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Activity guided isolation and mechanistic approach towards analgesic potential of Chenopodium mediated through opioidergic pathway

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Abstract: The current study is focused towards screening for its phytochemicals, phenolic and flavonoid contents of different species of Chenopodium. The plants were also screened for corroborating the traditional use of medicinal plants locally used for pain by determining the extract and their fractions for the *in-vivo* analgesic activity by using the modern scientific system. Among chloroform fractions, a high level of total phenolic contents was found in chloroform fraction of *Chenopodium ambrosioides* (ChAm-Chf) with 57.12±1.02 followed by *Chenopodium botrys (*ChBt-Chf) with 56.79±0.71. High content of flavonoids was found in chloroform fraction of *Chenopodium botrys (*ChBt-Chf) extract with 78.35±0.84 followed by *Chenopodium ambrosioides* (ChAm-Chf) with 75.20±0.81. The crude extract *Chenopodium album*, *Chenopodium botrys and Chenopodium ambrosioides* (ChAl-Crd, ChBt-Crd and ChAm-Crd) at 100 and 200 mg/kg, chloroform and ethylacetate fractions (ChAl-Chf, ChBt-Chf, ChAm-Chf, ChAl-Et, ChBt-Et and ChAm-Et) at 75 mg/kg caused significant inhibition ($P<0.05$, $P<0.01$, $P<0.001$, $n=8$) of the analgesic response induced by acetic acid, formalin and hotplate method. Mechanistically, the naloxone overturns completely the analgesic effects of beta-sitosterol (SN2) while partial reversal was observed by ursolic acid (SN1) indicating other possible mechanisms in association with opioid receptors.

Keywords: Chenopodium, pain, phenolics and flavonoids, *in-vivo* animal model.

INTRODUCTION

For centuries, the history of drug discovery and pharmacy has been the same with the history of ethnobotany and pharmacognosy. About 40% of the drugs emerge from natural products (Kayser, 2018). The role of traditional medicine is to deliver medicinally active plants in the drug discovery and development (Anand *et a*l., 2019). Many of these medicinal plants had been employed in management of pain lacking any marked undesirable effects and have been a dire interest to unearth new and safe analgesics from natural sources (Shoaib *et al*., 2016a). Use of medicinal plants is one of the most primary ways of combating diseases and relieving pain, and as the pharmaceutical industry is advancing, many synthetic analgesics have been introduced into pharmacology with many side effects alongside promoting analgesic capacity (Parsaei *et al*., 2016). Due to the various side effects and ineffectiveness of many conventional drugs, the quest for new drugs from natural origin has grown the momentum in recent years (Mohammad Hosseini *et al*., 2019).

Chenopodiaceae is a diverse family of about 102 genera and 1400 species (Ajayi *et al*., 2016). Species of Chenopodium genus are mostly herbaceous in nature and include more than 200 species which are native to Europe, America and Asia (Pakistan, India and China) (Sukhorukov *et al*., 2019). Species that fall in the genus of Chenopodium are reported for their biologically active secondary metabolites. The extract, fractions and isolated compounds from this genus are also reported for pharmacological potentials against various animal models (Ajayi *et al*., 2017, Kokanova-Nedialkova and Nedialkov, 2019).

In this study, an effort was carried out to investigate the analgesic effects of crude extract and fractions from the species of Chenopodium and their possible mechanisms involved followed by bioguided isolation of pure compounds responsible for analgesic activity.

MATERIALS AND METHODS

Chemicals and animals

$AICl_3$, Gallic acid, Folin-Ciocalteu reagent, Tween-80 were purchased from Sigma Aldrich Chemical Company, Germany. Diclofenac sodium, Tramadol, naloxone and indomethacin were purchased locally. Solvents and chemicals like methanol, ethanol, hexane, ethyl acetate, acetic acid used were of analytical grade extra pure purchased from E. Merck. Male Balb/C mice weighing 17-23gm 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

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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 (Pharm/EC/34-01/19), University of Malakand in compliance with provisions of the "Animal Bye-Laws 2008, Scientific Procedures Issue-I of the University of Malakand".

Plant material

The aerial parts of the plants of *Chenopodium album* L (ChAl), *Chenopodium botrys L (ChBt) and Chenopodium ambrosioides* L (ChAm) were collected in the month of June- July 2018 from the peripheral areas of University of Malakand, Pakistan and were authenticated. The plant specimens with voucher numbers ChAl-2018-177, ChBt-2018-178 and ChAm-2018-179 were submitted in the Herbarium, University of Malakand, Pakistan.

