Phytochemical screening and assessment of analgesic, Anti-inflammatory and hematological properties of the fruit of *Berberis baluchistanica*

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Abstract: Traditionally Berberis species have been used as anti-inflammatory, anti-rheumatic, analgesic and anti-anemic drugs. This study was aimed to determine chemical constituents and to assess analgesic, anti-inflammatory and hematological effects of the crude extract of the berries of *Berberis baluchistanica* to verify these folkloric claims. Phytochemical screening, carried out by using different chemical reagents and techniques like Thin Layer Chromatography (TLC) and Fourier Transform Infra-Red (FTIR) indicated presence of flavonoids, saponins, phytosterols and carbohydrates including reducing sugars. Analgesic and anti-inflammatory activities were assessed on mice by using acetic acid induced writhing method and formalin method. Potent anti-inflammatory and analgesic effects were observed during these experiments. The extract also showed anti anemic effect as it increased the levels of hemoglobin and red blood cells significantly. Increase in the platelet count was also noted. The extract of the berries was used at oral doses of 300 and 500 mg/kg during experiments. Anti-inflammatory and analgesic activities were determined by comparing with the standard i.e. aspirin 300 mg/kg. Both doses produced significant anti-inflammatory and analgesic activities at P□0.05. These activities were seemingly attributable to flavonoid and saponin contents of the drug. These results justify the folkloric claims that the drug could be used as good anti-inflammatory, antirehumatic, analgesic and anti-anemic drug. However, further chemical investigations on the drug are suggested for isolation and identification of compounds that could be safer and more effective than the currently available medicines in treating these disorders.

Keywords: *Berberis baluchistanica*, analgesic, anti-inflammatory, hematological, fourier transform infra-red, TLC, flavonoids, saponins, phytosterols.

INTRODUCTION

Inflammation is a part of body's defense mechanism that provides protection against infections, foreign noxious substances or any injury to tissues. Different inflammatory mediators like histamine, prostaglandins, kinins, cytokines, Nitric oxide and complements are released that cause typical inflammatory symptoms e.g. redness, swelling, heat and pain (Choi *et al*., 2012, Sherwood and Toliver-Kinsky, 2004). But over production and persistence of these mediators even after cessation of cause may lead to inflammatory disorders such as rheumatoid arthritis, atherosclerosis, autoimmune diseases, asthma, cancer etc. (Choi *et al*., 2012). Pain results from activation of affront nerves (Lawrence *et al*., 2002). The drugs frequently used to manage inflammation and associated pain, include non-steroidal anti-inflammatory drugs (NSAIDs), steroids and opioids. However, these drugs cause serious side effects, which can often limit their use for example NSAIDs can cause peptic ulcers. This necessitates the need for safer and effective anti-inflammatory and analgesic drugs (Lima *et al*., 2012). Herbal medicines are thought to be better compatible with human body with fewer side effects (Parekh *et al*., 2006). Berberine containing plants have been used as anti-inflammatory drugs. Brberis species are used to treat inflammatory disorders like rheumatism, bronchial infections, hepatic diseases and urinary complaints (Heron and Yarnell, 1998, Yeşilada and Küpeli, 2002). *Berberis baluchistanica* Ahrendt belonging to family Berberidaceae grows wild in Baluchistan province of Pakistan. Traditionally it is used as a good anti-inflammatory and hepato protective agent. Roots and sap of this plant is locally used with milk to treat pains and inflammatory disorders such as rheumatism (S.I. Ali, 1975, Tareen *et al*., 2010). As no research work has been reported to date on anti-inflammatory, analgesic and anti-anemic activities of this plant, the present study was carried out on the berries of this plant to verify these claims through approved methods.

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MATERIALS AND METHODS

The berries of *B. baluchistanica* were procured from the local market in Karachi, Pakistan. The berries were identified and voucher specimen (B-4047) was submitted to the herbarium of the University of Karachi, Pakistan. Berries were soaked in methanol for two weeks. After filtration the extract was dried under reduced pressure by rotary evaporator at 40°C. The percent yield of extract was found to be 8% (w/w). The extract was subsequently used in experiments.

Experimental animals

Swiss albino mice (25-30g) and white albino rats (150-200g) of either sex were employed in the experiments. Animals were allowed to access water and standard diet freely. Animals were acclimatized for one week prior to experiments. Permission was taken before experiments from the Ethical Committee of the University of Karachi (via letter PHC-204).

