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ANXIOLYTIC ACTIVITY OF *ABIES WEBBIANA* LEAVES ON BEHAVIOURAL MODELS OF ANXIETY IN RATS

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ABSTRACT

The present study has been conducted to assess the anxiolytic activity of chloroform extract of *Abies webbiana* leaves in rats using different anxiety model viz. Open field exploratory behavior, elevated plus maze [EPM] and elevated zero maze [EZM] tests. Chloroform extract orally at different dose levels once daily x 3days, while lorazepam [LR: 0.5 mg/kg,ip] was administered acutely. Chloroform extract of *A.webbiana* leaves [AW: 50 and 100 mg/kg, po.] showed anxiolytic effects on all the models of anxiety. The results indicate that AW and LR induced a significant increase in open field ambulation and slight increase in rearing and activity in center, whereas grooming and faecal droppings remain unchanged. In EPM, significant augmentation of open arm entries and time spent on open arms was recorded in AW treated rats. In EZM test, significant increase in time spent on open arms and entries in open arms was observed, whereas slight increase in head dips and stretched attend postures was also observed. The AW extract showed consistent and significant anxiolytic activity in all the paradigms of anxiety tests. The AW effects induced by chloroform extract were less marked than those of lorazepam.

KEYWORDS: *Abies webbiana: Anxiolytic activity: Behavioural paradigms.*

1. INTRODUCTION

Abies webbiana [Family: Pinaceae] is commonly known as Talisapatra tree in *Sanskrit* and popularly called Morinda in Hindi grown in the Himalayan region.[1,2] Leaves have been used as Ayurvedic remedy for fever, respiratory, asthma and inflammatory ailments.[3,4] *A. webbiana* leaves are known to possess analgesic, anti-inflammatory, barbiturates hypnosis, anti –ulcerogenic activities in rats, anti-stress activity in mice and hypotensive activity in dogs.[5,6,7,8] The organic compound is isolated from the leaves of *A. webbiana* include bioflavonoids[9], flavonoids[10], pindrolactone[11], (+)- pinitol[12] and Terephthalic acid dimethyl ester (TADE).[13] The pure compound (+)- pinitol and TADE of *A. webbiana* leaves also have anti-inflammatory activities in rats.[12,13]

The present investigation was conducted to assess the effect of chloroform extract of *Abies webbiana* leaves on behavioural test of anxiety in rats, in view of its medicinal importance in folklore medicine.

2. MATERIALS AND METHODS

2.1 Animal and Drug Administration

After approval of Institutional Animal Ethical Committee (IAEC), the present study was conducted in the Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi on inbred Albino rats (Wistar Strain)100-200g. They were kept in the departmental animal house in individual cages at an ambient temperature of 25 ± 1^0C and 45-55% relative humidity with 12h:12h light:dark cycles. They had fee access to standard rodent pellet diet and drinking water (Kinley) during the entire study period. The food was withdrawn 18 h prior to experimentation, however, water was allowed *ad libitum.*

Chloroform extract of *A.webbiana* leaves was dissolved in 3%Tween-80 prior to administration.AW extract was administered po. by using orogastric cannula in the doses of 50 and 100mg/kg once daily x3 days. Experiment were conducted on day 3rd, 1h after the last drug administration. Lorazepam [0.5mg/kg,ip] was used as standard anxiolytic agent and was administered 30 min prior to experimentation for comparison. Control groups were treated with vehicle [3%Tween-80].

2.2.1 Plant Material

The *A.webbiana* leaves obtained from the Regional Research Centre (Ay) Jammu-Tawi, India and identified in the Department of Dravy guna, IMS, BHU, Varanasi. A few mg of powdered drug was warmed with Chloral hydrate, washed and mounted in glycerine. A few mg of powder was cleared in 4% KOH, washed and mounted in glycerine. A few mg of powder was washed in plain water, a drop of KI –solution was added and mounted. Camera Lucida drawings were done for the salient features of the drug. The voucher specimens have been preserved.

2.2.2 Extraction

Dried powdered (2.9 kg) *A.webbiana* leaves were extracted by Chloroform and concentrated in a steam bath to a final yield of 40.0g (1.40% w/w). Chemical tests showed the presence of glycoside and steroids.

