Anti-nociceptive activity of seed extract of *Vernonia anthelmintica* willd

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Abstract: *Vernonia anthelmintica* is commonly known as kali ziri. Its seeds are used for several therapeutical purposes. Its seeds contain many constituents of medicinal importance as vernodlin, vernodalol, and vernolic acid. It is commonly used psoriasis and leucoderma or white leprosy. It is potent wormicidal agent. The present study was conducted on seed's extract of *V. anthelmintica* to determine its analgesic potency. The activity was conducted on mice by using acetic acid induced writhes,hot plate method and by tail flick method using water bath. The results of the writhing test were highly significant and comparable with Aspirin, which produced 26 and 20 writhes. The percentage of inhibition of writhes with the two doses of crude extract was 65.45% and 64.28% at 300mg/kg, while 83.63% and 71.42% at 500mg/kg, where as with Aspirin it was 52.72% and 28.57% in first and second phase respectively. Hot plate and tail flick method also indicated that vernonia has potent analgesic activity. The drug can be utilized as anti-nociceptive agent.

Keywords: *Vernonia anthelmintica*, kali ziri, anti-nociceptive, acetic acid induced writhes, tail flick method.

INTRODUCTION

Vernoniaanthelmintica is found throughout India from Kashmir to Ceylon, growing wild in hilly areas **(**Mhaskar *et al*., 2000). Seeds of *V. anthelmintica* containvernodalol, stigmastadienol, stigmasterol and stigmastarienol acetate as active constituents. Seeds also contain vernolic acid present in seed oil (Higgins 1968, Krewson *et al*., 1962)**.** Vernolic acid is a fatty acid which was isolated in 1954 for the first time (Gunstone, 1954; Krewson *et al*., 1962). Oil also has 6-8 percent unsaponifiable substance (Krewson *et al*., 1962).

The seeds have bitter hot and sharp taste. They have astringent action on the gastrointestinal tract and also used for ulcer treatment. Seeds are potent anthelmintic agent that causes purgation. Ingestion of seeds used in Malabar Coast for cough and flatulency. It is effective for leukoderma and skin diseases as an anti-inflammatory agent. It is used for asthma, kidney and liver disorders and to remove inflammation and blood from sores. Seeds are also used in powder formulation for snake bite.

MATERIALS AND METHODS

Plant material and extraction procedure

Seeds of *V. anthelmintica* were purchased from local market and identified by Prof. Dr. Mansoor Ahmad, Department of Pharmacognosy, University of Karachi and sample was deposited in the herbarium of the department (Herbarium No.001106-12).

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The seeds were powdered and soaked in ethanol for 15 days. Then it was filtered and the ethanol was evaporated under reduced pressure in rotary evaporator (BUCHI Rotavapor R-200). After evaporation a thick residue obtained that was used for the assay.

Animals

Male albino mice of weight 25g were purchased from animal house of HEJ Research Institute of Chemistry, University of Karachi. Animals were provided with food and water *ad-libitum*.

Analgesic activity

Acetic acid induced writhing

Acetic acid was used for assay of analgesic potential of crude seed extract. Mice weighing 25g were used for the activity. Group A received saline as control group. Group B and C received seed extract diluted in distilled water and administered orally in dose of 300 and 500mg/kg. Group D received standard drug Aspirin 300mg/kg orally. After 30 minutes of drug administration pain was induced by intraperitonial administration of 0.6% acetic acid. Number of writhes were observed by using the method of Koster and percent inhibition of writhes was calculated (Koster *et al.* 1959).

Hot plate test

For hot plate analgesic activity mice were placed on a hot plate (50±2ºC) and their reaction to heat was observed. When the animal raised and licked the front paws they were quickly removed from the hot plate and the time

period observed. Four groups of 5 mice each were used as group A (control), group B and C (treated) received 300 and 500mg/kg ethanolic seed extract respectively and group D (standard) received 300mg/kg Aspirin. The drug was diluted in distilled water and administered orally using stainless steel feeding tube. The observations were recorded after 30 minutes of drug administration (Dharmasiri *et al*. 2003).

