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Original article

In vivo pharmacological investigation of *Monothecca buxifolia* and *Bosea amherstiana* using animal modelsSaid Hassan^{a,b}, Bashir Ahmad^{a,*}, Shahid Ullah Khan^c, Huang Linfang^d, Syed Ishtiaq Anjum^e, Muhammad Javed Ansari^{f,g}, Khaista Rahman^h, Iftikhar Ahmadⁱ, Wasim Ullah Khan^j, Rabia Qamarⁱ, Shad Man^k, Ghulam Nabi^l, Ayesha Haleem Shah^m^a Center of Biotechnology and Microbiology, University of Peshawar, KPK, Pakistan^b Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei, China^c College of Plant Sciences and Technology/National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China^d Institute of Medicinal Plants Development, Peking Union Medical College Chinese Academy of Medical Sciences Beijing China^e Department of Zoology, Kohat University of Science and Technology, Kohat 26000, Pakistan^f Bee Research Chair, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia^g Department of Botany, Hindu College Moradabad, 244001, India^h College of Animal Sciences/State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, Chinaⁱ Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan^j School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou, China^k School of Life Sciences, Inner Mongolia University Hohhot, Inner Mongolia 010021, China^l The Key Laboratory of Aquatic Biodiversity and Conservation of the Chinese Academy of Sciences, Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan 430072, China^m Department of Biological Sciences Gomal University Dera Ismail Khan KP, Pakistan

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ABSTRACT

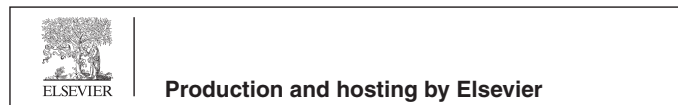
Experimental based evidence suggests that most of the medicinal plants possess wide-ranging pharmacological and biological activities that may possibly use in treatment of inflammation-related diseases. The current study was aimed to explore the acute toxicity, analgesic, sedative and antipyretic activities of *Monothecca buxifolia* and *Bosea amherstiana* in mice. *In vivo* experimental models were used in this study. Acute toxicity was evaluated for 24 h' interval at concentration of 500, 1000, 1500 and 2000 mg/kg. The analgesic activity was estimated by acetic acid induced writhing test. White wood apparatus enclosed in stainless steel was used for sedative experiment and antipyretic activity was evaluated in brewer's yeast induced hyperthermic at 50, 100 and 150 mg/kg i.p. Both plants were found safe at all tested doses. *Monothecca buxifolia* and *Bosea amherstiana* dose-dependently reduced abdominal constrictions in mice. Both plants exhibited significant ($P < 0.0001$) sedative effects in dose of 50, 150 and 150 mg/kg. Both plants markedly ($P < 0.0001$) reduced yeast induced hyperthermia. The inhibitions were dose-dependent and remained significant up to five hours of administration. These investigational results have linked a pharmacological indication for the traditional claim of the drugs to be used as an anti-inflammatory, analgesics and antipyretic agents.

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1. Introduction

Many plants synthesized constituents that are beneficial for maintaining human health. The man was completely dependent on medicinal plants prior to synthetic drug availability for treating diseases (Singh et al., 2008). Natural medications produced from plants extracts are being progressively utilized for treating wide number of clinical diseases, however relatively little information about their mode of actions are available. The World Health Organization (WHO) encourages the addition of herbal medication in

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health cares because of the tremendous potentials they have. Natural products like herbs, vegetables, and fruits became more popular in recent era due to increasing interest, public awareness consumers and scientific community (Thaipong et al., 2006). Epidemiologically evidence has suggested that natural products constituents show many pharmacological activities, like antiviral and anti-inflammatory effects (Uddin et al., 2011; Rauf et al., 2012).

