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Hypoglycemic, antistress, anxiolytic, and nootropic activity of roots of *Rubia cordifolia* Linn

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SUMMARY

The alcoholic extract of dried roots of *Rubia cordifolia* dose dependently reduced blood sugar level in alloxan treated rats. The extract also reduced ulcer index, plasma corticosterone in cold restrain stressed rats in dose related manner. The mice treated with alcoholic extract of *Rubia cordifolia* spent more time in the open arm of the elevated plus maze indicating anxiolytic activity. The extract also antagonized the amnesic effect of scopolamine in mice as indicated by reduced transfer latency in the elevated plus maze. Thus the plant bears potential for use in diabetes, stress, anxiety, and dementia.

Key words: *Rubia cordifolia;* Hypoglycemic; Anxiolytic; Antistress; Nootropic

INTRODUCTION

The *Rubia cordifolia* Linn (family: Rubiaceae) is a climbing plant growing in northwest Himalayas, and other hilly districts of India. Roots have antipyretic, analgesic, anthelmintic, anti-inflammatory activities and are also useful in leucoderma, urinary discharges, jaundice, and piles (Shastri, 1988). The methanolic extract of roots has anticancer activity (Advankar and Chitnis, 1982). Roots are used in folklore medicine for treatment of dropsy, paralysis, amenorrhoea, and visceral obstructions (Nadkarni, 1982). Tripathi and her associates (1995) have reported the lipoxygenase inhibitory activity of ethanolic extract of roots and the ethyl acetate fraction of this extract has maximum lipoxygenase inhibitory activity (Tripathi *et al.*, 1995). Roots are used in folklore medicine to cure ulcers (Mhaskar, *et al.*, 2000). The antioxidant property of alcoholic extract is due to rubiadin (Tripathi *et al.*, 1997). Ethanolic extract of the aerial parts of the plant has hypoglycemic activity (Singh and Ojha, 1991).

Most of the established nootropic plants like *Bacopa monnieri* L, *Asparagus racemosus* Linn, *Albizzia lebbeck, Panax ginseng* etc. are rich in saponins. Preliminary phytochemical work showed that roots of *R. cordifolia* also contain saponins. Diabetes patients have been shown to perform worse than non-diabetes subjects in a wide range of cognitive tests. Problems with memory and learning, problem solving, and mental and motor speed have been noted in diabetics (Mooradian *et al.*, 1988). Diabetes can be considered as a manifestation of oxidative stress. Stress alters the physiological homeostasis of the organism and elicits various endocrinal and visceral changes such as changes in plasma cortisone and gastric mucosal integrity (Ader, 1984). A wide variety of stressors, both physical and emotional, act *via* neural pathways to the hypothalamus to increase corticotrophin releasing hormone secretion (CRH) and hence adrenocorticotropic hormone (ACTH) and cortisol secretion (Vander *et al.*, 1990). In view of these observations,

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it was considered worthwhile to study the effect of alcoholic extract of roots of *R. cordifolia* on blood-glucose level, learning and memory and stress induced ulcers, and changes in plasma corticosterone.

MATERIALS AND METHODS

Extraction

The dried roots of *R. cordifolia* were purchased from commercial source. After identification by comparison with authentic sample the roots (500 g) were successively extracted with pet ether (60-80℃) (Yield, 0.96%) and alcohol (70% v/v) (Yield, 13%) using Soxhlet's extractor. Extracts were concentrated under vacuum. The alcoholic extract (AERC) was suspended in polyethylene glycol (PEG 400) and administered intraperitoneally (i.p.).

Animals

Albino mice (Swiss, 20-25 g of either sex) were housed into groups of 5-8 in standard laboratory conditions of temperature (25 ± 1℃), lighting (from 8 a.m. to 8 p.m.) with food and water *ad libitum*. All experiments were conducted during the light period of a 12/12 hours light/dark cycle. The Institutional Animal Ethical Committee approved the protocol of this study.

Drugs

The following drugs were used: alloxan (Burgoyne Burbidges, India), diazepam (Ranbaxy, India), piracetam (Uni-UCB, India), scopolamine (German Remedies, India). All drug solutions were prepared in distilled water immediately before the experiment and administered in a constant volume (5 ml/kg).