Phytochemical screening

Chemical composition of the extract was determined by performing various chemical identification tests (Brain and Turner, 1975, Purohit *et al*., 2007, Shaik, 2011, Sharma *et al*., 2013, da Rocha *et al*., 2013, Shah, 2009).

Thin layer chromatography

The crude extract was analyzed by thin layer chromatography using two mobile phases:

i. Chloroform : Methanol : Water (80:20:02)
ii. Ethyl acetate : Methanol : Water (100:16:13)

TLC was performed on the methanolic extract according to the method described by Stahl, 1969. (Stahl, 1969)

Fourier transform infrared analysis

Fourier transform infra-red spectrophotometric analysis was performed directly on the crude extract by Thermo Electron Corporation Nicolet Avatar 330 FT-IR, USA.

Analgesic activity

(1) By writhing Method

The modified method of Hunskaar and Hole (Hunskaar and Hole, 1987) was employed to measure the analgesic activity of the crude extract on mice. The extract was administered to mice orally. After half an hour, writhes were induced by injecting 1% acetic acid (10ml/kg body weight) into the peritoneal cavity. Writhes were counted for 30 minutes after the administration of acetic acid. Decreased writhing in test animals with regard to control group indicates analgesic activity. Mice were divided into 4 groups. Each group consisted of 6 mice. During the experiment, Group-A served as control group, Group-B and Group-C were administered 300mg/kg and 500mg/kg doses of the extract respectively, while Group-D received 300mg/kg of aspirin as standard. The extract and standard were administered orally after diluting with distilled water while mice of control group received saline.

(2) Formalin induced paw licking test

Swiss albino mice (each weighing 25-30gm) were divided into 4 groups of 6 animals each. 20µl of dilute formalin (2% sol.) was injected into the sole of left hind paw of mice 30 minutes after the oral administration of the drug and vehicle (Hunskaar and Hole, 1987, Rathi *et al*., 2003).

Two characteristic phases of intensive licking and biting of paw occurred. The first phase lasted from 0-5 minutes and the second phase lasting from 15-30 minutes of formalin injection. Time spent on licking was measured in seconds (or no. of licks) in the two phases for studying drug effects. The 1st phase corresponds to neurogenic pain while the later phase represents inflammatory pain.

Anti-inflammatory activity

Four groups of mice were formed each group consisted of 6 animals. First group serving as control received vehicle only. Mice of second and third groups were treated with the oral doses of extract (300mg/kg and 500mg/kg body weight respectively). The fourth group received 300mg/kg body weight of aspirin as standard. Inflammation was produced in the left hind paw by injecting 20µl of 2% formalin subcutaneously into the sole. Formalin was injected 30 to 40 minutes after the oral administration of the drug and standard. The increase in the size of paw due to edema was measured with the help of a Vernier Caliper. The increase in the size of paw was measured up to 4 hours starting after 30 minutes of formalin injection. Anti-inflammatory activity was measured as:

% inhibition of edema= (Vc-Vt)/Vc X 100

Where Vc and Vt represent the mean paw volumes of the control and the treated groups respectively (Rana, 2008).

Evaluation of hematological parameters

Albino rats of either sex weighing 150-200gm were divided into 3 groups of 6 rats each. Group-1 (Control group) was treated with distilled water only while Group-2 and Group-3 were treated with oral doses of 300mg/kg and 500mg/kg of the extract respectively for 28 days. On the completion of the treatment the blood samples were obtained and counting was done after dilution with Hayem' reagent. RBC's were counted with the help of Neubauer chamber observing under a light microscope. PCV (packed cell volume) was determined by hematocrit centrifuges and Microwintrobe hematocrit tubes. Sahli's hemometer was used to determine the quantity of hemoglobin. Turk's solution was used to make dilutions of blood prior to counting total leucocytes by means of Neubauer chamber. MCH (Mean erythrocyte volume), MCHC (mean corpuscular hemoglobin concentration) and mean corpuscular hemoglobin were also determined (Burnett *et al*., 2011, McGovern *et al*., 1955).

STATISTICAL ANALYSIS

Values of P≤0.05 were considered statistically significant. The data were calculated as ± S.E.M., evaluated by Dunnett after t-Test.