2.3. Behavioral Testing

2.3.1 Open –field Test [OFT]

The Open- field apparatus was made of plywood and consisted of squares [61 x 61cm]. The entire apparatus was painted black except for 6 mm thick white lines, which divided the floor into 16 squares. Open –field was lighted by 40W bulb focusing on to the field from a height of about 100 cm. The entire room, except the open field was kept dark during the experiment. Each animal was centrally placed in the test apparatus for 5 min and the following behavioral aspects were recorded.

[i]Ambulation: this was measured in terms of the number of squares crossed by the animal;

[ii]Rearing: number of times the animal stood on the hind limbs;

[iii]Self grooming: number of times the animal groomed facial region and licked/washed/scratched various parts of its body;

[iv]Activity in centre: number of central squares crossed by the animal; and

[v]Faecal droopings: number of faecal droppings excreted during the period.[14]

2.3.2. Elevated plus –maze test [EPM]

The maze had two opposite arms 50 x10 cm crossed with two enclosed arms of the same dimention but having 40 cm high walls. The arms were connected with a central square 10x10 cm, giving the apparatus shape of a plus sign. The maze was kept in a dimly-lit room and elevated 50 cm above the floor. Naïve rats were placed individually in centre of the maze, facing an enclosed arm. Thereafter, number of entries and time spent on the open and

enclosed arms were recorded during the next 5 min. An arm entry was defined when all four paws of the rat were in the arm. Observations were made by a neutral blind observer.[15]

2.3.3. Elevated Zero –maze test [EZM]

The maze comprised of a black perspex annular platform[105 cm diam,10cm width] elevated to 65 cm above the ground level, divided equally in to four quadrants. The two opposite quadrants were enclosed by a black Perspex wall[27 cm high] on both the inner and outer edge, of the platform, while the remaining two opposite quadrants were surrounded by perspex lip[1cm high] which served as a tactile guide to animal on these open areas. The apparatus was illuminated by dim white light arranged in such a manner as to provide similar lux levels in open and enclosed quadrants. Rats were placed on one of the enclosed quadrants for a 5 min test period. The maze was cleaned with 5%ethanol/water solution and dried thoroughly between test sessions. During the 5 min test period, time spend on open arms number of head dips over the edges of platform and number of stretched attend postures from closed to open quadrants were recorded. Animals were scored as being in the open area when all four paws were in the open quadrants and in the enclosed area only when all four paws had passed the open closed divide.[16]

2.4 Statistical Analysis

All the data was expressed as mean ± SD for each treatment group, analyzed by student's t-test followed by ANOVA.[17]

3. RESULTS AND DISCUSSION

3.1 Open –field exploratory behaviour [OFT]

The animal treated with both the doses of 50 &100 mg/kg, p.o. showed dose dependent significant increase in open field ambulation, rearing, self grooming and activity in centre in comparison to vehicle treated rats, evincing significant anxiolytic activity of AW. However, the open field faecal droopings remain unchanged. Lorazepam [LR] also induced significant anxiolytic activity and the effects were found to be more than that of AW extract [Table 1].

3.2 Elevated plus –maze behaviour [EPM]

The rats treated with both the doses of AW exhibited dose dependent significant increase in time spent and entries made in open arms and significant decrease in time spent/ entries in enclosed arms in comparison to control group of rats. These results also indicate significant

anxiolysis in rats by AW extract. LR caused more anxiolysis in comparison to AW extract[Table 2].

3.3 Elevated Zero –maze behaviour [EZM]

The rats treated with AW extract showed anxiolysis in the terms of significant increase in time spent in open arms, entries in open arms and number of head dips on elevated zero maze. However, the response stretched attend postures remain unchanged. LR also caused significant anxiolytic activity and the effects were comparable to that of AW extract [Table 3].