Acute toxicity

The AERC was administered in doses of 100, 200, 400 and 1000 mg/kg, i.p. to mice (n = 5) and the mortality was noted within 24 h in each group.

Motor toxicity

Mice were previously trained to remain on the rod (2.54 cm diameter) rotating at a speed of 25 rev/min for a period of 5 min. On the next day, the animals were randomly divided into groups of 5 each. The AERC (100, 200, or 400 mg/kg) or diazepam (1 mg/kg) or vehicle (5 ml/kg) was administered i.p. 30 min before the test and the time required falling off the rotating rod was noted for each animal (Dunham and Miya (1957). The animals were allowed to remain on the rod for 5 min. If the mouse does not fall in 5 min it was assigned a maximum time of 300 sec.

Assessment of blood glucose level

Blood glucose level was measured using the One Touch Glucometer (Johnson and Johnson Co. India) as described by Marks and Dawson (1965). Mice (n = 5) were treated with alloxan (200 mg/kg, s.c.) 24 h before AERC (100, 200 or 400 mg/kg) and after 60 min blood was withdrawn from retro-orbital plexus. One drop of blood was taken on glucometer strip and blood glucose level (mg/dl) was measured.

Assessment of antistress activity

Stress ulcers were induced in mice by subjecting them to cold restraint stress for 2 h at 4℃ (Ray *et al.*, 1992). Diazepam (1 mg/kg, i.p.) or AERC (25, 50 or 100 mg/kg i.p.) was administered 30 min before the animals (n = 5) were subjected to stress. Two hours after stress, the animals were sacrificed and severity of ulcers (Main and Whittle,1975), total acidity (Parmar *et al.*, 1984), and plasma corticosterone (Mattingly D. (1962) were assessed.

Assessment of anxiolytic activity

Elevated plus maze was used as described earlier by Pellow *et al.*, (1985). In brief, mice (n = 5) were placed individually in the center of elevated plus maze (25 × 5 cm, open arm and 25 × 5 × 20 cm, closed arm, elevated to the height of 70 cm) facing an enclosed arm. The time spent by mouse during

the next 5 min in open arm was noted. The mice were treated intraperitoneally with vehicle or AERC (25, 50 or 100 mg/kg) or diazepam (1 mg/kg) 30 min before the test.

Effect on learning and memory

Elevated plus maze (EPM) was used as described earlier (Kulkarni, 1992). In brief, mice (n = 5) were placed individually at the end of an open arm facing away from the central platform and the time it took to move from open arm to either of the enclosed arm (Transfer latency, TL) was recorded after 30 min, 24 h and 1 week. The mice were treated with vehicle or AERC (25, 50 or 100 mg/kg, i.p.) or piracetam (100 mg/kg, i.p.) 30 min before the test on first day. TL was expressed as retention scores after 24 h or 1 week for each mouse by calculating the inflexion ratio by the formula used previously by Jaiswal and Bhattacharya, 1992.

Inflexion ratio = $(L_1 - L_0) / L_0$

Where, L_0 = TL after 24 h or 1 week in sec. and L_1 = Initial TL in sec.

In another set of experiment, scopolamine antagonism was determined by administering scopolamine (0.3 mg/kg, i.p.) 30 min after AERC (100, 200 or 400 mg/kg, i.p.) or piracetam (100 mg/kg i.p.).

Statistical analysis

The data obtained were analyzed using One way analysis of variance (ANOVA) followed by Dunnett's test. Non-parametric data was analyzed by Kruskal - Wallis ANOVA, followed by Dunn's test. $P < 0.05$ was considered statistically significant.

RESULTS

Assessment of acute toxicity

Mortality was not observed in any group treated with AERC. However animals administered a dose of 1,000 mg/kg, i.p., were found sluggish.

Assessment of motor toxicity

Mice treated with vehicle were able to maintain equilibrium on the rotating rod for 247.25±35.58 minutes. AERC (200 mg/kg, i.p.) significantly ($P< 0.05$) decreased the fall off time to 114.5 ± 30.27 minutes. AERC (100 and 400 mg/kg, i.p.) decreased the fall off time to 206.6 ± 57.63 minutes and 224.8 ± 33.92 minutes. The decrease in fall off time could not reach statistical significance. Diazepam (1 mg/kg, i.p.) significantly ($P < 0.05$) decreased the fall of time. The observations are given in Table 1.

