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STUDIES ON ANALGESIC AND CNS DEPRESSANT ACTIVITIES OF ALHAGI MAURORUM MEDIC METHANOLIC EXTRACT ON MICE

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ABSTRACT

Alhagi maurorum (Leguminosae) is locally known as Seez/Shing. In Quetta Balochistan it is used for cure of various diseases. The aim of current study was to evaluate the effect of the methanolic extract of *A. Maurorum* on CNS (Central Nervous System) using various of neuropharmacological and analgesic activitiesin albino mice. *A. maurorum* methanolic extract was administered at the doses of 250 and 500mg/kg. *A. maurorum* methanolic extract at both doses showed significant CNS depressant activityin open field, cage crossing, rearing and traction test. In forced swimming test *A. maurorum* methanolic extract showed significant anxiolytic effect. Results of analgesic activity revealed that *A. maurorum* methanolic extract possessed significant analgesic effect on acetic acid induced writhing test and formalin test as compared with standard drug diclofenic sodium. The results of current study suggest that *A. maurorum* methanolic extract posse's significant CNS depressant and analgesic effects.

Keywords: *Alhagi maurorum*, Analgesic, CNS depressant

INTRODUCTION

Medicinal plants have great importance in human history. These have been identified and use as medicine from many years [1, 2]. According the World Health

Organization (WHO) 80 percent of the population of Asian and African countries currently uses herbal medicine for health care, because these are more affordable than modern medicines. In fact WHO also says that approximately 25% of modern drugs used in the United States have been derived from plants. All medicinal plant contains different types of chemical compound, the main phytochemical are divided into primary metabolites and secondary metabolites, and it is usually secondary metabolites which have the therapeutic properties [3]. Many of the modern medicines currently prescribed by physician have already a long history of use as herbal medicine like opium, digitalis and quinine [4]. Balochistan is the largest province [5,6], representing 43.6 per cent of the land mass of Pakistan and native home of many medicinal plants [7]. Many of the plants are identified as having medicinal characteristics and using for treatment of the different diseases [8].

Alhagi maurorum belongs to Papilionaceae (Leguminosae) family. In Balochistan Pakistan, it grows in Quetta, Kalat, Nichara, Rodenjo, Surab and Khuzdar. Locally it is known as Seez/Shing [9]. Literature revealed the presence of flavonoids, glycosides, alkaloids, saponins, tannins, steroids, anthrax quinones, coumarins, saponins, phlobatannins, and terpenoids in leaves and roots [10]. *A. maurorum* is used in folk medicine, as a purgative, diaphoretic, expectorant, diuretic used to treat piles, migraine, warts and rheumatism [10]. It is used for rheumatic pains, liver disorders, urinary tract infection and for various types of gastrointestinal discomfort. All parts of the plant drink, or incense, even though the cold water in which to treat haemorrhoids [11].

MATERIAL AND METHODS

Extract preparation

Plant material was collected from hazar Ganji Quetta Balochistan. Voucher specimen no. MD-180 was submitted in herbarium of Pharmacognosy department faculty of pharmacy and health sciences University of Balochistan Quetta. Plant material was dried under shade and soaked in methanol for 15 days. Solvent was evaporated under reduced pressure by using rotary evaporator. After evaporation of solvent, the dark green residue was obtained.

Animals

Female and Male (both) albino mice, were collected from the DUHS (Dow university of health sciences, Karachi) weighing about 20-25 gram. Animals were kept under standard environment, temperature was maintained at 25±1 °C, 12 hours dark / 12 hours in light cycle. The animals were adjusted to Lab environment for 7 days.

The mice had free access to feed and water, Prior to experimentation [12].

CNS depressant activities

The methanolic extract was assessed for its CNS depressant activities by using open field, traction test, cage cross method, rearing test and force swimming test. The animals were distributed into 4 following groups (5 mice in each group), Group 1 control (saline 5ml/kg treated), Group 2 & 3 *A. Maurorum* methanolic extract 250 & 500mg/kg treated and Group 4 standard drug (Diazepam) 2mg/kg treated group.

Open field activity (OFT)

Mice putted individually in plastic cube, walled (30 cm in height) and the floor was divided into twenty five (25) squares and length and width 10 cm. The mice after thirty minutes (30 min) following the adminsteration of drug placed in apparatus to determine exploratory and loco motor activities. After oral administration of the vehicle, plant extract (250 and 500mg/kg) and diazepam, animals were evaluated for (10 min) in order to record locomotor activity (expressed by squares crossing with the all four paws) [13, 14].