Preparation of extracts

The shade dried plants (2k g) were coarsely ground using a grinder followed by methanolic extraction (three times) and filtration through a piece of cloth. The filtrate was reduced to a semisolid mass using a rotary evaporator (Heidolph *Labo ROTA, Germany*) under reduced pressure at 45°C for a final extract as ChAl-Crd (19.8%), ChBt-Crd (24.6%) and ChAm-Crd (22.5%) respectively. The crude extracts were further fractionated with solvents to produce n-hexane, chloroform, ethylacetate, butanol and aqueous fractions.

Preliminary phytochemical tests

The crude extract of various plants was assessed for the determination of different phytoconstituents like saponins, alkaloids, terpenoids, flavonoids, glycosides and tannins by using standard qualitative methods described (Ali *et al*., 2017).

Determination of total phenolic and flavonoid content

Folin-Ciocalteu and aluminum chloride colorimetric methods were used for the determination of total phenolic and total flavonoid content, respectively according to the method reported (Pandey *et al*., 2016). The total phenolic content is represented as gallic acid equivalents through the calibration curve (Gallic acid in mg/g of extract). The total flavonoid content is represented as quercetin equivalents through the calibration curve (quercetin in mg/g of extract).

Pharmacological activities

Acute toxicity test

The acute toxicity in mice was performed in two phases at different dose concentration in various groups according to the Organization for Economic Cooperation and Development (OECD) guideline no. 423 (OECD, 2001). 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 to food and water (Xiao *et al*., 2019).

Writhing test by acetic acid

The extracts (ChAl-Crd, ChBt-Crd and ChAm-Crd) at a dose of 100 and 200 mg/kg and fractions (ChAl-Chf, ChBt-Chf, ChAm-Chf, ChAl-Et, ChBt-Et and ChAm-Et) at a dose of 75mg/kg body weight were administered via intraperitoneal route (i.p.) to each experimental group of 8 mice (n=8). Each experimental group was treated with 0.6% of acetic acid intraperitoneally (10 mL/kg, i.p.) 30 minutes after the administration of extracts, fractions and standard drug. The intensity of nociception was documented in a number of writhes produced within 30 minutes after administration of acetic acid (Shoaib *et al*., 2019).

Licking response by formalin

The mice in experimental groups of eight animals each (n=8) were treated intraperitoneally (i.p.) with crude extracts at a dose of 100 and 200 mg/kg and fractions at a dose of 75mg/kg body weight 1 hour before the treatment of animals with formalin (1%, 50µL) in respective groups in the right hind paw of animals. The treated paw of mice was experiential for 30 minutes in a plexiglass box and the time in seconds for paw licking by mice was documented in two phases, 0-5 min (neurogenic pain), and 15–30 min (inflammatory pain) (Shoaib *et al*., 2019).

Hot plate test and involvement of opioid system

The test was carried out according to the previously reported protocol by determining reaction time of mice as licking, shaking of the paw or jumping off from the hot surface maintain at 55±2°C. The data were recorded 30 minutes after intraperitoneal (i.p.) administration of crude extracts at a dose of 100 and 200 mg/kg and fractions at a dose of 75mg/kg body weight or vehicle (10mL/kg). Tramadol (20mg/kg, i.p.) and morphine (5mg/kg, i.p.) were given 30 minutes before the hot plate test. Naloxone (2mg/kg, i.p.) was used to assess the involvement of the opioid system (Eidi *et al*., 2016).

Bioguided isolation and characterization

The pharmacologically active fraction from potent specie was chromatographed by gravity column packed with silica with elution initially started from hexane and gradually increased the polarity with ethylacetate until it reached 100% ethylacetate to obtain major subfractions. These subfractions were tested for bioactivity using analgesic activity test models. The most potent subfraction was further chromatographed in a column using ethyl acetate, hexane system to yield compound SN-1 (49mg) and SN2 (64mg). The isolated compounds were screened for analgesic effect using a hot plate test and involvement of the opioid system (Eidi *et al*., 2016).

STATISTICAL ANALYSIS

For statistical analysis, GraphPad prism 5 version 5.01 software using one-way analysis of variance (ANOVA)

and Dunnett's test was used. The significance was set at $P<0.05$.