Inflammation is associated with protections of the body against infection, burns, allergens and toxic chemicals are considered as a basic physiological defense mechanism. If the inflammations are left uncontrolled, it will turn as an etiologic factor for many other chronic diseases (Kumar et al., 2015). It comprises a complex range of enzyme activation, intermediary proclamation, extravasation of the fluids, cell movement, tissues break down and its repairs (Vane and Botting, 1995). Previously, inflammation was considered as a sole disease caused by instabilities of body fluid, however, in the recent medical sciences, inflammation is considered as a vigorous process resultant from diseases or disturbance (Emamghoreishi et al., 2005). Currently, the inflammatory condition and related pain are succeeded by either narcotic, e.g., opioid or non-narcotics like salicylate and corticosteroid. Those synthetic medications are linked with some side effect (Gaddi et al., 2004).

Furthermore, these predictable medicines and drugs are either too expensive or toxic and not usually offered to the rural societies that constitute the key populace of the globe. So, the more effective and novel development of anti-inflammatory medications with a minimum side effect is essential. Plant origin medications have been using since ancient time with relatively minimum adverse effect. Therefore it is important to introduce novel therapeutic plant for cheaper drug development. Several herbal plants have been used to ameliorate the inflammation and related pain and fever with folk uses. Compounds that are isolated from plants can assist as a pattern for the production of new anti-inflammatory medications with higher therapeutic effects low toxicity level (Anilkumar, 2010). In developing countries, several medicinal plants have been used for the treatments of inflammation and pain. *Monothecha buxifolia* and *Bosea amherstiana*, are widely growing traditional plants in the northern hilly areas of Pakistan. These plants are used for wound healing and as antiseptic and for the gastrointestinal disorder treatment (Qureshi et al., 2007). The current study was conducted to determine the traditional use of *Monothecha buxifolia* and *Bosea amherstiana* as inflammation and related pain and fever.

2. Materials and methods

Diclofenac sodium was used as a standard drug. In writhing tests, the pain was induced by acetic acid (Merck Germany). Control group was administrated with normal sterile saline water throughout the experiments while normal saline was used to prepare methanolic extracts. The reference drug diclofenac sodium was administrated as an analgesic drug by the intraperitoneal administration with a concentration of 10 mg/kg. *Monothecha buxifolia* (MB) and *Bosea amherstiana* (BA) at a dose of (50, 100 and 150 mg/kg), was dissolved in normal saline and intraperitoneal administrated. Diazepam was used as a depressant drug for sedative activity while paracetamol was used as a reference drug for anti-pyretic evaluation.

2.1. Plant materials and extract preparation

Aerial parts of *Monothecha buxifolia* (MB) and *Bosea amherstiana* (BA) were collected from, Dir Lower, KPK, Pakistan, in March 2014 and September 2014 respectively. The plants samples were

authenticated by Dr. Jehandar Shah, Taxonomist; Ex-Vice Chancellor Shaheed Benazir University Sheringal, Pakistan.

The well-established protocol was followed to get a methanolic extract from the powder plant material (Ibrar and Muhammad, 2011; Khan et al., 2012). Extracts were concentrated under vacuum at 40 °C after filtration. The methanolic extract was screened for *in vivo* pharmacological assays using albino mice.

2.2. Animals

Albino rat (180–220 g) and mice (18–22 g) of either sex were attained from animal house, Animal Sciences Department, Quaid-e-Azam University, Pakistan. Standard laboratory protocols were provided to animals (25 °C light and dark cycle, i.e. 12/12 h) with standard quality water and food.

2.3. Acute-toxicity assay

The clinical trials for the determination of acute toxicity of both plants were done to evaluate the possibility of any type of acute toxicities. Mices of either sex were assessed by oral administering various dose (500, 1000, 1500 and 2000 mg/kg), whereas distilled water was provided to control group (1 mL/kg). Gross effects of first 4 h in all groups were observed then 24 h mortality was observed (Muhammad et al., 2013).

