (Av Cr.A), *Chenopodium ambrosioides* crude alkaloid (Ca Cr.A), *Vitex negundo* crude alkaloid (Vn Cr.A), *Peganum harmala* crude alkaloid (Ph Cr.A) and *Broussonetia papyrifera* crude alkaloid (Bp Cr.A) [21–23].

Drugs and standards
Analytical grade chemicals were used in the experiments. Acetylcholine was purchased from BDH, Poole, England. Other chemicals were of E. Merck grade. All the stock solutions were prepared in distilled water. Fresh dilutions were made in normal saline.

Animals
Either sex Balb/C mice (weight range: 25-30 g) were purchased from National Institute of Health Islamabad. They were housed in the animal house of the University of Malakand, Pakistan under a controlled environment (23-25 °C). The animals were fasted overnight before the start of the experiments. They were treated as per the principles mentioned in the "Animals Byelaws 2008 of University of Malakand (Scientific Procedures Issue-I)". Ethical Committee of the Department of Pharmacy, constituted under the approved Animals Byelaws 2008 of University of Malakand, endorsed the study protocols.

Acute toxicity
Acute toxicity study for crude alkaloids was carried out as per procedure reported by Lorke [24]. Male mice were selected by random sampling technique and three groups for individual test sample for two phases were used for the determination of LD_{50}. In the initial phase, the mice were orally treated with 125, 250 and 500 mg/kg of the alkaloidal extract. All test samples were tested in similar manner. The groups were observed for possible mortality in 24 h. In the second phase, the mice were treated with the test alkaloids extract in doses of 750, 1000 and 1250 mg/kg. Death toll was noted in 24 h. LD_{50} was calculated [24, 25].

Acetic acid-induced writhing
Analgesic activity was carried out in mice. Writhings were induced using acetic acid. Briefly, Balb/ C male mice were divided into different groups having six animals in each group. The animals were pretreated with

Shoaib *et al. BMC Complementary and Alternative Medicine* (2016) 16:178

Page 3 of 8

crude alkaloids of various medicinal plants at a dose of 50 mg/kg (i.p.). Diclofenac sodium (50 mg/kg, i.p.) was used as standard analgesic agent. The test samples and standard drugs were administered 1 h before of intraperitoneal injection of 1 % (v/v) acetic acid (0.1 ml/10 g). Five minutes after the injection (i.p) of acetic acid, counted the number of writhing following 10 min. Negative control group received carboxy methyl cellulose (CMC) 0.5 % and vehicle only [26].

Formalin test

This test was performed by the method of assessing the licking response of formalin-induced edema in paw of mice. 20 μl of 1 % formalin prepared in 0.9 % saline was administered (s.c) to the dorsal hind paw and immediately placed in the transparent box for observing the licking response. The duration of reaction time (paw licking or biting) was determined between 0 and 5 min (first phase) and 15–30 min (second phase). Animals in different groups were treated test samples (50 mg/kg, i.p), indomethacin (10 mg/kg, i.p) and morphine (5 mg/kg, i.p), 30 min prior to administration of formalin. Naloxone (2 mg/kg, i.p) was administered 20 min prior to treatment of animals with test samples and standards. Control animals received the vehicle (0.1 ml/10 g). The reaction time of the animals in respective groups was compared to control group and expressed as percent inhibition [27].

Tail immersion method

The animals were divided into groups of six animals each. To prelabelled group (CMC 0.5 %), morphine (5 mg/kg), and crude alkaloids at a dose of 50 mg/kg of different medicinal plants were given. Time for tail withdrawal from the water was measured before and after drug treatment in a regular interval of 15, 30, 45, 60, 75 and 90 min by immersing the tail tips (1-2 cm) of the mice in water bath thermostatically maintained at temperature of 55 ± 1 °C with a cut-off time of immersion at 10 s. The actual flick response of mice was measured by stop watch and results were compared with control and standard group [27].

Statistical analysis

Data are presented as mean ± SEM. Analysis of variance and Dunnett's test is statistically manipulated with GraphPad prism software version 5.01.

Results

After 24 h of observation, in acute toxicity test, no behavioral and physical changes in mice were observed at doses below 1250 mg/kgbody weight for crude alkaloids.