The present study indicate that Chloroform extract of *A.webbiana* treatment caused significant dose related anxiolysis in rats tested on all the behavioral paradigms viz Open field exploratory behavior [OFT], elevated plus maze behaviour [EPM] and elevated zero maze behaviour [EZM] tests. However, the anxiolytic activity of AW was found to be less marked than that of the common benzodiazepine anxiolytic agent lorazepam. *Abies pindrow* has been reported to exhibit antistress-adaptogenic activity besides having hypoglycemic, anti-inflammatory, analgesic and hypnosis potentiation activities.[5,6] Ethanolic extract of AW leaves have also been shown to inhibit cold restrained stress induced ulcer in rats.[6] The pure compound (+)- pinitol and TADE of *A. webbiana* leaves also have anti-inflammatory activities in rats.**[12,13]** Observed anxiolytic activity of AW leaf extract in the present study may be attributed to the antistress activity of AW reported earlier.[6] Ethanolic extract of *Abies pindrow* leaves are reported to contain various types of bioflavonoids,[9] flavonoids,[10] pindrolactone,[11] (+)- pinitol[12] and Terephthalic acid dimethyl ester (TADE).[13] Benzodiazepines are known to possess both antistress and anxiolytic properties.[18,19] Therefore, further investigation are warranted to elucidate the role of possible neurotransmitters underlying anxiolytic effect of AW leaves with isolate pure constituents of *A.webbiana,* to pinpoint the involvement of different chemical entities in view of its medicinal importance in folklore medicine.

Table 1: Effect of Chloroform extract of *Abies webbiana* and Lorazepam [LR] on open field exploratory behaviour in rats.[Values are mean± SD from 6 animals in each group].

Treatments mg/kg,po./ip.	Ambulation	Rearings	Self groomings	Faecal droppings
Vehicle	41.37±3.12	6.83±2.13	6.35±2.01	4.15±1.31
AW 50	54.00±4.00^{aa}	8.00± 1.41	7.50±1.04	3.00±0.63
AW 100	65.33±3.14^{aa,b}	9.66±1.03	8.83±1.94	2.33±1.36
LR 0.5	78.13± 4.33^{aa,b}	12.53±1.35$^{aa,b.c}$	10.37±3.37^{aa,b}	1.77±1.07^{aa}

Superscripts ^{abc} indicate statistical significant respectively in comparison to vehicle.

AW [100 mg/kg] and LR treatments $^{b, c \text{ and } aa, bb}$ denote p <0.05 and <0.01 respectively.

Table 2: Effect of Chloroform extract of *Abies webbiana* and Lorazepam [LR] on The elevated plus maze behaviour in rats.[Values are mean± SD from 6 animals in each group]

Treatments mg/kg,po./ip.	Time spent on (sec)		Entries on	
	Enclosed arms	Open arms	Enclosed arms	Open arms
Vehicle	178.35±7.68	78.60±9.16	7.87±1.79	2.99±1.21
AW 50	157.90±5.45^{aa}	91.65±3.96	10.16±0.98	5.16±1.17
AW 100	152.78±3.72^{aa,bb}	99.05±4.65	11.66±1.36	6.83±0.75
LR 0.5	141.30±3.78^{aa,cc}	125.65±3.62^{aa,bb}	10.01±1.56	7.48±1.66^{aa}

Superscripts ^{abc} indicate statistical significant respectively in comparison to vehicle.

AW [100 mg/kg] and LR treatments $^{aa,bb \text{ and } cc}$ denote p <0.05 and <0.01 respectively.

Table 3: Effect of Chloroform extract of *Abies webbiana* and Lorazepam [LR] on the elevated zero maze behaviour in rats.[Values are mean± SD from 6 animals in each group].

Treatments mg/kg,po./ip.	Time spent in open arms (sec)	Head dips	Stretched attend posture	Entries in open arms
Vehicle	47.81±5.28	9.07±1.64	4.07±1.62	3.97±1.77
AW 50	57.90±5.51	10.83±0.68	3.66±0.81	6.66±0.81
AW 100	61.45±5.13 ^{aa}	12.00±1.41	3.16±1.16	8.16±1.17
LR 0.5	72.56±4.21^{aa}	12.90±1.21	3.48±1.03	10.92±2.17^{aa,bb}

Superscripts ^{a} and ^{b} indicate statistical significant respectively in comparison to vehicle.

AW [100 mg/kg] and LR treatments $^{aa \text{ and } bb}$ denote <0.01.

CONCLUSION

The results of Chloroform extract of *A.webbiana* treatment caused significant dose related anxiolysis in rats tested on all the behavioral paradigms. Pure isolates of active principles need testing toward identifying neurotransmitters drug therapy for anxiolytic activity.

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