Rearing

Central excitatory behaviour is measured through rearing test. Number of upright standings at hind limbs while keeping forelimbs against the wall of the beaker or in free air were counted and measured as perceptions. These rearing perceptions are counted for 10 minutes [15].

Cage crossing test (CCT)

In order to measure the cage crossing capacity of the mice, they were kept in plastic cage of 26x 26 x26 dimension, and the number of movements when the mice moved from one end to the other was recorded [12].

Traction test (TT)

Mice were trained to walk a one meter long iron rod before conducting the test. The traction test was later on conducted to measure the muscle relaxant activity of the mice. Time (in seconds) was recorded during which the mice travelled across the rod [16].

Forced swim test (FST)

Forced swimming test is conducted to know the antidepressant potential of any drug. To conduct the test, animals were divided in 4 groups i.e. Control (drug non-treated group), 250 and 500mg/kg and standard drug (diazepam) treated group. Animals were forced to swim in a 25cm high and 10 cm wide cylindrical apparatus. The apparatus was filled with water which was 19 cm deep at 25 ± 1°C. Total time of the activity was recorded which consist of time spent while swimming actively and remaining immobile during the test. Immobility means to stop movement in stationary water. The time when mice

remain immobile denotes antidepressant potential of the drug [17, 18].

Analgesic activity

Acetic acid induced writhing test

Acetic acid induced writhing test is conducted in mice in order to assess the analgesic potential of the plants [11]. As a test solution, 0.6 % acetic acid is administered intra-peritoneally 30minutes after the administration of saline treated (control group), 50mg/ kg Diclofenac Sodium treated group, *A. maurorum*250 & 500 mg/kg treated group. After 30 minutes of the administration of test solution, number of writhes were counted. Analgesic activity was measured on the basis of decrease number of writhes [14].

Formalin test

Another test which was performed to assess the analgesic activity of the plant was formalin test. In this test 20 µl of 1% formalin, diluted in distilled water was injected in mice subcutaneously (dorsal hind paw) using a micro-syringe (26-gauge needle), after 30 minutes of administration of 50mg/kg Diclofenac Sodium treated group, saline treated (control group), and 250 & 500 mg/kg *A. Maurorum* treated group orally. Mice were observed after their return to the chamber. Time during which the animal licked the injected paw or leg was recorded. First (0-5 minutes after the administration of formalin) and late phase (15-30 minute) after the injection of formalin) was recorded [12, 18].

RESUTLTS

Open field Activities

For the control group the open field activities were 151.28 $\pm$ 8.35, for *A. maurorum* crude extract 250mg /kg treated group the open field activities was 123.16 + 3.25, in *A. maurorum* crude extract 500mg/kg treated group the open field activities were 111.46$\pm$ 3.00and Diazepam 2mg/kg number of open field activities were 54.22+1.80.

Cage crossing activities

For the control group the number cage crossings were 42.22$\pm$1.53, for *A. maurorum* crude extract 250mg/kg number of cage crossings were 32.2 $\pm$ 1.28,*A. maurorum* crude extract 500mg/kg number of cage crossings were 19.4$\pm$ 3.49 and Diazepam 2mg/kg treated group number of cage crossings were 18.58$\pm$ 0.85.

Rearing

For the control group the rearing activities were 38.92 $\pm$ 0.03, for *A.maurorum* crude extract 250mg /kg the rearing activities were22.68 $\pm$ .51, in *A. Maurorum* crude extract 500mg/kg the rearing activities were 20.7$\pm$ 0.57 and Diazepam 2mg/kg rearing activites were15.5$\pm$ 0.73.

Traction time

For the control group the traction time was 36.9 $\pm$ 0.83,for *A.maurorum* crude extract

250mg /kg treated group the traction time was 21.26 ± 1.14, in *A. maurorum* crude extract 500mg/kg the traction time was 19.66± 0.92 and Diazepam 2mg/kg results traction time was 15.74± 1.06.

Forced Swimming test

In this test, for the control group the mobility time was 144.32 ±0.93 (seconds) and immobility time was 215.68+0.93 (seconds), for 250mg /kg the mobility time was 121.36+0.06 and immobility time was 159+0.04 (seconds) in 500mg/kg the mobility time was 111.6+0.49 and immobility time was 248.4+0.06 (seconds) and Diazepam 2mg/kg mobility time was 97.26+0.68 and immobility time was 226.512+0.15(seconds).