The effect of crude alkaloids in the acetic acid induced writhing model is mentioned in Fig. 1. The compounds antagonized abdominal constrictions at a dose of 50 mg/kg i.p., maximum pain alleviation (78.45 %, $P < 0.001$, $n = 6$) was observed for *Woodfordia fruticosa* crude alkaloid followed by *Peganum harmala* crude alkaloid (72.13 %, $P < 0.001$, $n = 6$). Other alkaloids also produce protection against acetic acid to 63.50, 61.99, 51.10 and 43.03 respectively for *Broussonetia papyrifera* crude alkaloid, *Adhatoda vasica* crude alkaloid, *Vitex negundo* crude alkaloid and *Chenopodium ambrosioides* crude alkaloid. The standard diclofenac sodium produce significant protection of 86.42 %, $P < 0.001$, $n = 6$ at a dose of 50 mg/kg i.p.

The results of the crude alkaloids in post formalin induced flinching behavior are presented in Table 1 and Fig. 2. The compounds produced significant reduction in noxious stimulation in both phases. Pretreatment of test
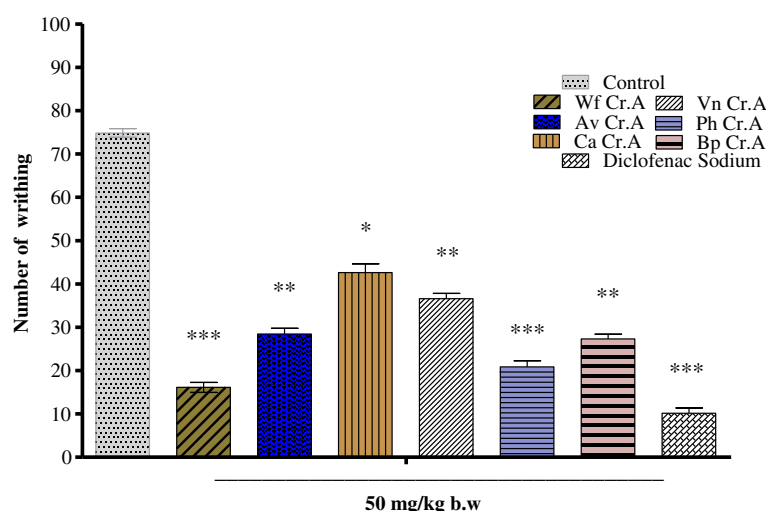

Fig. 1 Analgesic activity of crude alkaloids using acetic acid induced writhing model. All the values are expressed as mean ± SEM ($n = 6$). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to control group

Shoaib *et al. BMC Complementary and Alternative Medicine* (2016) 16:178

Page 4 of 8

Table 1 Effects of crude alkaloids and standardson formalin-induced paw-licking response in mice

Treatment/Dose	Licking time (Sec)		Inhibition (%)	
	1st Phase	2nd Phase	1st Phase	2nd Phase
Control (2 % Tween 80)	48.83 ± 1.627	72.83 ± 1.287	———	———
Wf Cr.A 50 mg	22.35 ± 1.231***	20.12 ± 1.163***	54.22	72.37
Av Cr.A 50 mg	28.31 ± 1.347**	31.45 ± 1.342***	42.14	56.81
Ca Cr.A 50 mg	34.75 ± 1.441*	44.39 ± 1.568*	28.68	39.04
Vn Cr.A 50 mg	33.35 ± 1.604*	38.93 ± 1.195**	31.82	46.54
Ph Cr.A 50 mg	24.85 ± 1.289***	24.53 ± 1.391***	49.16	66.31
Bp Cr.A 50 mg	27.80 ± 1.468**	33.79 ± 1.416**	43.09	53.60
Indomethacin (10 mg)	39.83 ± 1.541	18.66 ± 1.542***	18.43	74.37
Morphine (5 mg)	6.416 ± 1.165***	2.83 ± 1.260***	86.86	96.11