2.4. Acetic acid induced writhing's test

The analgesic evaluation of methanolic extracts of MB and BA were estimated by acetic acid induced writhing's test (Koster, 1959). Albino mice of each sex were randomly divided into four groups comprising of six in each. Normal saline at concentration of 10 mL/Kg was injected to Group I as control, diclofenac sodium as a standard drug was injected to Group II at concentration of 10 mg/kg, while different concentrations of methanolic extracts (50, 100 and 150 mg/kg), through i.p route were injected respectively. Acetic acid (1%) was injected after 30 min after the above treatment. After five minutes the abdominal writhes were counted for the periods of ten minutes.

2.5. Sedative effect

White wood apparatus enclosed in stainless steel was used in this experiment, divided by black lines in different squares. Mices of either sex were used for this activity. Mices were familiarized under red light prior to experiment for one hour in lab condition with feed supply. The animals used for experiment were treated with a reference drug diazepam (0.5 mg/kg i.p.) normal saline (control), methanolic extracts (50, 100 and 150 mg/kg i.p.). Each mouse was putted in the center of wood box after 30 min of the treatment and counted down the lines crossed by each animal (Muhammad et al., 2013).

2.6. Antipyretic activity

Antipyretic activity against yeast induced pyrexia of methanolic extracts of *Monothecha buxifolia* and *Bosea amherstiana* in mices were evaluated according to the previously described procedure with slight modification (Metowogo et al., 2008). Animals were kept fasting for overnight and were divided into five groups consisting of 6 mices in each group. By subcutaneous pyrexia was induced at concentration of 2 mL/100 g of 20% aqueous suspensions of brewer in yeasts. Group I with normal saline (2 mL/kg) kept as a control treatment. Group 2, 3 and 4 were administrated with methanolic extracts (50, 100 and 150 mg/kg *p.o.*) respectively, while group 5 was served with paracetamol (Standard drug)

100 mg/kg p.o. The rectal temperature was recorded after 24 h of inducing of pyrexia. The animals were treated according to protocols with plants extracts, standard and control. The rectal temperature was measured at the interval of 30, 90 and 150 min after drug and samples administrations.

2.7. Statistical analysis

The data generated was statistically analyzed by computer software program JMP. SAS (Version 7.0. SAS, USA), followed by Student's *t*-test.

3. Results

3.1. Acute toxicity

Both plants were found safe for all tested dose. All treated animals were found normal during the first four hours and after 24 h assessment.

3.2. Acetic acid induced test/analgesic activity

Monotheca buxifolia and *Bosea amherstiana* (50, 100 and 150 mg/kg) dose-dependently reduced abdominal constrictions in mice. The reduction was significantly ($P < 0.0001$) when compared with control (Figs. 1 and 2). The effect of the extracts was comparable to that of diclofenac sodium (10 mg/kg) the standard drug.

3.3. Sedative effect

The sedative effect, the open field test of MB and BA are given in Figs. 3 and 4. Diazepam the standard drug used exhibit maximum sedative effect which was significantly ($p < 0.0001$) compared with the control group. Both plants exhibit ($p < 0.0001$) sedative effects in dose of 50, 150 and 150 mg/kg, however, these should be considered mild to moderate sedative as the sedation induced was for less standard drug diazepam.

3.4. Antipyretic test

Both plants markedly ($P < 0.0001$) reduced yeast induced hyperthermia. The dose-dependent inhibitions remained significant up to five hours (Table 1). The maximum effect against pyrexia was observed at *Monotheca buxifolia* 150 mg/kg and *Bosea*

amherstiana 150 mg/kg, while, the antipyretic effect of paracetamol was 37.67 ± 0.44 . The pyrexia inhibition temperature is shown in Table 1.