Analgesic activities

Writhing activities

For the control group the number of writheswere87.54 ± 2.93,for treated *A. maurorum* crude extract 250mg /kg the mean number of writhes were 52.2±0.45,in *A. maurorum* crude extract 500mg/kg the number of writhes were 42.5± 0.97and diclofenac sodium50 mg/kg treated group number of writhes were 30.1± 1.32.

Formalin test

Phase 1

For the control group number of biting and licking in first phase was 41.88±0.40 and time spent on licking and biting was 32.88±0.50 seconds, number of licking and biting of *A. maurorum* 250 mg/kg treated group was 27.74±1.65, and duration of biting and licking was 26.5.±0.86, number of licking and biting of *A. maurorum* 500 mg/kg treated mice was 23.3±0.74 and time spent of licking and biting was 18.94±0.52. When treated with diclofenac Sodium50 mg/kg number of biting and licking was 16.9±0.92 and duration was 13.7±1.25 seconds.

Phase 2

For the control group number of biting and licking in first phase was 56.88±0.79 and duration of biting and licking was 83.8±1.32 seconds, number of licking and biting of *A. maurorum* 250 mg/kg treated mice was 35.42±0.68and time spent of licking and biting was 34.74±1.9 seconds, number of biting and licking of *A. maurorum*500 mg/kg treated mice was 27.8±0.47and duration of biting and licking was26.34±2.04 seconds. When treated with Diclofenacsodium 50mg/kg number of licking and biting were23.3±0.70 and time spent was 22.1±0.37 seconds.

Table No 1: Effect of *A. maurorum* crude extract on neuropharmacological activities of mice.

S. No.	Treatment	Open field activities Mean±SEM	Rearing activities Mean±SEM	Cage crossing activities Mean±SEM	Mean Traction time (Seconds)±SEM
1	Control	151.28±8.35	38.92± 0.03	42.22±1.53	36.9± 0.83
2	*A. maurorum* crude extract 250mg/kg	123.16±3.25*	22.68± .51*	32.2 ± 1.28*	21.26± 1.14*
3	*A. maurorum* crude extract 500mg/kg	111.46± 3.00	20..7± 0.57*	19.4± 3.49*	19.66± 0.92*
4	Diazepam 2mg/kg	54.22±1.80**	15.5± 0.73**	18.58± 0.85**	15.74± 1.06**

All values are mean ± SEM; n=5; * = Significant (*P*<0.05), ** = highly significant (*P*<0.01).

Table No 2: Effect of *A. Maurorum* crude extract on Forced Swimming test of mice

S. No.	Treatment	Mean mobility time (seconds)±SEM	Mean immobility time (seconds)±SEM
1	Control	144.32.+0.93	215.68+0.93
2	*A. maurorum* crude extract 250mg /kg	121.36+0.06*	159+0.04*
3	*A.maurorum* crude extract 500mg/kg	111.6+0.49*	248.4+0.06*
4	Diazepam 2mg/kg	97.26+0.68**	226.512+0.15**

All values are mean ± SEM; n=5; * = Significant (*P*<0.05), ** = highly significant (*P*<0.01).

Table No 3: Effect of *A. Maurorum* crude extract on acetic acid induced writhing testof mice

S. No.	Treatment	Mean number of Writhes±SEM
1	Control	87.54± 2.93
2	*A maurorum* crude extract 250mg/kg	52.2±0.45*
3	*A maurorum* crude extract 500mg/kg	42.5± 0.97*
4	Diclofenac Sodium 50mg/kg	30.1± 1.32**

All values are mean ± SEM; n=5; * = Significant (*P*<0.05), ** = highly significant (*P*<0.01).

Table No 4: Effect of *A. Maurorum* crude extract on formalin testof mice

Treatment	Dose mg/kg orally	First Phase Mean No. of observations ±S.E.M		Second Phase Mean No. of observations ±S.E.M	
		Number of Licking & Biting	Time Spent (Seconds)	Number of Licking & Biting	Time Spent (Seconds)
Control	0.5ml Distil water	41.88±0.40	32.88±0.50	56.88±0.79	83.8±1.32
Crude extract of *A. maurorum*	250 mg/kg	27.74±1.65	26.5.±0.86*	35.42±0.68*	34.74±1.9*
	500mg/kg	23.3±0.74*	18.94±0.52*	27.8±0.47*	26.34±2.04*
Diclofenac Sodium	50mg/kg	16.9±0.92**	13.7±1.25**	23.3±0.70**	22.1±0.37**

All values are mean ± SEM; n=5; * = Significant (*P*<0.05), ** = highly significant (*P*<0.01).