All the values are expressed as mean ± SEM. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ when compared to control group (one way ANOVA followed by Dunnetts: compare all vs control test)
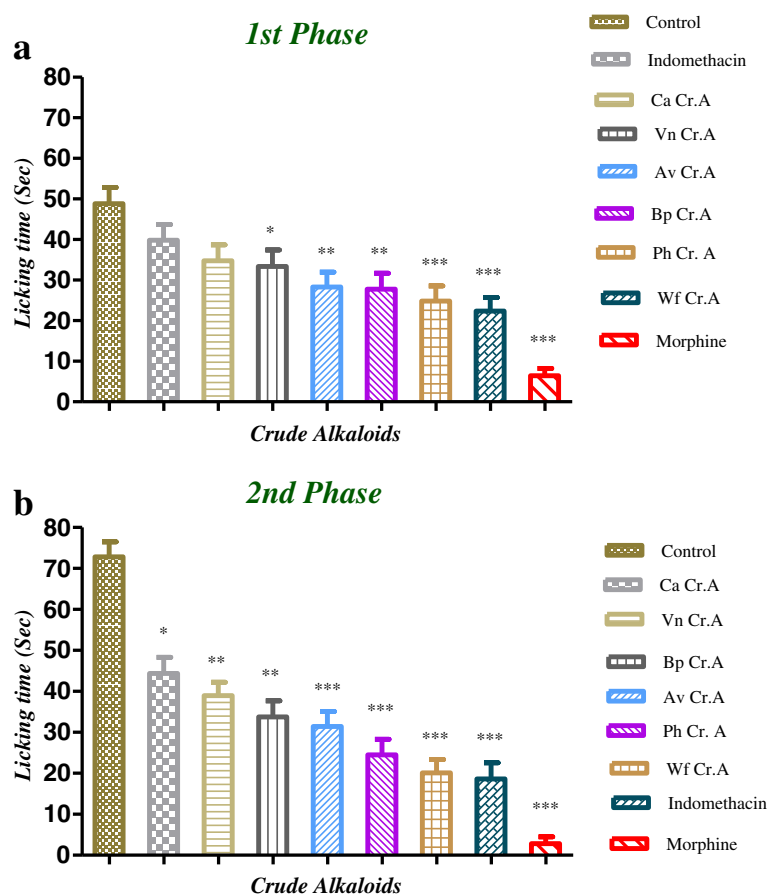

Fig. 2 a Analgesic effect of extracted crude alkaloids on formalin induced licking response in first phase of the test. Values are mean ± SEM, $n = 6$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ significantly different compared with control. **b**: Analgesic effect of extracted crude alkaloids on formalin induced licking response in second phase of the test. Values are mean ± SEM, $n = 6$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ significantly different compared with control

Shoaib *et al. BMC Complementary and Alternative Medicine* (2016) 16:178

Page 5 of 8

samples at the dose of 50 mg/kg i.p. showed marked attenuation of noxious stimulation in both phases in which *Woodfordia fruticosa* crude alkaloid (Wf Cr.A) was dominant with 72.37 and 54.22 % in the both phases, respectively. Similar tendency was produced by *Peganum harmala* crude alkaloid (Ph Cr.A) with 66.31 and 49.16 % control in the 1st and 2nd phases respectively (Fig. 2a and b). The other crude alkaloids also produced moderate to good reduction in noxious stimulation in both phases. The standard indomethacin at a dose of 10 mg/kg produced significant effects of 74.37 % in second phase while less effects were produced in first phase. The centrally acting standard morphine at a dose of 5 mg/kg significantly reduced the noxious stimulation in both phases upto 96.11 % ($P <$ 0.001, $n = 6$) and 86.86 % ($P < 0.001$, $n = 6$) respectively.

In thermal nociception model, the tail immersion test has been carried out for finding the central effect of crude alkaloids. In this test, *Woodfordia fruticosa* crude alkaloid at 50 mg/kg (i.p) showed its highest analgesic response at all time points (15-90 min) in comparison with alkaloids of other plants. The results of these crude alkaloids are shown in Table 2. Morphine was used as reference at a dose of 5 mg/kg, i.p.

The analgesic response of alkaloids was significantly antagonized by naloxone at all the time intervals. The crude alkaloids extracted from different medicinal plants exhibited good to significant analgesic effect in tail immersion test, indicating the involvement of both spinal and supraspinal analgesic pathways.

