International Journal of Research Studies in Biosciences (IJRSB)
Volume 4, Issue 2, February 2016, PP 7-12
ISSN 2349-0357 (Print) & ISSN 2349-0365 (Online)
http://dx.doi.org/10.20431/2349-0365.0402002
www.arcjournals.org

Acute - Toxicity, Anti-Inflammatory and Anti-Diarrhoeal Activity of *Ailanthus excelsa* in Mice and Rats

Dr. R. K. Singh

Associate-Professor of Zoology, Faculty of Life –Sciences,
Dr. C.V. Raman University, Kargi -road, Kota, Bilaspur, Chhattisgarh, India
rk20singh@gmail.com

Abstract: *Ailanthus excelsa (Stem bark) are used in folk medicine for a treatment of inflammation, arthritis, expectorant, stimulant and asthma. Acute toxicity study of A. excelsa stem bark was performed on mice (Swiss) and albino rats (Wistar strain),given single doses 1,10, 50, 100 ,1000 and 2000mg/kg,po. showed normal behavior and no mortality up to 14 days except 500,1000 and 2000mg/kg dose 20%, 60.0 % &100% mortality recorded respectively. A. excelsa (10- 50x7days or 10,50 and100mg/kg, po single dose), pretreatment time , 60 min showed significant sub-acute anti-inflammatory protection against Cotton pellet induced granuloma formation, no analgesic activity, pentobarbitone induced sleeping time reduced insignificantly, decreased swim stress immobility in mice indicating some degree of antidepressant activity. A. excelsa exhibited significant inhibitory activity against castor oil –induced diarrhoea and significant reduction in gastrointestinal motility in the activated charcoal meal in rats. The steroids, glycosides , alkaloids, and flavonoids present in the plant appear to be of chemotherapeutic interest.*

Keywords: *Ailanthus excelsa, Acute- toxicity, Anti-inflammatory, Diarrhoea, Swim– stress immobility, Motility*

1. INTRODUCTION

Ailanthus excelsa Roxb *(* Family: Simaroubaceae*)* is commonly known as *Aralu,* which is found in various parts of India . *Ailanthus* species is worldwide distributed from India, Sri Lanka, Japan, China, Australia and Sudan. In relation to India it grows in Gujrat , Rajasthan, Bihar, Orrisa, M.P. and Maharastra, Karnataka and Tamilnadu **[1]**. *A. excelsa* Roxb stem bark have employed as a folk medicine remedy for inflammation and rheumatoid **[2,3]** antipyretic, antifertility, antifungal, anti-malarial and antibacterial activities **[4,5]**. *A. excelsa* has a role in treatment of diabetes **[4]**. Ethanolic extracts of *A. excelsa* reported phytotoxic, cytotoxic and insecticidal activities **[4]**. *A. excelsa* root bark showed antioxidant activity,anti-cancer activity **[5,6]** Three flavonoids and two sitosterols were isolated from leaves and stem bark of *A. excelsa* **[7]**.This genus was found to contain flavonoids ,quercetin , alkaloids, anthraquinones **[8]** , oils, steroids and glycosides **[9]**. Species of *A. excelsa* from leaves isolated flavonoids with anti-oxidant properties, **[9]**.

The present investigation was conducted to study in detail *A. excelsa* (Stem bark) 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, NRIADD, Kolkata on inbred Albino mice (Swiss) 15-20g and Albino rats (Wistar Strain)100-200g .They were kept in the departmental animal house in individual cages at an ambient temperature of 26 ± 3^0C and 60- 70% relative humidity with 12h:12h light: dark cycles. They had fee access to standard rodent pellet diet (NIN, Hyderabad) and drinking water (Kinley) during the entire study period. The food was withdrawn 18h prior to experiment /surgical procedure, however, water was allowed *ad libitum.*

2.2.1. Plant Material

The *Ailanthus excelsa* (Stem bark) was obtained from the Regional Research Centre (Ay) Patna,Bihar,India and identified in the Department of Pharmacognosy, NRIADD, Kolkata, peripheral

Institute of Central Council for Research in Ayurvedic Sciences, Govt.of India. 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 (500g) *A. excelsa* were extracted by ethanol, and concentrated in a steam bath to a final yield of 80.0 g (16.0% w/w). Chemical tests showed the presence of glycosides, steroids, alkaloids and flavonoids.