4. Discussion

The present study indicates that *Monotheca buxifolia* and *Bosea amherstiana* has noticeable anti-inflammatory, analgesic and antipyretic effects with a rational safety profile. The anti-inflammatory protocols are a well-established and used for assessing therapeutic agents for their analgesic effect. The reduction in abdominal constrictions was significantly when compared with control. The induced pain due to liberating endogenous constituents as well as another pain mediator such as arachidonic acid via cyclooxygenase, and biosynthesis of prostaglandin (Khan et al., 2010). This pain pattern is generally used for the evaluation of peripheral analgesic assay due to its response and sensitivity to the containing compounds at dose with no effect in other procedures approaches. The locally peritoneal receptors might be the main cause of abdominal writhing (Mbiantcha et al., 2011). The sensation of pain is caused by phospholipid in tissue via cyclooxygenase (COX), and producing prostaglandins specifically PGF2 α and PGE2, in peritoneal fluids the level of increase may occur in lipoxygenase production (Khan et al., 2010). The pain and inflammations may cause due to prostaglandins and lipoxygenase products by rising capillary penetrability. The writhing inhibiting substances have the analgesic effect preferable by inhibiting synthesis of prostaglandin, a fringe mechanism of inhibiting pain (Duarte et al., 1988). Concerning the outcomes of our plants extract in acetic acid induced abdominal constrictions evaluation, a significance inhibition of writhing's reflex was noted. These results strongly endorse that both plants have peripheral analgesic effects and their way of act may be facilitated through inhibitions of locally peritoneal receptor which may be the contribution in inhibition of cyclooxygenase potentials.

Diazepam belongs to the group benzodiazepine a depressant of nervous system and is used for sleeping disorders. The binding site of benzodiazepine is GABA receptor type ionosphere complex. It reduce the activity, balance the excitement and calm down the recipient. The drug diazepam used as a reference reduced the onset and enhance the interval of barbiturate induced sleep and lower down exploratory activity owning potential as sedative (Hossain et al., 2016a). Pyrexia induced by Brewer's yeast is recommended as an appropriate test for the screening of medicinal plants as well

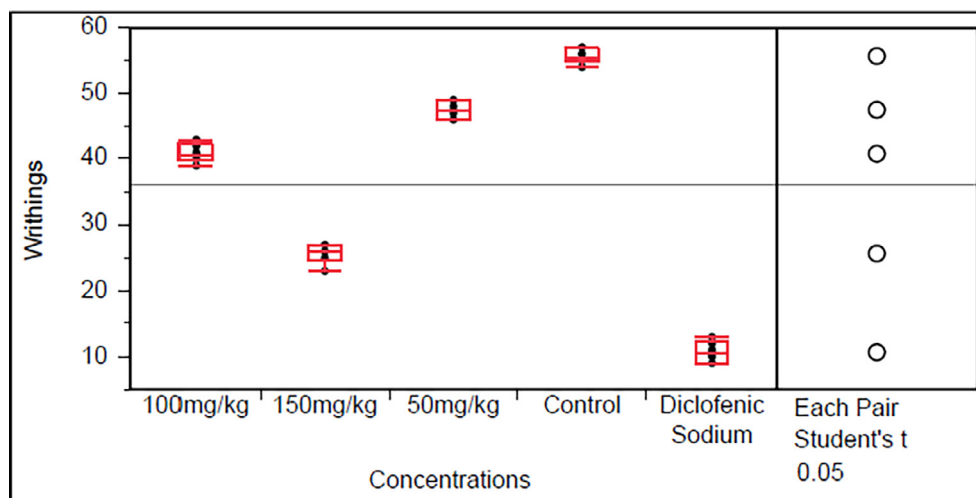


Fig. 1. Effect of *Monotheca buxifolia* 50, 100 and 150 mg/kg in acetic acid induced test (Data are reported as mean \pm S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Student's *t* test with significance value $P > 0.0001$).