Upon the administration of the nonselective opioid receptor antagonist naloxone, the analgesic response of crude alkaloids was antagonized.

Discussions

Plants have been in continuous use for treatment of various ailments since creature of human beings. Today many of the current drugs are from natural sources [28]. Pain management sometimes requires more than one drug therapy. Thus the practice of polypharmacy carries risks of adverse drug reactions and or side effects. Therefore, search for new drugs with the same therapeutic efficacy with relatively less frequency of side effects is the need of the time [29, 30].

This study helped us in understanding the possible mechanisms of potential analgesic effect of the test alkaloids that work through inhibition of central nervous system and peripheral nervous system. The abdominal constriction induced by acetic acid is thought to be due to the involvement of peripheral mechanisms, while tail immersion test is thought to be due to central mechanisms [31]. Formalin test is used for both peripheral and central mechanisms [32].

Acetic acid-induced writhing is a well-recommended model to assess the analgesic proprieties of drugs having analgesic and anti-inflammatory activity. Acetic acid performs its action by release of endogenous mediators like prostaglandins from arachidonic acid through cyclooxygenase enzymes. These prostaglandins stimulate the nociceptive neurons [33, 34] with induction of pain sensation. The abdominal constriction response may also be due to the activation of local peritoneal receptors [35] and involved prostanoids mediators.

NSAIDs possess analgesic activity by inhibiting the cyclooxygenase pathway and synthesis of the prostaglandins that confirm the involvement of peripheral mechanism in inhibition of pain [36]. Thus NSAIDs in writhing model are used as positive control for inhibition of pain that works through peripheral mechanism [37, 38]. Therefore, the analgesic action of crude alkaloids seems to be due to inhibition of cyclooxygenase or 5-lipoxygense pathways that may be attributed to inhibition of inflammatory cytokines and interleukins [39].

The formalin test model is used to investigate on the ability to exert peripheral and or central analgesic effects as it assays biphasic characteristics, labeled as the early and late phases that occur as a result of formalin administration [40]. The early phase is a neurogenic pain resulting from an acute response towards direct action of formalin on nociceptors within the intraplantar region, while the late phase is considered as an inflammatory-mediated pain resulting from a tonic response due to the release of inflammatory mediators [41]. There may be activation of the neurons in the dorsal horns of the spinal cord as well [42, 43]. Generally, the peripherally-acting drugs like aspirin blocks the synthesis of prostaglandins,which inhibit the late phase only, while narcotic analgesics (centrally-acting) inhibit nociception in both phases of the formalin test [44, 45].

Tail immersion test is selective for morphine like compounds. It tests the reflex responses involved through the spinal cord for possible sensation of nociceptive stimuli [46]. The brain and spinal cord play an important role in central pain mechanism. Dorsal part of spinal cord is rich withsubstance P, endogenous opioids, somatostatin, and other inhibitory hormones which are the targets of pain and inflammation [47]. The results of the tail immersion test indicated that antinociceptive effect of crude alkaloids and standard morphine was reversed by naloxone suggests the involvement of opioid receptors at level of spinal cord. It has been suggested that μ2- and δ- opioid receptors are involved in spinal mechanism [48]. Therefore, it can be anticipated that the central analgesic effects of extracted crude alkaloids may be prominent on μ-opioid receptors.

The crude alkaloidal extractsof different medicinal plants possess analgesic potentials possibly through inhibion of central and peripheral pain mediators. The

Shoaib *et al. BMC Complementary and Alternative Medicine* (2016) 16:178

Page 6 of 8

Table 2 Analgesic effects of crude alkaloids and standard drugs using tail flick method