2.3.1. Acute-Toxicity Studies on Mice

Albino mice (Swiss: 2M+3F=5) weiging 15-20g were given graded doses of *A. excelsa* at the dose level of 1,10, 50, 100 , 200,500,1000 and 2000mg/kg,po and with Control. These animals were fasted 18 h prior to the experimentation. Both the test and control groups were received in a same volume of drug or vehicle control as per body weight. Experiments were conducted as per OECD guidelines-423(Acute-Oral Toxicity-Single Dose) **[10].** The animals were kept in observation for 96h upto 14 days for any gross behavioral changes and mortality. The animals were observed for symptoms ie writhing pilo-erection, salivation fur, lacrimation, convulsion, hyperreactivity etc continuously for the first 4h after dosing. The numbers of survival were noted after 24h.These animals were then maintained and observed daily for 14 days for further any toxicity. Complete postmortem was done on all survivors or if any animal found dead or moribund condition during the study period. Histopathological examination was performed on all collected tissues of individual animals.

2.3.2. Acute-Toxicity Studies on Rats

Albino rats (Wistar strain: 2M+3F=5) weiging 100-120g were given graded doses of *A. excelsa* at the dose level of 1, 10,50, 100,200,500, 1000 and 2000mg/kg,po and with Control. These animals were fasted 18 h prior to the experimentation. Experiments were conducted as per OECD guidelines-423(Acute-Oral Toxicity-Single Dose) **[10]** . The animals were kept in observation for 96 h upto 14 days for any gross behavioral changes and mortality.

2.4. Anti –Inflammatory Activity (Cotton Pellet Granuloma Pouch)

Albino rats (Wistar Strain) weighing 150-200g were divided into 4 groups (N=6).Group I received double distilled water DDW(Control) , Group II received Diclofenac sodium (13.5mg/kg,po) served as Standard Control and Group III and IV was given *A. excelsa* at the dose level of 10 & 50mg/kg, po X 7days.Cotton pellet was weighed 20mg sterilized and in a hot air oven at 120^0C for 2h, then implanted bilaterally in region of rat under light ether anesthesia and stitched properly **[11]** . At the end of drug treatment cotton pellet were taken out by dissection, placed in petri dish and placed in 70^0C oven for over- night and weight after cooling. Increase in the dry weight of the pellets was taken as measure of granuloma formation and compared with Control and Standard Control. Cotton pellet induced granuloma the average weight of the pellets of the Control group and Standard control as well as of the test group was calculated. The percent change of granuloma weight relative to control group was determined.

2.5. CNS Activity

2.5.1. Analgesic Activity (Writhing-Test)

The male mice (N=6 in each group) were pretreated with *A. excelsa* at the dose level (10,50 & 100mg/kg,po) and Control group received (DDW) 60 min prior to the experiments. The writhing induced by freshly prepared 0.6% Acetic acid (ip) in mice within 3-10 min. The number of writhing of the abdominal musculature and extension of the hind limbs were recorded for 10 min **[12]**.

2.5.2. Swim Stress Immobility in Mice

The mice (N=5 in each group) pretreated with *A. excelsa* at the dose level (10,50 and 100mg/kg,po) and Control group received (DDW) 60 min prior to the experiments. Mice were made to swim in a 7x8x24 inch Perspex cage filled with water at 30^0C for 15 min **[13]**. The immobility phase of each mouse was recorded and compared with that of the control.

2.5.3. Pentobarbitone (PB) hypnosis

The male rat (N=6 in each group) were pretreated with *A. excelsa* at the dose level (10,50 and100 mg/kg,po) and Control group received (DDW) 60 min prior to the experiments. Each animal was

injected with pentobarbitone sodium (35mg/kg,ip) to study any significant change in sleeping time by *A.excelsa* **[14].** The experiments were conducted at an ambient temperature of $20^0 \pm 2^0$ C.