RESULTS

Phytochemical analysis

The test samples of ChAl-Crd, ChBt-Crd and ChAm-Crd were tested for the identification of different phytoconstituents. The results revealed the presence of moderate to strong quantity of major phytoconstituents like alkaloids, flavonoids, phenloics, glycosides, tannins, saponins, fats and fixed oils etc. in ChAl-Crd, ChBt-Crd and ChAm-Crd**.**

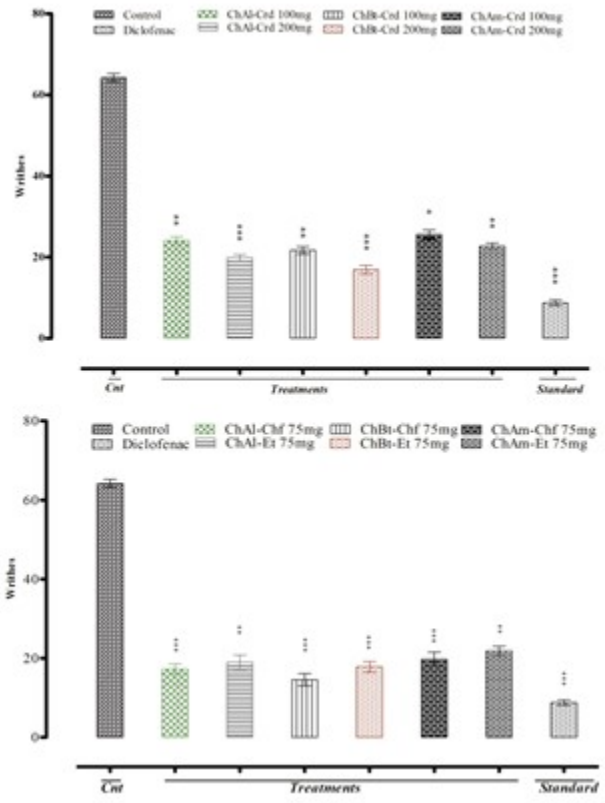

Fig. 1: a) Acetic acid induced writhing model for analgesic effects of crude extracts. b: Acetic acid induced writhing model for analgesic effects of chloroform and ethylacetate fractions. Mean ±SEM (n=8). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *vs* control group.

Determination of total phenolics and total flavonoids contents

The Folin-Ciocalteu reagent and aluminium chloride methods were used for the determination of total phenolics and flavonoids contents respectively in all the samples. The values of phenolic and flavonoid contents were summarized in table 1. The values of total phenolic contents ranged from 25.40±0.82 to 36.28±0.72 in crude extracts. Among chloroform fractions, high level of total phenolic contents was found in ChAm-Chf with 57.12±1.02 followed by ChBt-Chf with 56.79±0.71. ChAl-Chf was found with low phenolic contents of 55.91±0.67.

Similarly the total flavonoids contents of crude extracts ranged from 32.06±0.96 to 41.05±1.04. High content of flavonoids was found in ChBt-Chf extracts with 78.35±0.84 followed by ChAm-Chf with 75.20±0.81. Similar results were produced in the analysis of ethylacetate fractions for total phenolics and total flavonoids contents.