Treatment/Dose	Time in Sec (Tail Flick)/Response (%)					
	15 min	30 min	45 min	60 min	75 min	90 min
Control (2 % Tween 80)	0.78 ± 1.30	0.88 ± 1.21	0.98 ± 1.021	0.93 ± 1.381	0.86 ± 1.025	0.92 ± 1.401
Wf Cr.A 50 mg	1.01 ± 1.12* (22.70 %)	1.38 ± 1.112* (36.23 %)	1.68 ± 1.131** (41.66 %)	2.13 ± 1.124** (56.33 %)	2.41 ± 1.163*** (64.31 %)	2.15 ± 1.138*** (57.20 %)
Av Cr.A 50 mg	0.92 ± 1.20 (15.21 %)	1.14 ± 1.411* (22.52 %)	1.41 ± 1.161** (30.49 %)	1.48 ± 1.201** (37.16 %)	1.65 ± 1.206*** (47.87 %)	1.57 ± 1.282*** (41.40 %)
Ca Cr.A 50 mg	0.89 ± 1.30 (12.35 %)	1.09 ± 1.411* (19.85 %)	1.35 ± 1.161** (27.40 %)	1.39 ± 1.231** (33.09 %)	1.53 ± 1.226*** (43.79 %)	1.49 ± 1.218*** (38.25 %)
Vn Cr.A 50 mg	0.90 ± 1.10 (13.81 %)	1.12 ± 1.521* (21.75 %)	1.38 ± 1.611** (28.98 %)	1.40 ± 1.421** (33.80 %)	1.55 ± 1.261*** (44.69 %)	1.51 ± 1.618*** (39.27 %)
Ph Cr.A 50 mg	0.97 ± 0.82 (19.58 %)*	1.23 ± 1.135* (28.45 %)	1.70 ± 1.141** (42.35 %)	2.08 ± 1.511** (55.28 %)	2.33 ± 1.133** (63.09 %)	2.04 ± 1.123** (54.90 %)
Bp Cr.A 50 mg	0.90 ± 0.97 (13.81 %)	1.11 ± 1.411* (20.42 %)	1.37 ± 1.261** (28.46 %)	1.42 ± 1.301** (34.50 %)	1.57 ± 1.237*** (45.22 %)	1.53 ± 1.218*** (39.90 %)
Standard (Morphine 5 mg)	1.54 ± 1.24** (49.35 %)	2.11 ± 1.066** (58.29 %)	4.52 ± 1.038*** (78.31 %)	6.12 ± 1.054*** (84.80 %)	4.98 ± 1.309*** (82.70 %)	4.74 ± 1.074*** (80.59 %)
Wf Cr.A (50 mg) + Naloxone (2 mg)	0.82 ± 0.83	0.81 ± 1.165	0.93 ± 1.049	0.99 ± 1.308	0.87 ± 1.029	0.83 ± 1.461
Av Cr.A (50 mg) + Naloxone (2 mg)	0.96 ± 0.91	0.86 ± 1.036	0.88 ± 1.131	0.94 ± 1.047	0.89 ± 1.381	0.84 ± 1.069
Ca Cr.A (50 mg) + Naloxone (2 mg)	0.84 ± 1.08	0.91 ± 1.044	0.84 ± 1.045	0.89 ± 1.642	0.94 ± 1.263	0.78 ± 1.422
Vn Cr.A (50 mg) + Naloxone (2 mg)	0.94 ± 1.03	0.88 ± 1.051	0.81 ± 1.521	0.83 ± 1.342	0.86 ± 1.061	0.87 ± 1.356
Ph Cr.A (50 mg) + Naloxone (2 mg)	0.81 ± 0.69	0.96 ± 1.031	1.05 ± 1.271	0.97 ± 1.535	0.91 ± 1.251	0.93 ± 1.034
Bp Cr.A 50 mg + Naloxone (2 mg)	0.88 ± 1.124	0.81 ± 0.89	0.94 ± 1.034	0.86 ± 1.324	0.93 ± 1.304	0.94 ± 1.263
Morphine (5 mg) + Naloxone (2 mg)	0.76 ± 0.82	0.87 ± 1.336	0.96 ± 1.204	0.95 ± 1.641	0.89 ± 1.035	0.97 ± 1.432

All the values were expressed as mean ± SEM. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ when compared to control group (one way ANOVA followed by Dunnetts: compare all vs control test)

antinociceptive activity confirms traditional uses of the aforementioned medicinal plants for the management of pain.

Conclusion

Crude alkaloids of *Woodfordia fruticosa* and *Peganum harmala* showed prominent analgesic potentials that requires further work for isolation of pharmacologically active constituents.