Tail flick or tail immersion test

Tail immersion method was used for analgesic activity by the same method as Owoyele *et al*. 2004. Mice were divided in to four groups of five animals each. Group A (control group) received normal saline orally. Group B and C (treated groups) received ethanolic seed extract in 300mg/kg and 500mg/kg concentrations and group D (standard group) received 300mg/kg Aspirin orally.

In this method tail was immersed in water heated at 50± 2°C in water bath. The time period in which tail flicked out from water bath was recorded. The observations were made for five hours at every 30 minutes interval. Percentage response was calculated by the formula,

$$\frac{C-T}{C}\times 100$$

STATISTICAL ANALYSIS

Results are expresses as mean ± SEM. Statistical analysis was carried out using Student t-test. The level of significance was considered at $p \leq 0.05$.

RESULTS

Effect of crude extract on acetic acid induced writhing in mice

Peripheral analgesic activity of crude extract of *V. anthelmintica* was assessed by acetic acid induced writhing method as given in experimental part. The results were expressed in table 1and fig. 1. The extract of *V. anthelmintica* showed prominent analgesic activity that is 83 and 71% inhibition in first and second phase while for Aspirin percentage inhibition was 52 and 28 in first and second phase respectively.

Effect of crude extract on Hot plate Analgesiometer in mice

Results of analgesic activity performed by hot plate method at 50±2ºC are given in table 2 and shown in fig. 2. *V. anthelmintica* showed analgesic activity(39.6 sec) that is highly significant analgesic activity as compared to the standard drug that is aspirin (28.6 sec).

Effect of crude extract by tail flick method in mice by water bath

The analgesic activity of crude extract of *V. anthelmintica*was also determined by tail flick method in mice using water bath. The results are mentioned in table 3 and fig. 3. *V. anthelmintica* (5.8 sec) showed analgesic activity comparable to the aspirin (4.8 sec).

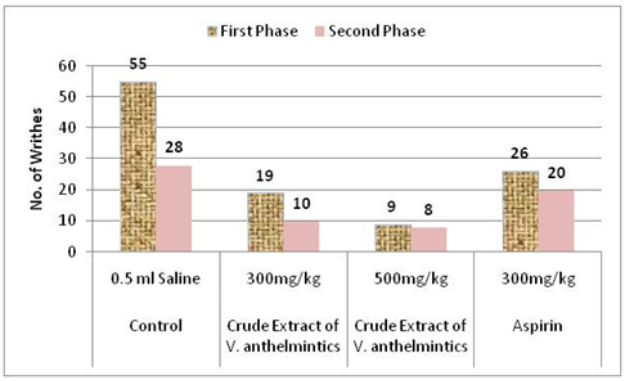

Fig. 1: Effect of crude extract on acetic acid induced writhing in mice.

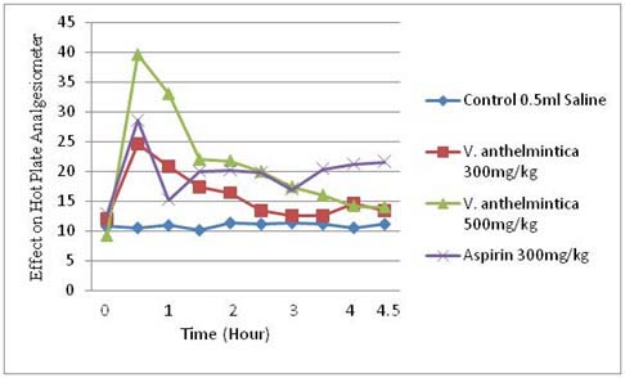

Fig. 2: Effect of crude extract on Hot plate Analgesiometer in mice.

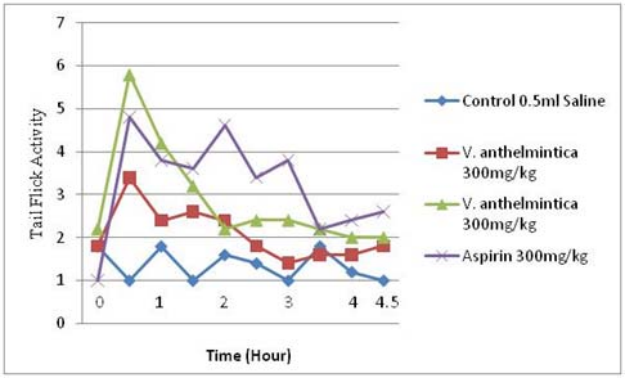

Fig. 3: Effect of crude extract by tail flick method in mice by water bath.