RESULTS

Phytochemical screening

The results obtained from FTIR and phytochemical analysis of the drug can be used for identification of this plant in future. The results are shown in table 1 and fig 1. TLC results are shown in table 2 and table 3.

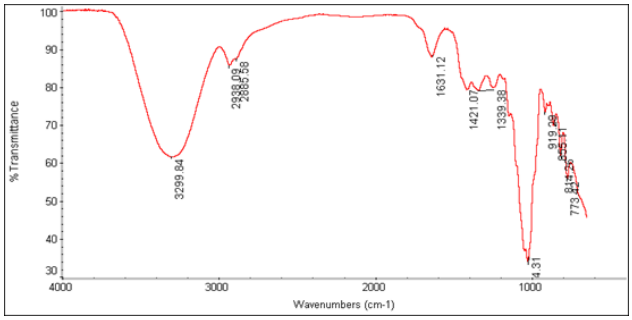
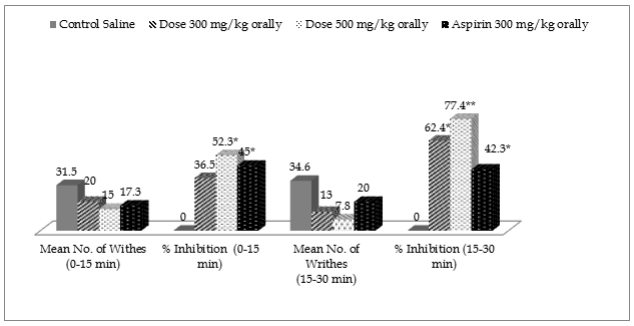


Fig. 1: FTIR of *B. baluchistanica*



Results are shown as Mean ± SEM, *Significant at P□0.05; **Highly significant at p□0.01

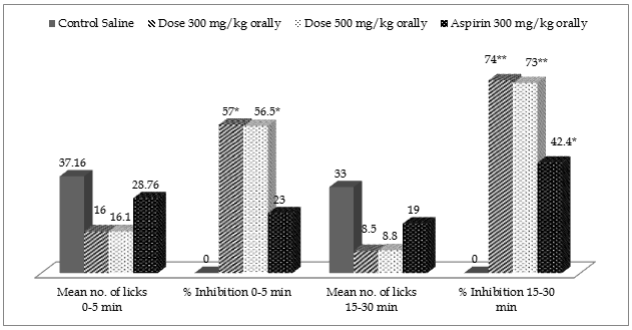
Fig. 2: Analgesic Activity, *B. baluchistanica* Acetic Acid Method

Phytochemical screening showed the presence of different compounds like carbohydrates, flavonoids, Saponins and phytosterols in the drug (table 4).

Analgesic activity

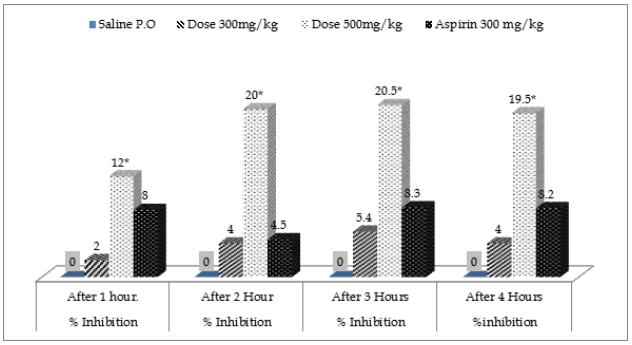
The crude extract of the berries of *B. baluchistanica* was evaluated for its analgesic property by inducing writhes with acetic acid i. p. According to results shown in fig. 2, the extract of *B. baluchistanica* exhibited good analgesic activity in a dose dependent manner. The lower dose (300mg/kg) produced 36.5% analgesia during the 1st phase (0-15 minutes) in comparison to 45% by the standard drug Aspirin. Whereas in the 2nd phase (15-30minutes) it produced even a greater analgesic effect, i.e. 62.4% as compared to 42% by the standard. The higher dose i.e. 500mg/kg reduced the pain sensation by 52.3% and 77.4% in the 1st and 2nd phase respectively.