Abbreviations

Av Cr.A, *Adhatoda vasica* crude alkaloid; Bp Cr.A, *Broussonetia papyrifera* crude alkaloid; Ca Cr.A, *Chenopodium ambrosioides* crude alkaloid; CMC, Carboxy methyl cellulose; Ph Cr.A, *Peganum harmala* crude alkaloid; s.c, subcutaneous; Vn Cr.A, *Vitex negundo* crude alkaloid; Wf Cr.A, *Woodfordia fruticosa* crude alkaloid.

Availability of data and materials

The data sets supporting the conclusions of this article are presented in this main paper. Plant materials used in this study have been identified at the University of Malakand and voucher specimens are deposited Herbarium of Botanical garden.

Authors' contributions

MS: Supervision of whole project. SWAS: Extraction of alkaloids. NA: Supervision of the pharmacological tests and manuscript preparation.IS: Collaborated in manuscript preparation. S: Performed the pharmacological tests. MG: Performed the pharmacological tests. MNT: Interpret the data. SA: Collaborated in statistical interpretation. A, WA, AU: Performed the pharmacological tests. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not relevant.

Shoaib *et al. BMC Complementary and Alternative Medicine* (2016) 16:178

Page 7 of 8

Ethics approval and consent to participate

Author details

[1]Department of Pharmacy, University of Malakand, Malakand, Khyber Pakhtunkhwa, Pakistan. [2]Department of Pharmacology, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Khyber Pakhtunkhwa, Pakistan. [3]Department of Physics, University of Sargodha, Punjab, Pakistan. [4]Pharmacology Section, PCSIR Laboratories Complex, Peshawar, Khyber Pakhtunkhwa, Pakistan. [5]Department of Statistics, University of Malakand, Malakand, Khyber Pakhtunkhwa, Pakistan.