DISCUSSION

Medicinal plants are good source for treating certain disorders. Different species of Vernonia have analgesic as well as anti-inflammatory potential. One of the specie as *Vernonia nigritiana* Oliv. has certain steroids with anti-inflammatory activity (Vassallo *et al.*, 2013). Main constituents of *V. anthelmintica* are vernadalol and

Table 1: Effect of crude extract on acetic acid induced writhing in mice

Treatment	Dose mg/kg orally	Mean No. of Writhes ± S.E.M		Inhibition (%)	
		First phase	Second phase	First phase	Second phase
Control	0.5 ml Saline	55± 1.86	28 ± 0.951	-	-
Crude extract of V. anthelmintica	300mg/kg	19±0.709	10±1.098	65.45**	64.28**
	500mg/kg	09±1.002	08±0.838	83.63**	71.42**
Aspirin	300mg/kg	26± 1.382	20± 1.186	52.72**	28.57*

Table 2: Effect of crude extract on Hot plate Analgesiometer in mice

Variation flicking time with ± SEM (Time in sec at 55 ± 1ºC)										
Group	0hr	0.5hr min	1hr	1.5hrs	2hrs	2.5hrs	3hrs	3.5hrs	4hrs	4.5hrs
Control	10.8± 0.802	10.6 ± 0.929	11.0 ± 0.709	10.2± 0.862	11.4 ± 0.750	11.2 ± 0.862	11.4 ± 0.750	11.2 ± 0.862	10.6± 0.929	11.2 ± 0.862
V. anthelmintica (300mg/kg)	12 ±0.709	24.6** ±0.814	20.8** ±1.533	17.4* ±0.680	16.4* ±0.929	13.4 ±1.032	12.6 ±0.929	12.6 ±0.929	14.6 ±0.680	13.4 ±1.032
V. anthelmintica (500mg/kg)	9.2 ±0.862	39.6** ±1.367	33** ±1.268	22** ±0.002	21.8** ±0.972	20** ±0.709	17.4* ±0.680	16* ±0.709	14.2 ±1.070	14.0 ±0.951
Aspirin 300mg/kg	12.8 ±0.972	28.6** ±0.814	15.2* ±1.886	20.0* ±1.051	20.2** ±1.160	19.8* ±1.022	17.0* ±1.307	20.4** ±1.079	21.2** ±0.862	21.6** ±0.814

Table 3: Effect of crude extract by tail flick method in mice by water bath

Variation flicking time with ± SEM (Time in sec at 55 ± 1ºC)										
Group	0hr	0.5hr min	1hr	1.5hrs	2hrs	2.5hrs	3hrs	3.5hrs	4hrs	4.5hrs
Control	1.8 ±0.089	1.00 ±0.070	1.80 ±0.089	1.00 ±0.070	1.6 ±0.118	1.4 ±0.138	1.00 ±0.054	1.8 ±0.089	1.2 ±0.145	1.0 ±0.054
V. anthelmintica (300mg/kg)	1.80 ±0.145	3.4* ±0.164	2.4 ±0.152	2.6 ±0.217	2.4 ±0.145	1.8 ±0.145	1.4 ±0.109	1.6 ±0.158	1.6 ±0.152	1.8 ±0.145
V. anthelmintica (500mg/kg)	2.2 ±0.130	5.8** ±0.138	4.2* ±0.141	3.2* ±0.152	2.2 ±0.130	2.4 ±0.217	2.4 ±0.210	2.2 ±0.158	2.00 ±0.141	2.00 ±0.122
Aspirin 300mg/kg	1.00 ±0.054	4.8* ±0.243	3.8* ±0.235	3.6* ±0.130	4.6* ±0.340	3.4* ±0.130	3.8* ±0.235	2.2 ±0.130	2.4 ±0.192	2.6 ±0.221

Values represent the Mean ± SEM. N = 5; The results are expressed at P≤0.05 and P≥0.05Statistically significant from control and standard drug. *Significant, **Highly significant.

vernolic acid. The analgesic activity of *Vernonia anthelmintica* was determined by three different methods that is acetic acid induced writhing, hot plate method and tail flick on water bath. Mice were taken in four different groups to observe the analgesic activity of the crude extract of *V. anthelmintica* at the dose of 300mg/kg and 500mg/kg and Standard drug i.e. Aspirin at the dose of 300mg/kg. The results showed that crude drug at the dose of 300mg/kg and 500mg/kg had significant analgesic activity, which is comparable to the standard drug i.e. Aspirin at the dose of 300mg/kg.