DISCUSSION

Purpose of the study was to investigate the CNS depressant effect of methanolic extract of *A. maurorum*. Significant CNS depressant effects were produced by *A. maurorum* in traction, cage crossing, open field, and rearing tests. Results of the forced swimming test were also significant. Presence of glycosides, saponins, tannins, steroids, carbohydrates and flavonoids were reported in previous phytochemical analysis [10]. Various reports demonstrate that flavonoid, glycoside, and alkaloid rich plants possess antiepileptic, anxiolytic, and

sedative properties. This effect is due to their affinity for GABAergic receptors in the central nervous system where they make a complex by direct or indirect modulation of these receptors [19]. Tannins also have been found attributing to the nonspecific CNS depression [20]. It has been confirmed through experimental observation that saponins, glycosides, carbohydrates, flavonoids and such other phytochemicals are responsible for the hypnotic and sedative effects of the plant, *A. maurorum* inhibition of the pain produced by methanolic extract of the plant was significantly dose dependent. However, effect of the standard drug was more than the extract. Writhing is basically a manifestation of peripheral pain arising after acetic acid administration [21]. Administration of acetic acid stimulates the release of certain pain mediating chemicals such as, Serotonin, Bradykinin, Substace P, Prostaglandin E2, and Histamine, which stimulate the neurons responsible for nociception. [22, 23], substance P, serotonin, histamine, bradykin in which further causes stimulation of nociceptive neurons. It has been found that the pain is inhibited by the extract when the release of prostaglandins is stopped. The exact mechanism of pain inhibition is quiet similar to non-steroidal anti-inflammatory drugs (NSAIDS) [24]. Formalin-induced rat paw oedema is a well-known and standard experiment phase which develops in a few hours is attributed to the release of histamine, serotonin and kinins [25, 26, 27]. The chronic phase is accompanied by the release of prostaglandins [28]. Since activity was observed in both phases of formalin-induced oedema, and this might be due to the Serotonin, Histamine, or Prostaglandin inhibition. Although the effect on chronic phase is more pronounced and this is probably indicative of the inhibitory effects of *A. maurorum* on prostaglandin synthesis or release and this effect may attributed to the presence of various phytochemical constituents.

CONCLUSION

A. maurorum is used in traditional medicine in Quetta Balochistan. Results revealed that plant extract possess CNS depressant and analgesic effects and can be utilized for nervous disorders and pain management. However, extensive research is yet to be done to isolate active constituents responsible for these effects.

REFERENCES

[1] Taprell LC, Heniphill, Cobiac L (2006). Health benefits of herbs and spices. *The past, the present, the future., 185*(4) :4-24

[2] Lai PK, Roy J (2004). Anti-microbial and chemopreventic

properties of herb and spices. 11(11): 1451-60

[3] Fabrical DS and Farnsworth NR (2001). The value of plant used in traditional medicine for drug discovery. Ehp (environmental health perspective). 109(1):69-75

[4] Meskin and Mark. (2002). Phytochemical in nutrition and health CRC Press. p123

[5] Zaidi SF, Muhammad JS, Usmanghani K, Sugiyama (2015) Pharmacological ins and outs of medicinal plants against Helicobacter pylori: A review. *Pak J Pharm Sci., 28*(3): 1171-6.

[6] Zaidi MA, Crow SA (2012). Cytotoxicity of four medicinal plants of Pakistan. *Pakistan J Botany* 1(44):395-7.

[7] Ahmad M, Muhammed S, Mehjabeen, Jahan N, Jan SU and Qureshi ZR (2014) Anti-dermatitis, anxiolytic and analgesic effects of *Rhazya stricta* from Balochistan. *Pak J Pharm Sci. 27*(3): 481- 486.

[8] Syed RS, Hassan G, Rehman A and Ahmed I (2006) Ethnobotanical studies of the flora of district Musakhel and Barkhan in Balochistan, Pakistan. *Pak J Weed. Sci Res. 12*(3): 199-211.

[9] Tareen RB, Bibi T, Khan MA, Ahmad M, Zafar M, Hina S (2010). Indigenous knowledge of folk medicine by the women of Kalat and Khuzdar Regions of Balochistan, Pakistan. *Pakistan J Bot 42*(3):1465-85.

[10] Ahmad N, Bibi Y, Raza I, Zahara K, Khalid N, Bashir T, Tabassum S (2015) Traditional uses and pharmacological properties of Alhagi maurorum: A review. *Asian Pacific Journal of Tropical Disease,* 5(11):856-61.