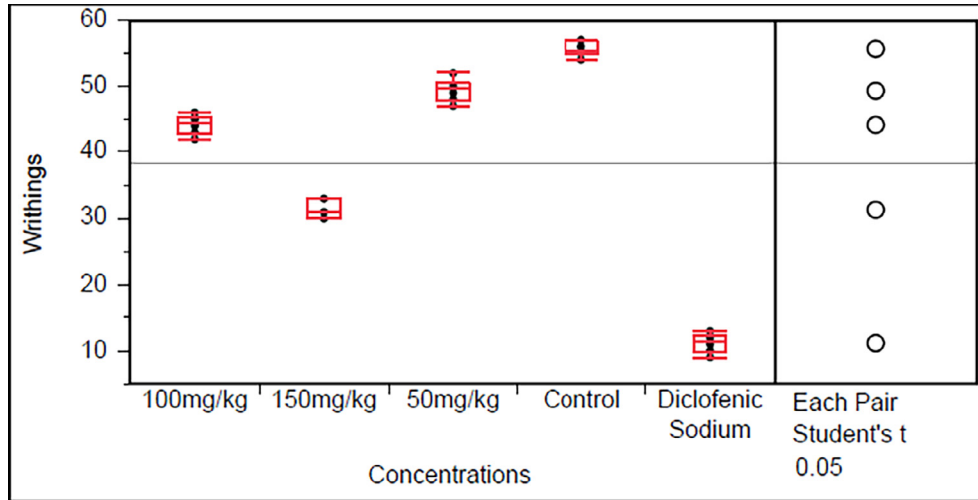


Fig. 2. Effect of *Bosea amherstiana* 50, 100 and 150 mg/kg in acetic acid induced test (Data are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Student's *t* test with significance value $P > 0.0001$).

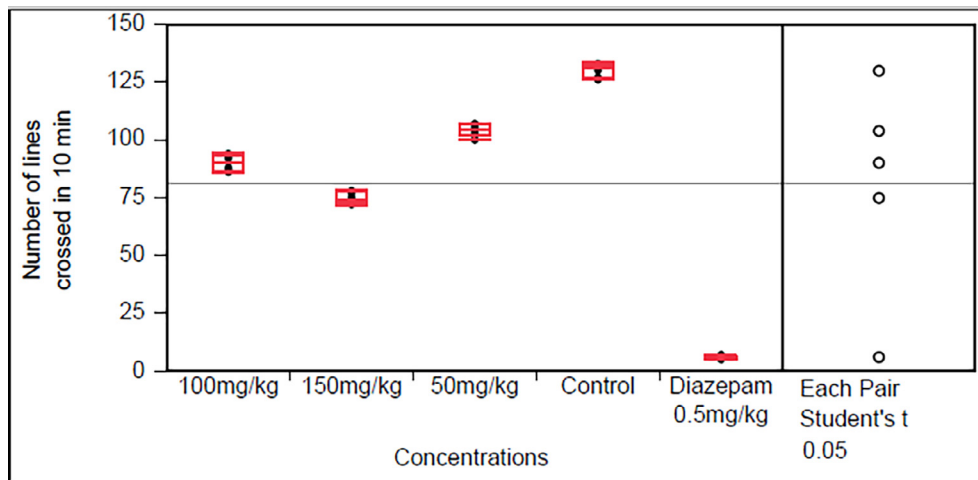


Fig. 3. Sedative activity of *Monothecha buxifolia* (Data are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Student's *t* test with significance value $P > 0.0001$).