Assessment of blood glucose level

After 24 h alloxan (200 mg/kg, s.c.) significantly increased blood glucose level from 65.4 ± 9.53 mg/dl to 147.2 ± 8.21 mg/dl. AERC (100, 200 and 400 mg/kg, i.p.) given 24 h after alloxan decreased the blood glucose level significantly to 84.8 ± 3.58 mg/dl, 77.75 ± 3.30 mg/dl and 108.8 ± 7.83 mg/dl respectively when measured after 1 h of AERC treatment. The observations are given in Table 2.

Table 1. Effect of alcoholic extract of *R. cordifolia* on fall off time in mice using Rotarod test

Treatment (mg/kg)	Falling time (sec) (mean ± SEM)
Vehicle	247.25 ± 35.58
AERC (100)	206.6 ± 57.63
AERC (200)	114.5 ± 30.27*
AERC (400)	224.8 ± 33.92
Diazepam (1)	99.2 ± 9.11*

n = 5 , * $P < 0.05$.
The results were analyzed by ANOVA followed by Dunnett's test.

Table 2. Effect of alcoholic extract of *R. cordifolia* on blood glucose level in mice treated with alloxan

Treatment (mg/kg)	Blood glucose level (mg/dl)
Vehicle	65.40 ± 9.53
Alloxan (200,s.c.) (After 24 hrs.)	147.2 ± 8.21[@]
Alloxan (200,s.c.)+AERC (100) (After 1 hr.)	84.80 ± 3.58**
Alloxan (200,s.c.)+AERC (200) (After 1 hr.)	77.75 ± 3.30**
Alloxan (200,s.c.) +AERC (400) (After 1 hr.)	108.8 ± 7.83*

n = 5, *$P < 0.05$, ** $P < 0.01$, vs. alloxan treated group. [@]$P < 0.01$, vs. vehicle. The results were analyzed by ANOVA followed by Dunnett's test.

Table 3. Effect of alcoholic extract of *R. cordifolia* on ulcer index, acidity and plasma corticosterone concentration in rats

Treatment (mg/kg)	Ulcer index in mm (mean SEM)	Acidity (meq/l/100g)	Plasma corticosterone (mcg/100ml blood)
Rats without stress	0.4 ± 0.06	4.2 ± 0.56	1.35 ± 0.29
Rats with stress	1.34 ± 0.06[#]	26.81 ± .01[£]	9.6 ± 0.12[£]
AERC (100)	0.57 ± 0.08	5.72 ± 1.06	3.18 ± 0.42[@]
AERC (25)+stress	0.94 ± 0.01[$]	12.54 ± 0.74**	4.7 ± 0.43**
AERC (50)+stress	0.81 ± 0.09[$]	8.36 ± 1.28**	2.46 ± 0.18**
AERC (100)+stress	0.67 ± 0.05[$]	6.32 ± 0.93**	2.32 ± 0.16**
Diazepam (1)+stress	0.64 ± 0.09[$]	5.97 ± .84**	2.46 ± 0.14**

n = 5, ** $P < 0.01$, vs. stress control (Student's *t* test).
[@] $P < 0.05$, [£] $P < 0.01$, vs. without stress (Student's *t* test).
[$] $P < 0.05$, vs. stress control (Mann-Whitney U test)
[#] $P < 0.05$, vs. without stress (Mann-Whitney U test).

Assessment of antistress activity

In vehicle treated animals, cold restraint stress (CRS) significantly increased ulcer index. AERC (25, 50, and 100 mg/kg, i.p.) significantly ($P < 0.05$) decreased ulcer index in a dose-dependent manner in animals under CRS. Diazepam (1 mg/kg, i.p.) significantly decreased ulcer index in animals under CRS as compared to stress control animals. In vehicle treated animals, CRS significantly ($P < 0.01$) increased total acidity. AERC (100 mg/kg, i.p.) *per se* increased acidity insignificantly. AERC (25, 50, and 100 mg/kg, i.p.) significantly decreased acidity in a dose-dependent manner in animals under CRS. Diazepam also significantly ($P < 0.01$) decreased total acidity as compared to stress control animals. Cold restraint stress significantly ($P < 0.01$) increased plasma corticosterone level in vehicle treated animals. AERC (100 mg/kg, i.p.) *per se* significantly ($P < 0.05$) increased plasma corticosterone level. AERC (25, 50, and 100 mg/kg, i.p.) significantly ($P < 0.01$) decreased plasma corticosterone level in animals under CRS. Diazepam (1 mg/kg, i.p.) also significantly ($P < 0.01$) decreased plasma corticosterone level in animals under CRS. The observations are given in Table 3.