[11] Neamah NF. A pharmacological evaluation of aqueous extract of Alhagi maurorum (2012). *Global J Pharmacol*. 6(1):41-6.

[12] Jabbar A, Muhammad S, Razaque G, Qadir A, Younis M, Ahmad N, Baloch I A and Mustafa G (2016). Studies on neuropharmacological and analgesic effects of *Periploca aphylla* extract in mice. *Pure. Appl. Biol., 5*(4): 1207-1215.

[13] Kennett GA, Dicknison SL and Curzon G (1985). Central serotonergic responses and behavioral adaptation to repeated immobilization: The effect of corticosterone synthesis inhibitor metyrapone. *Eur. J. Pharmacol.*, *119*: 143-152.

[14] Qureshi, M., Muhammad, S., Siddiqui, F. A., Baig, I., & Ahmad, M. (2017). Phytochemical and biological assessments on Lipidium meyenii (maca) and Epimidium sagittatum (horny goat weed). *Pakistan Journal of Pharmaceutical Sciences*, *30*(1).29 -36.

[15] Arsalan M, Muhammad S, Qadir A, Younis M, Marvi, Razque G, Shahwani NA, Jabbar A, Sarwar G (2017). Phytochemical and pharmacological studies on *Microcephala lamellata* (bunge.) Pobed methanolic extract in mice. *IJBPAS,* 6(4): 623-634

[16] Sarwar G, Muhammad S, Marvi, Ahmad N, Qadir A, Jabbar A, Muhammad, Arsalan M, Mustafa G, Khattak MA (2017) Evaluation of neuropharmacological and analgesic effects Cones of *Juniperus excelsa* (M. Bieb) of ziarat, Balochistan IJBPAS 6(4): 749-761.

[17] Turner RA (1965). Screening methods in pharmacology, Academy Press, New York. P.113

[18] Marvi, Iqbal J, Muhammad S and Ahmad M (2017). Evaluation of acute toxicity, sedative and analgesic effects of *Taverniera glabra* methanolic extract on mice. *Pak J Pharm Sci, 29* (6): 2335-2341.

[19] Moniruzzaman M, Rahman A, Ferdous A (2015) Evaluation of sedative and hypnotic activity of ethanolic extract of *Scoparia dulcis* Linn. *Evidence-Based Complementary and Alternative Medicine.* 12; 2015.

[20] Takahashi RN, de Lima TC, Morato GS (1986) Pharmacological actions of tannic acid; II. Evaluation of CNS activity in animals. *Planta medica. 52* (04): 272-5.

[21] Sanchez-Mateo C C, Bonkanka C X, Hern´andez-P´erez M, Rabanal R M (2006) Evaluation of the analgesic and topical anti-inflammatory effects of *Hypericum reflexum* L. fil. *J Ethnopharmacol*. 107:1–6. 26.

[22] Zahid H, Rizwani Ghazala Hafeez, Shareef Huma, Ahmed Maryam, Hina Bushra (2012). Analgesic and antipyretic activities of *Hibiscus schizopetalus* (mast.) hook. *Int J Pharm Pharm Sci.* 4(3):218-221. 28.

[23] Roger D, Simone J, Françoise D, Micheline F (1980) Release of prostaglandins E and F in an

algogenic reactions and its inhibition. *Eur J Pharmacol*. 61(1):17-24.

[24] Banerjee SA, Mukherjee AN, Chatterjee TK (2012). Evaluation of analgesic activities of methanolic extract of medicinal plant Juniperus communis Linn. *International Journal of Pharmacy and Pharmaceutical Sciences,* 4(5):547-50.

[25] Santos A, Calixto J (19997) Further evidence for the involvement of tachykinin receptor subtypes in formalin and capsaicin models of pain in mice. *Neuropeptides* 31:381–389.

[26] Doak G, Sawynok J (1997) Formalin-induced nociceptive behavior and edema: involvement of multiple peripheral 5-hydroxy-tryptamine subtypes. Receptorneur osci. 80: 939–949.

[27] Coruzzi G, Adami M, Guaita E, de Esch IJP, Leurs R (2007) Anti-inflammatory and antinociceptive effects of the selective histamine H4-receptor antagonists JNJ7777120 and VUF6002 in a rat model of carrageenan-induced acute inflammation. *Europ.J.Pharmacol.* 563:240–244.

[28] Yamamoto T, Nozaki-Taguchi N (2002) The role of cyclooxygenase-1 and-2 in the rat formalin test. *Anesthesia & Analgesia*. 94:962–967.