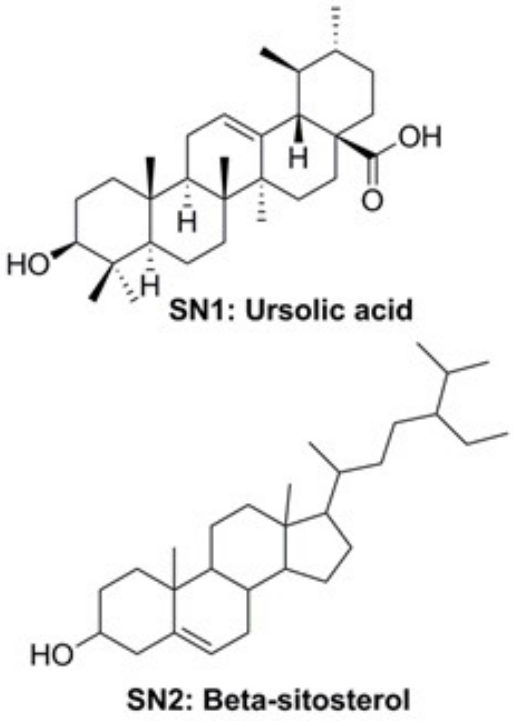

Fig. 2: Isolated compounds for analgesic activity

Acute Toxicity

Results from crude extract (ChAl-Crd, ChBt-Crd and ChAm-Crd) and fractions (ChAl-Chf, ChBt-Chf, ChAm-Chf, ChAl-Et, ChBt-Et and ChAm-Et) revealed no mortality even a maximum dose up to 1500 mg / kg (b.w) was orally administered. Hence, 100 mg/kg, 200mg/kg dose for crude extracts and 75 mg/kg for subsequent fractions were chosen then to evaluate pharmacological activities.

Writhing test

Fig. 1a and 1b represents the effect of administered crude extract (ChAl-Crd, ChBt-Crd and ChAm-Crd) and fractions (ChAl-Chf, ChBt-Chf, ChAm-Chf, ChAl-Et, ChBt-Et and ChAm-Et) on mice during the abdominal constriction model. The crude extracts impart a dose dependent significant inhibition of nociceptive effects at 100 and 200 mg/kg ($P<0.05$, $P<0.01$, $P<0.001$, n=8) and fractions at 75 mg/kg ($P<0.01$, $P<0.001$, n=8) induced by acetic acid when compared to control. The results

Table 1: Total phenolic and total flavonoid contents in Chenopodium species

Extracts sample	TPC (mg GAE/g)	TFC (mg QE/g)
ChAl-Crd	28.61 ± 0.41	38.94 ± 0.91
ChBt-Crd	36.28 ± 0.72	41.05 ± 1.04
ChAm-Crd	25.40 ± 0.82	32.06 ± 0.96
ChAl-Chf	55.91± 0.67	61.20± 1.02
ChBt-Chf	56.79 ± 0.71	78.35 ± 0.84
ChAm-Chf	57.12± 1.02	75.20± 0.81
ChAl-Et	49.15 ± 1.12	52.13 ± 0.86
ChBt-Et	44.95 ± 0.74	63.49 ± 1.13
ChAm-Et	47.51± 0.91	55.60± 0.87

Results are taken as mean ± SEM, n=3.

Table 2: Formalin-induced paw-licking response.

Treatment/Dose					
		1st Phase	2nd Phase	1st Phase	2nd Phase
Control	(2% Tween-80)	43.14±2.32	66.51±2.57	----	----
ChAl-Crd	100	20.65±2.31**	26.96±2.55*	52.20	59.43
	200	19.22±1.33***	24.38±1.34**	54.44	63.34
ChAl-Chf	75	19.35±1.25***	20.57±1.02***	55.13	69.03
ChAl-Et	75	21.54±1.85**	23.13±1.98**	50.09	65.24
ChBt-Crd	100	17.01±0.94***	23.83±2.45**	60.60	64.18
	200	15.79±1.68***	19.21±1.57***	63.39	71.11
ChBt-Chf	75	17.91±1.12***	18.85±1.31***	58.41	71.00
ChBt-Et	75	19.27±2.24***	21.67±2.24**	55.39	67.00
ChAm-Crd	100	21.86±1.92**	27.53±2.38*	49.36	58.61
	200	19.36±2.93***	23.13±1.76**	55.11	66.23
ChAm-Chf	75	18.88±1.58***	20.32±1.68***	56.21	69.45
ChAm-Et	75	20.55±2.83**	23.20±2.63**	52.39	66.11
Indomethacin	10	35.18±2.52	17.71±1.30***	18.43	74.37
Morphine	5	5.67±1.60***	2.59±1.01***	86.86	96.11

Mean ±SEM,n=8. *P<0.05, **P<0.01 and ***P<0.001 *vs* control group (one way ANOVA followed by Dunnetts: compare all vs control test).

revealed achievement of dose dependent pain relief, while writhing percent inhibition at all test doses is shown in (fig. 1a). Maximum inhibition (77.31%, P <0.001, n=8) was experienced at 75mg/kg dose of ChBt-Chf. The inhibitory effect of Diclofenac sodium (88.42%, P <0.001, n=8) was greater than crude extract and fractions.