Table 4. Effect of alcoholic extract of *R. cordifolia* on the time spent in open arm in the elevated plus maze in mice

Treatment (mg/kg)	Time spent in open arm (sec) (mean SEM)
Vehicle	77.00 ± 6.02
AERC (25)	53.75 ± 2.78
AERC (50)	94.00 ± 7.70
AERC (100)	102.00 ± 2.01*
Diazepam (0.5)	87.40 ± 7.53
Diazepam (1.0)	112.20 ± 6.38*

n = 5, * $P < 0.05$

The results were analyzed by ANOVA followed by Dunnett's test.

Assessment of anxiolytic activity

Vehicle treated mice spent 77.0 ± 6.02 sec (out of 300 sec) in the open arm. The changes produced by AERC 25 and 50 mg/kg, i.p. were not insignificant. However AERC (100 mg/kg, i.p.) significantly increased time spent in open arm. Diazepam (0.5 mg/kg, i.p.) though increased time spent in open arm, the effect could not reach statistical significance. Diazepam (1 mg/kg, i.p.) significantly increased time spent in open arm. The observations are given in Table 4.

Effect on learning and memory

In vehicle treated group, transfer latency decreased significantly from 32.33 ± 1.85 sec to 21.66 ± 2.18 sec and 14.66 ± 0.88 sec on day 2 and day 9 respectively. AERC (25 mg/kg) decreased transfer latency by 2.78 percent on day 2 while it was increased by 152.77% on day 9. AERC (50 mg/kg) decreased transfer latency by 11.77% and 30.75% on day 2 and day 9 respectively. AERC (100 mg/kg, i.p.) decreased transfer latency by 24.33 and 63.09 percent on day 2 and day 9 respectively. Inflexion ratio was significantly ($P < 0.05$) increased on day 9 as compared to vehicle. Piracetam (100 mg/kg) decreased transfer latency significantly ($P < 0.05$) by 40.06 and 67.49 percent on day 2 and day 9 respectively. It significantly ($P < 0.05$) increased inflexion ratio on day 9. Scopolamine (0.3 mg/kg) increased transfer latency by 146.88% on day 2 and decreased transfer latency by 15.97% on day 9. Scopolamine decreased inflexion ratio significantly ($P < 0.01$) on day 2 and day 9. AERC (100 mg/kg), when given in combination with scopolamine (0.3 mg/kg), significantly ($P < 0.01$) decreased transfer latency by 36.44 and 19.87% on day 2 and day 9 respectively. AERC significantly increased inflexion ratio on day 2 and day 9. AERC (200 mg/kg, i.p.) significantly ($P < 0.01$) decreased transfer latency by 24.04% and 14.83% on day 2 and day 9 respectively. It significantly increased inflexion ratio on day 2 and day 9.

AERC (400 mg/kg) when given in combination with scopolamine (0.3 mg/kg) significantly ($P < 0.05$) decreased transfer latency by 46.81% and 18.92% on day 2 and day 9 respectively. It significantly increased inflexion ratio on day 2 and day 9 respectively. The observations are given Table 5.