2.6. Castor Oil -Induced Diarrhoea in Rats

Albino rats (Wistar Strain) weighing 100-120g were divided into 5 groups (N=3M+3F in each group).Group I received double distilled water DDW(Control) , Group II received Diphenoxylate (5mg/kg,po) served as Standard Control and Group III, IV and V was given *A. excelsa* at the dose level of 10, 50 and 100mg/kg, po. were fasted for 18h prior to the experiment. One hour after treatment, each group of animals received 1ml of castor oil po and then defection was observed up to 4h. The presence of characteristic diarrhoeal droppings of individual animal was recorded **[15,16,17].**

2.7. Gastrointestinal Motility in Rats

Albino rats (Wistar Strain) weighing 100-120g were divided into 5 groups (N=3M+3F in each group).Group I received double distilled water DDW(Control) , Group II received atropine sulphate (0.1 mg/kg,ip) served as Standard Control and Group III, IV and V was given *A. excelsa* at the dose level of 10, 50 and 100mg/kg, po. were fasted for 18h prior to the experiment. Each animal was administered with 1ml of charcoal meal po (3% deactivated charcoal in DDW). After 30 min each animal was sacrificed and the intestinal distance moved by the charcoal meal from the pylorus was cut and measured and expressed as a % of the distance from pylorus to the caecum **[18].**

2.8. Statistical Analysis

All the data was analyzed by student's t-test followed by ANOVA.

3. RESULTS AND DISCUSSION

3.1. Acute-Toxicity Studies on Mice

All animals treated with different doses of *A. excelsa* showed normal behavior and No mortality was recorded up to 14 days, except 500,1000 and 2000mg/kg dose 20%, 60.0 %&100% mortality recorded respectively. After postmortem, histopathological examination was performed, actual route cause of mortality is higher exposure of dose.

3.2. Acute-Toxicity Studies on Rats

All animals treated with different doses of *A. excelsa* showed normal behavior and No mortality recorded up to 14 days , except 500, 1000 and 2000mg/kg dose 20%,40.0% and 60.0% mortality recorded respectively. After postmortem, histopathological examination was performed, actual route cause of mortality is higher exposure of dose.

3.3. Anti –Inflammatory Activity (Cotton Pellet Granuloma Pouch)

The results are summarized in Table 1. *A. excelsa* doses 10 and 50mg/kg significantly inhibited and compared with control and standard drug diclofenac sodium.

3.4. CNS Activity

3.4.1. *Analgesic Activity (Writhing-Test)*

The results are summarized in Table 2. All the doses 10, 50 and 100mg/kg showed insignificant writhing against 0.6% Acetic acid (ip) (No analgesic activity).

3.4.2. Swim- Stress Immobility in Mice

The results are summarized in Table 3. All the doses 10, 50 and 100mg/kg showed a tendency to decrease immobility significantly.

3.4.3. Pentobarbitone (PB) Hypnosis

The results are summarized in Table 4. All the doses 10, 50 and 100mg/kg showed insignificant decrease sleeping time.

3.5. Castor Oil -Induced Diarrhoea in Rats

The results are summarized in Table 5. All the doses of *A. excelsa* exhibited dose-dependent significant anti-diarrhoeal activity.

3.6. Gastrointestinal Motility in Rats

The results are summarized in Table 6. . All the doses of *A. excelsa* exhibited significantly the population of charcoal meal in rats. *A. excelsa*, apart from diverse uses in folk medicine, has recently

been shown to possess anti- inflammatory, analgesic and antioxidant properties. The acute toxicity studies indicate that *A. excelsa* have a significant margin of safety in mice and rats. The present study on sub- acute anti- inflammatory activity induced by cotton pellet granuloma pouch showed significant inhibition and compare with diclofenac sodium. The Porsolt swim stress immobility model is widely used to screen anti- depressant activity .The present finding indicate that decreasing swim stress immobility may reflect the presence of antidepressant activity **[13]**, further investigation is required to reach a definite conclusion. The data of the present study indicate that *A. excelsa* protects rat against anti-diarrhoeal activity and gastrointestinal motility .Therfore , the finding suggests a protective role of *A. excelsa* in anti-inflammatory activity [**2,3**] anti-diarrhoeal activity and intestinal motility [**19,20**]. This finding is justify the traditional use for the treatment of arthritis and management of diarrhoea.