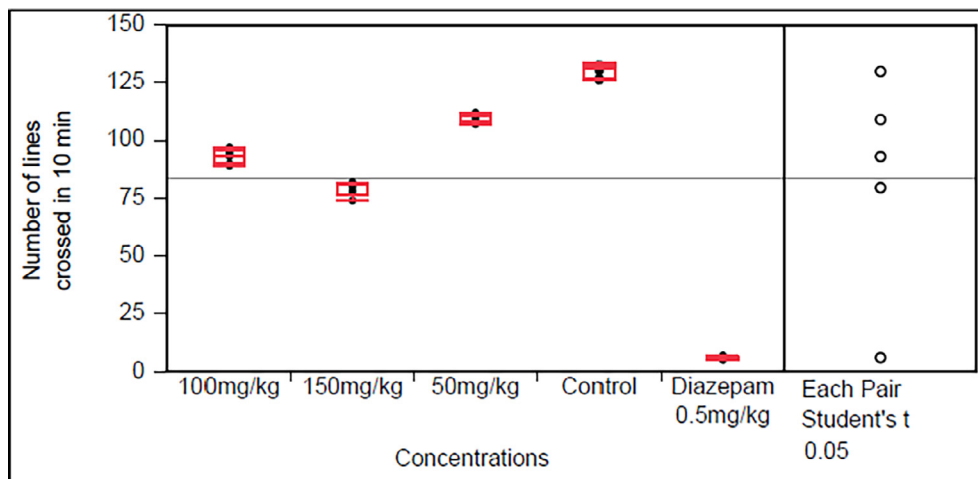


Fig. 4. Sedative activity of *Bosea amherstiana* (Data are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Student's *t* test with significance value $P > 0.0001$).

Table 1
Antipyretic activity of *Monothecha buxifolia* (MB) and *Bosea amherstiana* (BA).

Treatment	Dose	Normal (A)	24 h (B)	1 h (C1)	2 h (C1)	3 h (C1)	4 h (C1)	5 h (C1)
N. Saline	10 mL/kg	36.67 ± 0.44	39 ± 0.67	38 ± 0.67	38.67 ± 0.67	39.33 ± 0.67	38.33 ± 0.44	38 ± 0.67
Paracetamol	100 mg/kg	36.67 ± 0.44	38.67 ± 0.44	37.67 ± 0.44	37.33 ± 0.44	37.67 ± 0.56	37.67 ± 0.67	37.67 ± 0.44
MB	50 mg/kg	36.33 ± 0.44	39.33 ± 0.44	38.67 ± 0.44	38.67 ± 0.44	38.67 ± 0.67	38.67 ± 0.44	38.67 ± 0.44
MB	100 mg/kg	36 ± 0.67	39 ± 0.67	38.67 ± 0.44	38.33 ± 0.44	38 ± 0.67	38.33 ± 0.44	38 ± 0.00
MB	150 mg/kg	36 ± 0.67	38.67 ± 0.44	37.67 ± 0.44	37.67 ± 0.44	38.67 ± 0.44	37.67 ± 0.67	37.67 ± 0.89
BA	50 mg/kg	36.67 ± 0.44	38.33 ± 0.44	38 ± 0.67	38.33 ± 0.44	38.33 ± 0.44	39 ± 0.67	38.67 ± 0.44
BA	100 mg/kg	36.67 ± 0.44	38.67 ± 0.44	38.33 ± 0.44	39 ± 0.67	38.33 ± 0.44	38.67 ± 0.44	38.67 ± 0.44
BA	150 mg/kg	37.33 ± 0.44	38.33 ± 0.44	38.33 ± 0.44	38.33 ± 0.44	38.33 ± 0.44	38.33 ± 0.44	38.33 ± 0.44

Data are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Student's *t* test with significance value $P > 0.0001$.

as drugs formed synthetically for their antipyretic effects (Khan et al., 2009; Devi et al., 2003). Yeast-induced pyrexia is also known as pathogenic fever (Hossain et al., 2016b; Hossain et al., 2017). The intraperitoneal administration of *Monothecha buxifolia* and *Bosea amherstiana* significantly attenuating rectal temperature of yeast induced mice.

5. Conclusion

These investigational results have linked a pharmacological indication for the traditional claim of the drugs to be used as an analgesic, anti-inflammatory, and antipyretic agent. Supplementary studies on the probable mechanism of actions and isolation of active compounds responsible for such activities are presently under progress in our laboratory.

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