V. anthelmintica seeds also have other medicinal properties as evaluated by Fatima *et al*, in 2010. Ethyl acetate and isopropanol (1:1) fraction of *V. anthelmintica* seeds have antidiabetic and anti-hyperlipidemic activity that was determined on streptozotocin induced diabetic rats (Fatima *et al*, 2010). Effect of *Vernonia cinerea* L. and vernolide-A on cell-mediated immune (CMI) response was determined by Pratheeshkumar. *V. cinerea* extract and vernolide-A stimulate the CTL, NK cell, ADCC, and ADCC through enhanced secretion of IL-2 and IFN-γ (Pratheeshkumar*et al*, 2012). Extract of *Vernonia condensata* leaves has different uses in Brazilian folk medicine, which includes analgesic and antiinflamatory agent. Risso determined antinociceptive activity of *V. condensate,* evaluated by writhing test. Acetone and the ternary mixture, acetone-ETOH-ethyl acetate extracts showed higher margin of safety than aqueous extract (Risso*et al*, 2010). *Vernonia amygdalina* possesses several bioactive compounds and is used in traditional medicines of southwestern Uganda, along with other regions. Njan examined the antinociceptive potential of the aqueous leaf extract (50-200mg/kg) using three models of nociception (acetic acid-induced writhing, formal in test, and tail-flick

test), antiplasmodial activity, and toxicology of the extract, so *V. amygdalina* is traditionally use as an alternative therapy for malaria and the symptomatic relief of pain usually associated with malaria (Njan *et al*, 2008). On storage of plant material for long time it was found that antibacterial activity was retained in most species while COX-1 inhibition activity was lost rapidly (Stafford, *et al*, 2005). *Vernonia cinerea* methanol, chloroform and ether leaf extract have analgesic, antipyretic, anti-inflammatory effects (Iwalewa, 2003). Polar extract from leaves of *Vernonia condensata* have analgesic and anti-ulcerogenic effects. Frutuoso found that when polar fraction administered orally in acetic acid induced writhing test to rat, it gav its analgesic effect as well as when it is administered with indomethacin it gave synergistic analgesic effect and also inhibited ulcerogenic effect of indomethacin (Frutuoso, 1994). The recent study was conducted on crude extract of *V. anthelmintica* to evaluate its antinociceptive potential.

V. anthelmintica seed extract was evaluated for its analgesic potential by acetic acid induced writhing test. This activity was done using two different concentrations that were 300 mg/kg and 500mg/kg. The results were compared with control and standard group of animals. It was found that numbers of writhes were reduced to 19, 10 and 09, 08 in animals treated with 300mg/kg and 500mg/kg oral dose as compared to control group that were 55 and 28 in first and second halves. The percentages of inhibition of writhes were highly significant (table 1; fig. 1) as compared to standard drug that is aspirin (26 and 20 writhes). The percentage of inhibition of writhes in the first and second half of observation was 65.45 and 64.28 at 300mg/kg while 83.63 and 71.42at 500mg/kg. The percentage of inhibition of writhes with aspirin was 52.72 and 28.57.

The analgesic activity was also evaluated by Hot plate analgesiometer. The results in table 2 showed that *V. anthelmintica* has potent analgesic activity (39.6sec) that was higher than aspirin (28.6sec). Similar results observed when seed extract was analyzed on mice by tail flick method (table 3; fig. 3). *V. anthelmintica* has significant dose dependent analgesic potential (5.8sec), it was comparable with aspirin (4.8sec). The results showed that crude extract of *V. anthelmintica* had significant analgesic activity as the other species of Vernonia.

CONCLUSION

The seed extract of *Vernonia anthelmintica* has potent antinociceptive activity. The results show that analgesic activity at 300mg/kg oral dose of crude seed extract is comparative with aspirin that is standard pain killer. At higher dose the analgesic potential is more but it may toxic to animal however 300mg/kg dose is safe as well as effective as antinociceptive.