Analgesic activity was also determined by inducing inflammation on the hind paw of mice. The method is described in the experimental section. The results are shown in fig. 3. The lower dose (300mg/kg) caused 57% and 74% inhibition of pain in the 1st and 2nd phase respectively in comparison to 23% and 42% in the two phases respectively by the standard. 500mg/kg dose produced analgesia to almost similar extent. The activity increased with time in the 2nd phase fig. 3.



Results are shown as Mean ± SEM, *Significant at P□0.05; **Highly significant at p□0.01

Fig. 3: Analgesic Activity, *B. baluchistanica* Formalin Method



Results are shown as Mean ± SEM, *Significant at P□0.05; **Highly significant at p□0.01

Fig. 4: Anti-inflammatory Activity, *B. baluchistanica*

Anti-inflammatory activity

Anti-inflammatory activity of the extract was evaluated by inducing inflammation in the hind paw of mice by formalin after giving oral doses of the extract. Dose 500mg/kg produced significant anti-inflammatory activity (12%, 20%, 20.5% and 19.5% after 1st, 2nd, 3rd and 4th hour) as compared to Aspirin (8%, 8.5%, 8.3% and 8.2% at 1st, 2nd, 3rd and 4th hour respectively) used as standard drug (fig. 4).

Hematological effects

Hematological effects were noted by method described in the experimental section. The extract increased the hemoglobin, total RBC's, MCH, MCV and total platelet counts. Results are shown in table 16a. 500mg/kg increased the hemoglobin to 15.1±0.015 in comparison to control 13.5±0.14. Similarly, RBC count and platelet count were also raised i.e. 7.68 and 1195 respectively as

Table 1: FTIR Peaks, cm^{-1}, *B. baluchistanica*

No. of Peak	cm^{-1}	Functional group
1	3350-3250	OH broad band
2	2938-2885	C-H stretch
3	1631-1421	C=C aromatic
4	1339	C=O stretch
5	1024	C-O-C
6	919, 855, 814, 773	C-H out of plane bend

Table 2: TLC, *B. baluchistanica* TLC System: Ethyl acetate : Methanaol : Water (100:16:13)

Compounds	U.V Light 254 n.m.	U.V Light 366 n.m.	R_f Value
1	+	-	0.66
2	+	-	0.77
3	+	+	0.13

Table 3: TLC, Methanolic extract of *B. baluchistanica* TLC System: Chloroform : Methanol : Water (80:20:2)

Compounds	U.V Light 254 n.m.	U.V Light 366 n.m.	R_f Value
1	+	-	0.47
2	+	-	0.
3	+	+	0.13

compared to control (6.8 and 887 respectively). The WBC count was slightly decreased table 5.

DISCUSSION

The study reports for the first time on anti-nociceptive, anti-inflammatory and anti-anemic effects of the fruit of the plant *B. baluchistanica.*

Inflammation is a body's natural defensive response to noxious stimuli, tissue injury or infections (Lumeng and Saltiel, 2011). However, persistent and excessive inflammation can damage different tissues and organs causing redness, swelling and pain. Large quantities of prostaglandins, reactive oxygen species and cytokines (like IL-1β, IL-6 and TNF-α) are produced in an inflammatory response by monocytes and macrophages. It has been indicated that inflammation is involved in the development of different diseases like, cardiovascular, neurological, dental, intestinal and renal disorders. It is also involved in ageing and diseases like diabetes, obesity, multiple sclerosis, pancreatitis and cancer (Lumeng and Saltiel, 2011, Wyss-Coray and Rogers, 2012, Janero, 1990, Grivennikov *et al*., 2010, Jenny, 2012, Hoque *et al*., 2012, Marchant *et al*., 2012, Kuek *et al*., 2006). Different herbal drugs are used for the treatment of inflammation and different types of pains. Keeping in view the folkloric claims, we studied the analgesic and anti-inflammatory effects of the berries of *B. baluchistanica* in animal models (in mice). Two methods were employed to evaluate the possible central and peripheral analgesic activities, acetic acid induced writhing method and formalin induced licking method.