Formalin test

A dose-related inhibitory effect was produced by crude extract (ChAl-Crd, ChBt-Crd and ChAm-Crd) in formalin induced nociception by significantly impeding both the neurogenic and inflammatory phases (P<0.05, P<0.01, P<0.001, n=8) of licking test at 100 and 200 mg/kg when compared with control (table 2). Similarly the fractions of Chenopodium (ChAl-Chf, ChBt-Chf, ChAm-Chf, ChAl-Et, ChBt-Et and ChAm-Et) also produced significant response and ChBt-Chf (P<0.001, n=8) was found to be more potent than the rest of fractions. However, the phase second antinociceptive effect of the test model was more obvious. Animals pretreated with morphine (5 mg/kg) inhibited significantly both phases to 86.86% (P<0.001, n=6) and 96.11% (P<0.001, n=6) respectively of paw licking responses.

Hot plate test and involvement of opioid system

The central analgesic effect of the samples is commonly assessed by hot plate test. A significant inhibition was shown by crude extract of Chenopodium ((ChAl-Crd, ChBt-Crd and ChAm-Crd)) as compared to the control group (P<0.01 and P<0.001, n=8). Tramadol and morphine as a positive control demonstrated 72.58% (P<0.001, n=8) and 81.19% (P<0.001, n=8) inhibitory effects respectively at a therapeutic dose as compared to control. The maximum inhibition of 69.97% (P<0.001, n=8) and 67.60% (P<0.001, n=8) for ChBt-Chf and ChAl-Chf respectively, was observed at 75mg/kg for each compound (table 3). Animals treated with naloxone produced significant reduction in the analgesic activity of tested samples and standard indicating the involvement of opioid receptors in analgesic response (table 3).

Table 3: Analgesic response using hot plate method.

Treatment/Dose (mg)		0 min	30 min	60 min	90 min	120 min	At 60 min
Control (2% Tween 80)		3.31±1.15	3.29±1.01	3.35±0.38	3.41±1.02	3.35±1.39	---
ChAl-Crd	100	3.52±0.95	5.48±1.10	7.51±0.55**	7.01±1.19	6.83±1.14	55.28%
	200	3.40±1.13	6.39±1.09	8.75±0.67**	7.02±1.05	7.33±0.74	61.67%
ChAl-Chf	75	3.62±0.61	8.57±1.14	10.35±0.72***	9.66±1.15	8.21±1.07	67.60%
ChAl-Et	75	3.68±1.06	7.71±0.94	9.66±0.88**	8.16±0.95	7.38±0.97	65.31%
ChBt-Crd	100	3.35±1.03	6.21±0.91	7.83±0.75*	7.52±1.31	6.11±1.18	57.19%
	100	3.19±1.39	6.02±1.13	9.16±0.77**	88.72±1.07	7.58±1.34	63.41%
ChBt-Chf	75	3.51±1.05	8.61±1.38	11.16±1.29***	10.88±1.19	8.02±0.96	69.97%
ChBt-Et	75	3.11±1.05	8.09±0.81	10.16±0.93***	9.58±0.89	7.11±1.06	67.01%
ChAm-Crd	100	3.41±1.35	4.48±1.10	6.51±0.79*	6.31±1.19	5.83±1.14	48.76%
	100	3.29±1.13	5.91±1.09	7.58±0.65*	6.92±1.05	6.31±1.41	55.78%
ChAm-Chf	75	3.32±0.96	7.75±0.74	8.66±0.71**	8.06±1.15	7.21±1.07	61.30%
ChAm-Et	75	3.21±1.16	6.71±0.81	8.33±0.59**	7.76±0.93	7.09±1.11	59.76%
Tramadol	20	3.27±0.98	9.86±1.02	12.23±1.39***	10.98±1.37	9.88±0.78	72.58%
Morphine	5	3.31±1.08	15.93±9.91	17.83±1.67***	15.57±1.02	12.11±0.86	81.19%
ChAl-Crd +Naloxone	100+2	3.42±0.51	3.01±0.95	3.97±0.69	3.57±1.61	3.47±1.19	---
	200+2	3.45±1.27	3.15±0.61	4.18±0.91	3.10±1.23	3.53±0.92	---
ChAl-Chf+Naloxone	75+2	3.29±1.15	3.11±0.91	4.10±1.23	3.71±0.61	3.41±1.39	---
ChAl-Et+Naloxone	75+2	3.47±0.61	3.15±0.61	4.01±1.15	3.16±1.91	3.18±0.91	---
ChBt-Crd +Naloxone	100+2	3.18±0.52	3.07±1.11	3.97±1.11	3.41±0.72	3.31±0.89	---
	200+2	3.64±1.19	3.11±0.82	3.48±0.82	3.21±1.09	3.03±1.13	---
ChBt-Chf+Naloxone	75+2	3.03±0.92	3.19±1.12	3.67±1.11	3.19±1.18	3.39±1.16	---
ChBt-Et+Naloxone	75+2	3.01±0.72	3.31±0.89	3.71±0.91	3.01±1.05	3.56±0.91	---
ChAm-Crd +Naloxone	100+2	3.19±1.12	3.37±0.61	3.11±0.89	3.39±1.01	3.31±0.89	---
	200+2	3.14±1.09	3.48±1.11	3.47±0.91	3.29±1.31	3.11±1.05	---
ChAm-Chf+Naloxone	75+2	3.58±1.11	3.10±1.23	3.13±0.92	3.42±0.96	3.55±0.91	---
ChAm-Et+Naloxone	75+2	3.47±1.13	3.37±1.19	3.22±1.22	3.19±0.98	3.12±1.16	---
Tramadol+Naloxone	20+2	3.29±1.31	3.11±1.05	3.71±1.01	3.14±1.11	3.18±0.91	---
Morphine+Naloxone	5+2	3.35±0.67	3.21±0.91	3.62±1.04	3.41±0.89	3.37±1.01	---