References

1. Merskey H. Pain terms: a list with definitions and notes on usage. Recommended by the IASP subcommittee on Taxonomy. Pain. 1979;6:249–52.
2. Unruh A, Strong J, Wright A. Introduction. In: Strong J, Unruh AM, Wright A, Baxter GD, Wall PD, editors. Pain: a textbook for therapists. London: Churchill Livingstone; 2002.
3. Hewitt DJ, Hargreaves RJ, Curtis SP, Michelson D. Challenges in analgesic drug development. Clin Pharmacol Ther. 2009;86:447–50.
4. Hanson GR, Venturelli PJ, Fleckenstein AE. Drugs and society. 10th ed. Boston, Mass: Jones and Bartlett; 2011.
5. Fan SH, Noraisah AA, Dayang FB. Evaluation of analgesic activity of the methanol extract from the galls of Quercus infectoria (Olivier) in rats. J Evid Based Complementary Altern Med. 2014;2014:1–6.
6. Rates SMK. Plants as source of drugs. Toxicon. 2001;29:603–13.
7. Zainul AZ, Hijaz MS, Manraj SC, Arifah AK, Teh LK, Mohd ZS. Antinociceptive activity of methanolic extract of Muntingia calabura leaves: further elucidation of the possible mechanisms. BMC Complement Altern Med. 2014;14:63–75.
8. Asie S, Majid M, Sima N, Manijeh M. Evaluation of anti-inflammatory and analgesic activity of the extract and fractions of Astragalus hamosus in animal models. Iran J Pharm Res. 2015;14:263–9.
9. Roberts MF, Wink M. Introduction. In: Roberts MF, Wink M, editors. Alkaloids: biochemistry, ecology, and medicinal applications. Springer Street, New York: Plenum Press; 1998.
10. Amir MK, Rizwana AQ, Faizan U, Syed AG, Asia N, Sumaira S, Muhammad KL, Muhammad YL, Shafiq UR, Ishtiaq H, Waheed M. Phytochemical analysis of selected medicinal plants of Margalla hills and surroundings. J Med Plants Res. 2011;5:6017–23.
11. Yogesh B, YV, Sumitra C. Brine shrimp cytotoxicity, anti-inflammatory and analgesic properties of Woodfordia fruticosa Kurz flowers. Iran J Pharm Res. 2012; 11: 851-861.
12. Wahid AM, Suyog DM, Suraj BJ, Ajinkya MP, Mukhtar SK, Madhukar RV. Evaluation of antiinflammatory and analgesic activities of ethanolic extract of roots Adhatoda vasica Linn. Int J PharmTech Res. 2010;2:1364–8.
13. Ibironke GF, Ajiboye KI. Studies on the anti-inflammatory and analgesic properties of Chenopodium ambrosioides leaf extract in rats. Int J Pharm. 2007;3:111–5.
14. Zhenga CJ, Tangb WZ, Huanga BK, Hana T, Zhanga QY, Zhanga H, Qin LP. Bioactivity-guided fractionation for analgesic properties and constituents of Vitex negundo L. seeds. Phytomedicine. 2009;16:560–7.
15. Loubna F, Amine L, Rachida A, Ahmed B, Abderrahman C. Evaluation of the analgesic effect of alkaloid extract of Peganum harmala L. Possible mechanisms involved. J Ethnopharmacol. 2008;115:449–54.
16. Lin LW, Chen HY, Wu CR, Liao PM, Lin YT, Hsieh MT, Ching H. Comparison with various parts of Broussonetia papyrifera as to the antinociceptive and anti-inflammatory activities in rodents. Biosci Biotechnol Biochem. 2008;72:2377–84.
17. Irene MV, Arlyn CS. Cassiaindoline, a new analgesic and anti-inflammatory alkaloid from Cassia alata. Z Naturforsch C. 2009;64:335–8.
18. Kaleem WA, Muhammad N, Qayum M, Khan H, Khan A, Aliberti L, Vincenzo DF. Antinociceptive activity of cyclopeptide alkaloids isolated from Ziziphus oxyphylla Edgew (Rhamnaceae). Fitoterapia. 2013;91:154–8.
19. Fagner CL, Jaime RF, Hermann FC, Paula RRS, Andrea SC, Alan BC, de Almeida RN, Dias Cda S, Bozza PT, Piuvezam MR. Curine, an alkaloid isolated from Chondrodendron platyphyllum inhibits prostaglandin E2 in experimental models of inflammation and pain. Planta Med. 2014;80:1072–8.
20. Muhammad N, Shrestha RL, Adhikari A, Wadood A, Khan H, Khan AZ, Maione F, Mascolo N, De Feo V. First evidence of the analgesic activity of govaniadine, an alkaloid isolated from Corydalis govaniana Wall. Nat Prod Res. 2014;29:430–7.
21. Harborne JB. Phytochemical methods. 2nd ed. New York: Chapman and Hall; 1984.
22. Mahboba BN, Rabia OA, Nabil AS, Asma YAN. Evaluation of antibacterial and antioxidant activities of Artemisia campestris (Astraceae) and Ziziphus lotus (Rhamnacea). Arab J Chem. 2010;3:79–84.
23. Shammary AAH, Malik AASS, Saeidy MAA. Evaluation of analgesic activity and toxicity of alkaloids in Myristica fragrans seeds in mice. J Pain Res. 2013;6:611–5.
24. Lorke D. A New approach to practical acute toxicity testing. Arch Toxicol. 1983;53:275–87.