REFERENCES

Dharmasiri MG, Jayakody JR, Galhena G, Liyanage SS and Ratnasooriya WD (2003). Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitexnegundo*. *J. Ethnopharmacol.*, **87**: 199-206.

Fatima SS, Rajasekhar MD, Kumar KV, Kumar MT, Babu KR and Rao CA (2010). Antidiabetic and antihyperlipidemic activity of ethyl acetate: Isopropanol (1:1) fraction of *Vernonia-anthelmintic*a seeds in streptozotocin induced diabetic rats. *Food Chem. Toxicol.*, **48**(2): 495-501.

Frutuoso VS, Gurjão MR, Cordeiro RS and Martins MA (1994). Analgesic and anti-ulcerogenic effects of a polar extract from leaves of *Vernonia condensata*. *Planta Med*., **60**(1): 21-25.

Gunstone FD (1954). Fatty acids. Part II. The nature of the oxygenated acid present in *Vernonia anthelmintica* (Willd.) seed oil. *J. Chem. Soc.,* 1611-1616.

Higgins JJ (1968). *Vernonia anthelmintica*: A potential seed oil source of epoxy acid. I. Phenology of seed yield. *Agron. J*., **60**: 55-58.

Higgins JJ and White GA (1968). *Vernonia anthelmintica*: A potential seed oil source of epoxy acid. II. Effects of cultural practices, seed maturity and after-ripening conditions on germination. *Agron. J.,* **60**: 59-61.

Iwalewa EO, Iwalewa OJ and Adeboye JO (2003). Analgesic, antipyretic, anti-inflammatory effects of methanol, chloroform and ether extracts of *Vernonia cinerea* less leaf. *J. Ethnopharmacol*., **86**(2-3): 229-234.

Koster R, Anderson M and De Bear EJ (1959). Acetic acid for analgesic screening. *Fed. Proceed,* **18**: 412-416.

Krewson CF, Ard JS and Riemenschneider RW (1962). *Vernonia anthelmintica* trivernolin, 1, 3- divernolin and vernolic (epoxyoleic) acid from the seed oil. *J. Amer. Oil Chem. Soc*., **39**: 334-340.

Mhaskar KS, Blatter E and Caius JF (2000). Kirtikar and Basu's illustrated, Indian Medicinal Plants. Their usage in Ayurvedic and Unani Medicines. Volume 6.3rd ed. Sri Satguru Publications. A division of Indian Books Center, Delhi, India, pp.1832-1834.

Njan AA, Adzu B, Agaba AG, Byarugaba D, Díaz-Llera S and Bangsberg DR (2008). The analgesic and antiplasmodial activities and toxicology of *Vernonia amygdalina*. *J. Med. Food*, **11**(3): 574-81.

Owoyele BV, Olaleye SB, Oke JM and Elegbe RA (2004). Anti-inflammatory and analgesic activities of *Nothospondias staudtii*. *Nigerian J. of Physiol. Sci.,* **19**(1-2): 102-105.

Pratheeshkumar P and Kuttan G (2012). Modulation of cytotoxic T lymphocyte, natural killer cell, antibody-dependent cellular cytotoxicity and antibody-dependent complement-mediated cytotoxicity by *Vernonia cinerea* L. and vernolide-A in BALB/c mice via

enhanced production of cytokines IL-2 and IFN-γ. *Immunopharmacol Immunotoxicol*., **34**(1): 46-55.

Risso WE, Scarminio IS and Moreira EG (2010). Antinociceptive and acute toxicity evaluation of *Vernonia condensata* Baker leaves extracted with different solvents and their mixtures. *Indian J. Exp. Biol*., **48**(8): 811-816.

Stafford GI, Jäger AK and van Staden J (2005). Effect of storage on the chemical composition and biological activity of several popular South African medicinal plants. *J. Ethnopharmacol*., **97**(1): 107-115.

Vassallo A, De Tommasi N, Merfort I, Sanogo R, Severino L, Pelin M, Della Loggia R, Tubaro A and Sosa S (2013). Steroids with anti-inflammatory activity from *Vernonia nigritiana* Oliv. and Hiern. *Phytochemistry*, **96**: 288-298.