Table 4: Analgesic response using hot plate method.

Treatment/Dose (mg)		0 min	30 min	60 min	90 min	120 min	At 60 min
Control (2% Tween 80)		3.31±1.15	3.29±1.01	3.35±0.38	3.41±1.02	3.35±1.39	---
ChBt-Chf-43	75	3.29±1.61	7.31±1.03	8.40±0.91**	7.86±1.05	7.01±0.97	60.12%
ChBt-Chf-44	75	3.14±1.01	7.91±0.91	9.95±0.87***	9.11±1.09	8.22±1.01	66.33%
ChBt-Chf-45	75	3.31±1.09	6.11±0.87	8.13±1.02**	7.61±0.87	6.97±1.11	58.79%
SN1	10	3.28±1.01	7.11±1.04	9.71±0.81***	8.01±0.91	7.81±0.78	65.49%
SN2	10	3.34±0.87	8.17±0.97	11.03±0.92***	8.91±1.05	8.04±1.01	69.62%
ChBt-Chf-43+Naloxone	75+2	3.29±1.15	3.12±0.91	4.12±1.13	3.61±1.11	3.39±1.19	---
ChBt-Chf-44+Naloxone	75+2	3.41±0.81	3.17±0.97	4.03±1.05	3.26±1.01	3.21±0.89	---
ChBt-Chf-45+Naloxone	75+2	3.23±0.97	3.31±1.21	3.71±1.11	3.31±0.98	3.29±1.06	---
SN1+Naloxone	10+2	3.31±1.26	4.73±1.08	5.71±0.89	6.03±1.02	5.51±0.91	---
SN2+Naloxone	10+2	3.38±1.01	3.28±1.13	3.23±1.02	3.41±1.16	3.58±1.12	---

Mean±SEM, *P<0.05, **P<0.01 and ***P<0.001 ANOVA followed by Dunnett's multiple comparison test

Table 4 represents the effect of administered subfractions and isolated compounds of ChBt-Chf on mice during the abdominal constriction model. A significant inhibition was shown by crude extract of Chenopodium (ChBt-Chf-43, ChBt-Chf-44 and ChBt-Chf-45) as compared to the control group (P<0.01 and P<0.001, n=8). The maximum inhibition of 60.12% (P<0.01, n=8), 66.33% (P<0.001, n=8) and 58.79% (P<0.01, n=8) for ChBt-Chf-43, ChBt-Chf-44 and ChBt-Chf-45 respectively, was observed at 75mg/kg (table 4). Animals treated with naloxone produced significant reduction in the analgesic activity of tested samples and standard indicating the involvement of opioid receptors in analgesic response (table 4).

The isolated compounds SN1 and SN2 were also tested for analgesic activity using hot plate model (table 4). It is

observed from the data that SN2 showed good results (69.62% ($P<0.001$) in comparison to SN1 (65.49% ($P<0.001$). Mechanistically the naloxone reverses completely the analgesic effects of SN2 while partial reversal was observed upon analyzing SN1 indicating other possible mechanisms in association with opioid receptors.