25. Niaz A, Hina A, Aslam K, Ghayour A, Wadood AS, Muhammad N, Muhammad J. Antispasmodic and antidiarrhoeal activity of the fruit of Rosa moschata (J). BMC Complement Altern Med. 2014;14:485–90.
26. Koster R, Anderson M, Beer EJ. Acetic acid for analgesic screening. Fed Proc. 1959;18:412–8.
27. Pandurangan SB, Paul AS, Savarimuthu I, Ali AA. Antinociceptive, immunomodulatory and antipyretic activity of nymphayol isolated from Nymphaea stellata (Willd.) flowers. Biomol Ther. 2013;21:391-97.
28. Bonito MC, Cicala C, Marcotullio MC, Maione F, Mascolo N. Biological activity of bicyclic and tricyclic diterpenoids from Salvia species of immediate pharmacological and pharmaceutical interest. Nat Prod Commun. 2011;8:1205–15.
29. Alfieri A, Maione F, Bisio A, Romussi G, Mascolo N, Cicala C. Effect of a diterpenoid from Salvia cinnabarina on arterial blood pressure in rats. Phytother Res. 2007;7:690–2.
30. Maione F, Cicala C, Musciacco G, De Feo V, Amat AG, Ialenti A, Mascolo N. Phenols, alkaloids and terpenes from medicinal plants with antihypertensive and vasorelaxant activities: a review of natural products as leads to potential therapeutic agents. Nat Prod Commun. 2013;4:539–44.
31. Paulino N, Dantas AP, Bankova V, Longhi DT, Scremin A, Decastro SL, et al. Bulgarian propolis induces analgesics and antiinflammatory effects in mice and inhibits in-vitro contraction of airway smooth muscle. J Pharmacol Sci. 2003;93:307–13.
32. Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain. 1992;51:5–17.
33. Collier HO, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the Mouse. Br J Pharmacol Chemother. 1968;32:295–310.
34. Dhara AK, Suba V, Sen T, Pal S, Nagchaudhuri AK. Preliminary studies on the anti-inflammatory and analgesic activity of the methanolic fraction of the root extract of Tragia involucrate. J Ethnopharmacol. 2000;72:265–8.
35. Bentley GA, Newton SH, Starr J. Studies on the anti-nociceptive action of a agonist drugs and their interaction with opioid mechanisms. Br J Pharmacol. 1983;79:125–34.
36. Duarte I, Nakamura M, Ferreira S. Participation of the sympathetic system in acetic acid-induced writhing in mice. Braz J Med Biol Res. 1988;21:341–3.
37. Vane JR, Botting RM. The mechanism of action of aspirin. Thromb Res Rev. 2003;110:255–8.
38. Biswal S, Das MC, Nayak P. Antinociceptive activity of seeds of Trigonella foenumgraecum in rats. Indian J Physiol Pharmacol. 2003;47:479–80.
39. Reibero RA, Vale ML, Thomazzi SM, Pascholato ABP, Poole S, Ferreira SH, et al. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur J Pharmacol. 2000;387:111–8.
40. Malmberg AB, Yaksh TL. Antinociceptive actions of spinal nonsteroidal anti-inflammatory agents on the formalin test in the rat. J Pharm Exp Ther. 1992;263:136–46.
41. Verma PR, Joharapurkar AA, Chatpalliwar VA, Asnani AJ. Antinociceptive activity of alcoholic extract of Hemidesmus indicus R.Br. in mice. J Ethnopharmacol. 2005;102:298–301.
42. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain. 1987;30:103–14.
43. Tang L, Chen Y, Chen Z, Blumberg PM, Kozikowski AP, Wang ZJ. Antinociceptive pharmacology of N-(4-chlorobenzyl)-N′-(4-hydroxy-3-iodo-5-methoxybenzyl) thiourea, a high-affinity competitive antagonist of the transient receptor potential vanilloid 1 receptor. J Pharm Exp Ther. 2007;321:791–8.
44. Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test: characteristic biphasic pain response. Pain. 1989;38:347–52.
45. Mohammad I, Mohamad Y, Mohammad ZS, Teh LK, Norizan A, Nik FNA, et al. Activity-guided isolation of bioactive constituents with antinociceptive

Shoaib *et al. BMC Complementary and Alternative Medicine* (2016) 16:178

Page 8 of 8

activity from *Muntingia calabura* L. Leaves using the formalin test. Evid Based Complement Alternat Med. 2013;2013:1–9.

46. Manju B, Jagtap AG. Exploring the possible mechanisms of action behind the antinociceptive activity of *Bacopa monniera*. Int J Ayurveda Res. 2011;2:2–7.

47. Curdy CRM, Scully SS. Analgesic substances derived from natural products (natureceuticals). Life Sci. 2005;78:476–84.

48. Jinsmaa Y, Okada Y, Tsuda Y, Sasak Y, Ambo A, Bryant SD, et al. Novel 2′,6′ dimethyl-l-tyrosine-containing pyrazinone opioid mimetic A agonists with potent antinociceptive activity in mice. J Pharmacol Exp Ther. 2004;309:1–7.