Table1. *Anti-inflammatory effects of A. excelsa by Cotton Pellet- induced Granuloma pouch Weight. Values are mean± SE Weight (mg. Figures in parentheses indicate number of animals used.*

Treatment (mg/kg ,po)	N	Weight of dry Cotton Pellet Granuloma	% inhibition
Control (DDW)	6	26.31 ± 0.02	-
DICLOFENAC SODIUM 13.5	6	11.24 ± 0. 06[a]	57.27[b]
A. excelsa 10	6	18.13± 0.03[a]	31.09[b]
50	6	10.64 ± 0.01[a]	59.56[b]

[a]*p <0.001 in respect to control,* [b]*p < 0.001 in respect to Standard Control*

Table2. *Analgesic effects of A. excelsa by Writhing test induced by 0.6% Acetic acid in albino mice. Values are mean ± SE% writhing. Figures in parentheses indicate number of animals used.*

Treatment mg/kg,po	N	% Writhing
Control (DDW)	6	32.16± 1.61
A. excelsa 10	6	35.51± 4.87[NS]
50	6	41.32± 7.51[NS]
100	6	44.27±8.64[NS]

NS= Not significant in respect to control.

Table3. *Effect of A. excelsa on Swim stress (900s duration) in albino mice. Values are mean ± SE immobile phase (s). Figures in parentheses indicate number of animals used.*

Treatment mg/kg,po	N	Swim stress immobile phase (s)
Control (DDW)	5	327.16± 32.13
A. excelsa 10	5	180.49± 26.07[a]
50	5	147.21± 23.18[a]
100	5	139.33± 20.15[a]

[a]*p<0.001 in respect to control.*

Table4. *Effect of A. excelsa on Pentobarbitone - induced sleeping time in male albino rat. Values are mean ± SE sleeping time (min). Figures in parentheses indicate number of animals used.*

Treatment mg/kg,po	N	Pentobarbitone- induced sleeping time (min)
Control (DDW)	5	114.75± 17.32
A. excelsa 10	5	116.80± 27.08[NS]
50	5	89.60± 17.04[NS]
100	5	121.50± 19.53[NS]

NS= Not significant in respect to control.

Table5. *Effect of A. excelsa on Castor oil- induced diarrhoea in rats. Values are mean ± SE defecation. Figures in parentheses indicate number of animals used.*

Treatment mg/kg,po	N	Castor oil- induced defecation
Control (DDW)	6	6.10 ± 0.53
Diphenoxylate 5	6	1.42 ± 0. 45[a]
A. excelsa 10	6	3.07± 0.67[a]
50	6	2.23 ± 0.56[a]
100	6	1.89 ± 0.31[a]

[a]*p<0.01 in respect to control.*

Table6. *Effect of A. excelsa on gastrointestinal motility in rats. Values are mean ± SE % movement of charcoal meal. Figures in parentheses indicate number of animals used.*

Treatment mg/kg,po	N	% Movement of Charcoal meal
Control (DDW)	6	90.25± 2.27
Atropine sulphate (0.1mg/kg,ip)	6	47.16± 2.03[a]
A. excelsa 10	6	70.89± 2.19[a]
50	6	49.54±1.89[a]
100	6	39.67±1.98[a]

[a]*p <0.001 in respect to control.*

4. CONCLUSION

Acute toxicity of *A. excelsa* has safe up to the doses of 200 mg/kg and caused no mortality and normal behavior. The results of the present study reveal that significant anti- inflammatory activity, swim stress immobility anti- diarrhoeal activity and gastrointestinal motility. *A. excelsa* is rich in glycoside, alkaloids and flavanoids. Pure isolates of active principles need testing toward identifying steroids, glycosides, alkaloids and flavonoids present in the plant appear to be therapeutic interest for chronic arthritis treatment and management of diarrhoea.