Two compounds (SN1 and SN2) were bio-guided isolated from the subfraction (ChBt-Chf-44) chloroform fraction of C. botrys (ChBt-Chf) and their structures were determined with spectroscopic data (fig. 2). Compounds SN1 and SN2 were ursolic acid and beta-sitosterol, respectively and spectral data is well correlated with previously reported literature data (Labib *et al.,* 2016; Shoaib *et al.,* 2016b; Kahraman *et al.,* 2019).

DISCUSSION

Pain is an unpleasant sensation and may be either physical or mental depending on its source of origin. Analgesic is those drugs or agents, which reduce or block the sensation of pain temporarily. Several synthetic and plant origin analgesics are being tested for their efficacy and potency on different animal models for pain (Patel *et al*., 2016).

Using acetic acid induced writhing model, the analgesic action of crude extract and fractions, subfractions and isolated secondary metabolites was observed and this model is used as a sorting tool for estimation of peripheral and central analgesic response (Mannan *et al*., 2017). Therefore, in the current literature study, the inhibition of writing response shows the peripheral antinociceptive effect of crude extracts and fractions is in a supplement to its central effect.

In the formalin-induced test, the injected paw is observed in two different phases for licking response due to induced pain. The first phase is a neurogenic pain caused as a result of direct chemical stimulation of nociceptors. While in the second phase the pain is because of inflammation manifested by mechanisms of central sensitization and a union of stimuli-inflammation of the peripheral tissues (Bannister *et al*., 2017). Our current literature study shows that (table 2) crude extract and fractions lessened the number of paws licking remarkably in both neurogenic and inflammatory pain responses ($P<0.05$, $P<0.01$ and $P<0.001$, $n=8$). Although in the second phase, this effect was more assertive. The results obtained from hot plate and formalin test further give the confirmation of the central antinociceptive effects. While in the late phase, the observed analgesic effect is primarily attributed by inhibiting the inflammatory mediators (Shoaib *et al*., 2019).

In this study, classical pharmacological models of pain have been employed to ascertain peripheral and central mechanism in the antinociceptive response of extract and fractions. The latency time in the hot plate was increased ($P<0.05$, $P<0.01$ and $P<0.001$, $n=8$) by crude extract and fractions indicating the possible central antinociceptive effects (table 3 and table 4). This method is specific just for the analgesics that centrally act, while the agents acting peripherally are inert (Alabi *et al*., 2019). Naloxone, an antagonist of non-selective opioid receptors has the ability to invert the antinociceptive effect of crude extracts and fractions indicated by the results obtained from the hot plate model. Participation of opioid receptors of the spinal and supraspinal system may result in the central antinociceptive response of testing plants. This surely shows that the antinociceptive effect of crude extracts and fractions involves activation of opioid receptor.

Many plant extracts and isolated compounds from these have been proved to be analgesic by evaluating through models reported for analgesic activity (Bhuiyan *et al*., 2020). Crude alkaloidal fractions from medicinal plants are reported to possess analgesic response (Shoaib *et al*., 2016). Therapeutic potential of flavonoids isolated from the extract of medicinal plants has been screened for its potential central and peripheral analgesic activity. These have been found beneficial in the management of pain in animal models that is supported by preclinical and clinical evidences (Rajamanickam and Rajamohan, 2020; Ferraz *et al*., 2020).

From the above discussion, it is concluded that the secondary metabolites like flavonoids, alkaloids, terpenoids and sterols can be used as a prime source of pain killer and thus used as a helpful tool for the management of pain. The present study confirms the presence of such type secondary metabolites from its qualitative and quantitative results and analgesic potentials are possibly due to the presence of these types of components. As per previous literature, it is reported that medicinal plants are extensively used for the management of inflammation and pain (Napagoda *et al*., 2018, Forouzanfar and Hosseinzadeh, 2018). Thus the current study of medicinal plants warrants its traditional use as painkiller agents. Furthermore, extensive scientific work is demanded to explore the bioactive components from these medicinal plants that are used as a key source for combating pain.

CONCLUSION

The current study of Chenopodium species warrants its traditional use as analgesic agents by isolating compounds from Chenopodium species responsible for the management of pain via opioidergic pathway.

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