Table 4: Phytochemical Analysis of *B. baluchistanica*

Phyto-constituents	Result
Alkaloids	-
Carbohydrates	+
Amino acids	-
Protein	-
Flavonoids	+
Tannins	-
Saponins	+
Phytosterols	+

Acetic acid induced writhing test is a widely used method for evaluating anti-nociceptive effects (Shibata *et al*., 1989). Acetic acid injected into the peritoneal cavity, causes writhes or painful sensation by releasing inflammatory substances like prostaglandins, serotonin and cytokines which in turn stimulate peripheral pain receptors (Manjavachi *et al*., 2010). This nociceptive effect can be blocked by both NSAIDs and centrally acting analgesics e.g. morphine. In this test, the drug produced potent analgesic effects at both doses (300mg/kg and 500mg/kg) compared with Aspirin used as standard. This test however, cannot indicate whether the anti-nociceptive effect is due to central and or peripheral actions (Godoy *et al*., 2004).

Formalin test is a well-established method that can differentiate between central and peripheral anti-nociceptive effects (Hunskaar and Hole, 1987). Formalin test consists of two phases. The initial phase consisting of first 5 minutes after formalin injection reflects neurogenic pain which is centrally mediated non inflammatory pain due to release of substance-P or bradykinin, it originates via direct stimulation of peripheral pain receptors. The

Table 5: Hematological Effects of *B. baluchistanica*

Hematological Parameters	Control	Dose300mg/Kg	Dose500mg/Kg
Hb	13.5±0.14	13.9±0.06	15.1±0.015*
Total RBC	6.8±0.06	6.88±0.08	7.68±0.09*
MCH	19.8±0.4	20.4±0.2	19.7±0.16
Hematocrit	36.3±0.196	37.3±0.3	42.1±0.2*
MCV	53.3±0.26	55±0.43	42.1±0.36
Platelets	887±6.78	1053±5	1195±3.4*
Total WBC	12.7±0.93	11.8±0.1	9.8±0.1*
Neutrophils %	8±0.23	16±1*	22±0.7*
Lymphocytes %	92±1.06	84±0.63	78±0.44*

second phase called inflammatory nociceptive phase consisting of 15-30 minutes and is due to releases of prostaglandins and bradykinin (Shibata *et al*., 1989). Centrally acting drugs like morphine can inhibit both phases while the NSAIDs or steroidal drugs can inhibit only the second phase (Tjølsen *et al*., 1992).

In our study, the berries of *B. baluchistanica* showed significant analgesic activity both at central as well as peripheral level. According to results obtained, the drug caused pain inhibition in both phases but greater inhibition of pain was observed in the second phase, which is also known as inflammatory phase and is typically blocked by NSAIDs (Shibata, Ohkubo *et al*., 1989). The drug also showed good anti-inflammatory activity in Formalin edema test. These results suggest that the drug contains types of constituents that not only can act via central opioid receptors to cause central analgesia but can also block formation of prostaglandins via cyclooxygenase to cause peripheral analgesia. It is important to note that preliminary phytochemical analysis of the drug revealed presence of saponins, phytosterols and flavonoids and interestingly all of these constituents have already been shown to possess anti-inflammatory and analgesic properties in various studies (Tjølsen *et al*., 1992, Chandel and Rastogi, 1980, Cheeke *et al*., 2006, Capra, 1972). This suggests that our results are in consistence with the out-come of these studies. During hematological examination, the drug was found to be anti-anemic as it increased the level of hemoglobin and erythrocytes in a dose dependent manner. These findings supported the folkloric use of the drug in the treatment of anemia. These anti anemic effects can be related to phytosterols and saponins present in the drug as previous studies have shown that β-sitosterol (a phytosterol) and saponins may enhance erythropoiesis and hemoglobin level (Unakalamba *et al*., 2013, Efraim *et al*., 1999). Platelet count was also increased appreciably by the drug, which means the drug can be useful in conditions characterized by thrombocytopenia for example dengue fever.

CONCLUSION

During our study, carried out on the berries of *B. baluchistanica* significant analgesic, anti-inflammatory and erythropoietic activities were noted which substantiated the folkloric claims that the drug can be used as good analgesic, anti-inflammatory, antirehumatic and anti-anemic drug. Furthermore, the phytochemical screening along with TLC and FTIR revealed the presence of phytosterols, saponins and flavonoids, which have been linked to these effects in previous studies. The drug also increased the platelet count greatly and can be effective in treating conditions like dengue fever where the platelet count is low. Further research on this drug may result in the isolation and structure characterization of compounds actually responsible for the therapeutic effects of the drug.

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