DISCUSSION

Stress is known to affect the central nervous system (CNS) to various extents. It has been shown that

Table 5. Effect of alcoholic extract of R. cordifolia on learning and memory in mice using elevated plus maze

Treatment (mg/kg)	Transfer latency in sec. (mean SEM)			Inflexion ratio (mean SEM)	
	Day 1	Day 2	Day 9	Day 2	Day 9
Vehicle	32.33 ± 1.85	21.66 ± 2.18*	14.66 ± 0.88**	0.50 ± 0.06	1.21 ± 0.14
AERC (25)	18.00 ± 0.76	17.5 ± 0.53	27.25 ± 0.87	0.77 ± 0.14	0.33 ± 0.21
AERC (50)	25.50 ± 0.71	22.5 ± 0.70	17.66 ± 0.68	0.15 ± 0.11	0.44 ± 0.08
AERC (100)	37.00 ± 1.05	28 ± 0.53	13.66 ± 0.97	0.35 ± 0.09	2.18 ± 0.29#
Piracetam (100)	20.33 ± 1.05	12.16 ± 0.47**	6.6 ± 0.50**	0.71 ± 0.08	2.19 ± 0.28#
Scop.(0.3)	83.6 ± 4.55	122.80 ± 13.55	70.25 ± 2.68	-0.29 ± 0.05#	0.24 ± 0.05#
AERC (100)+ Scop. (0.3)	66.4 ± 6.32	24.2 ± 2.45**	13.2 ± 1.15**	1.81 ± 0.28£	4.46 ± 0.94@
AERC (200)+ Scop. (0.3)	59 ± 8.31	14.2 ± 2.15**	8.75 ± 0.85**	3.57 ± 0.82@	5.72 ± 1.26£
AERC (400)+ Scop. (0.3)	50.2 ± 5.17	23.5 ± 3.79*	9.5 ± 1.02*	1.20 ± 0.15@	4.72 ± 1.36@

n = 5, * $P < 0.05$, ** $P < 0.01$ vs. Day 1. # $P < 0.05$ vs. vehicle. £ $P < 0.05$ vs scopolamine, @ $P < 0.01$ vs. scopolamine

The results were analyzed by ANOVA followed by Dunnett's test.

stress induces physical (Hurst *et al.*, 1976) and mental disorders Cooke and Hole (1983). Stress activates all the systems that raise plasma glucose concentration, which explains why stress exacerbates the symptoms of diabetes (Vander *et al.*, 1990). AERC significantly decreased the blood glucose level elevated by alloxan. The U-shaped dose-effect curve obtained may be because of increased concentration of glucocorticoids in the blood as AERC increases plasma corticosteroid level, which results in increased blood glucose level (Vander *et al.*, 1990). Alloxan destroys the pancreatic β-cells (Kershbaum *et al.*, 1968) and raise blood glucose concentration. AERC lowered blood sugar levels in alloxanized mice, an indication that the extract has expancreatic effects. The biologically active constituent(s) has neither been known nor did the exact mode of action of the hypoglycemic effect determined; nonetheless, this observation is consistent with the use of *R. cordifolia* in folklore diabetes management.

There seems to be an increased release of stress hormones (glucocorticoids) from the adrenals in humans or animals exposed to stress (Kershbaum *et al.*, 1968; Hill and Wynder, 1974; Balfour, 1980; Cam and Bassett, 1983; Balfour *et al.*, 1986; Wilkins *et al.*, 1987). The results emanated in the present study indicated that AERC possessed significant antistress activity. AERC decreased plasma corticosterone levels. Diabetes can be considered as a manifestation of oxidative stress. An increased prevalence of psychiatric disorders, in particular depression and anxiety disorders, has been reported in both insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM)(Lustman *et al.*, 1986). Depression may result from an inability to cope with stress of diabetes, but may also result from direct effects of

diabetes on the CNS or a combination of both (Lustman *et al.*, 1992). AERC (50 and 100 mg/kg) showed increase in time spent in open arm. AERC significantly increased time spent in open arm. These observations are in congruent with those described by Pellow *et al.* (1985).

The animals made amnesic with scopolamine were also used for the study. Lenegre *et al.*, (1988) used scopolamine, diazepam and electroconvulsive shock to produce amnesia in mice. In the present study we used scopolamine (0.3 mg/kg, i.p.) to produce memory impairment. Amnesic effect associated with scopolamine resulted in significant deterioration of learning and memory in mice in elevated plus maze model. AERC prevented the scopolamine-induced amnesia in elevated plus maze model. Further studies will elucidate the mode of nootropic activity of *Rubia cordifolia*.Thus, it is concluded that the alcoholic extract of *Rubia cordifolia* is safe till 1,000 mg/kg, i.p. and it possessed hypoglycemic, antistress, anxiolytic, and nootropic activity. AERC bears potential for further studies.

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