ACKNOWLEDGEMENTS

The work was financed by Central Council for Research in Ayurvedic Sciences, Department of AYUSH, Ministry of Health & Family Welfare, New-Delhi, Government of India.

REFERENCES

[1] Chopra,R.N. Chopra,I.C,HandaK.L. ,Kapur,L.D. Chopra's Indigenous Drugs of India,2nd Edn,UN Dhar and Sons,Pvt. Ltd, Calcutta,India .p408 (1958)

[2] Kirtikar,K.R. , Basu,B.D. Indian Medicinal Plants .Vol I ,International Book Distributors, Dehradun, India, p 371-372 (1995)

[3] Kirtikar,K.R., Basu,B.D. Indian Medicinal Plants .2nd Edn , Mohan Basu, Publisher, Allahabad,India (2003)

[4] Lavhale, M.S., Mishra, S.H. Nutritional and therapeutic potential of *Ailanthus excelsa:* A review.Pharmcognosy Rev., 1,105-113. (2007).

[5] Lavhale, M.S., Kumar,S., Mishra ,S.H.,Sitasawad ,S.L. A novel triterpenoid isolated from the root bark of *Ailanthus excelsa* Roxb (Tree of Heaven).AECHL-1 as a potential anti cancer agent PLoS One,4:e5365-E5365.(2009)

[6] Kumar ,D.,Bhat ,Z.A.,Singh,P., Shah,M.Y., Bhujbal,S.S. *Ailanthus excelsa* Roxb ,is Really a Plant of Heaven .Int. J. Pharmacol.6 (5),535-550 ISSN1811-7775 (2010).

[7] Khan,S.A., Shamsuddin, K.M. Quassinoids from *Ailanthus excelsa* .Ind.J.Chem,16B,1045-1046 (1978).

[8] Khan,S.A., Shamsuddin, K.M.Isolation and structure of13,18-dehydroexcelsin,aquassinoid and glaucarubol from *Ailanthus excelsa* .. Phytochemistry 19,2484- 2485.(1980).

[9] Yoganandam,G.P.,Periyanayagam,K., IIango, K.Protective effect of ethanolic extract of stem bark of *Ailanthus excelsa* Roxb against CCL_4 induced hepatotoxicity in rats .Drug Invention Today,1,28-31(2009).

[10] M. J. Derelanko and M.A. Hollingeer, Hand Book of Toxicology ,2nd Edn Chapter "Acute , Subchronic and Chronic toxicology eds. Auletta C.S., p. 69, (2002) (CRC Press, New York, Washigaton DC).(2002)

[11] Meir ,R.,Schuler,W. and Desaulles,P., Experientia,6 ,469.(1950).

[12] Fukawa, K., Kawano,O., Hibi,M,, Misaki, N.,Ohba, S.and Hatanaka, T.,J.Pharmacol.Meth.4,251. (1980).

[13] Porsolt, R.D., Bertin A,Blavet, N.Daniel, M. and Jalfare, M.,Immobility induced by forced swimming in rats.Effects of agents which modify central catecholamine and serotonin activity.Eur.J. Pharmacol.57, 201-210.(1979).

[14] Ikeda,H.,Take,Y.,Itoh,Y.,Nagawa,Y. Jap. J. Pharmacol, 23, 77. (1973).

[15] Valette,G.,Salvant,R. Les causes de Paction de l'huile de ricin.Bull Sci. Pharmacol. 43, 1-11(1936)

[16] Gaginella,T.S. Haddad, A.C.,GO,V.L.W.,Phillips,S.F.. Cytotoxicity of ricinoleic acid (Castor-oil) and other intestinal secretagogues on isolated intestinal epithelial cells. J. Pharmacol.Exp.Ther., 201,259-266.(1977).

[17] Mascolo,N, Izzo,A.A.,Autore, G. ,Barbato,F., Capasso,F. Nitric oxide and Casor oil-induced diarrhea. J. Pharmacol.Exp.Ther.268, 291-295. (1994).

[18] Macht,D.I.,Barbara-Gose,J. J.Am.. Pharm. Asso., 20,558. (1931).

[19] Mascolo,N, Izzo,A.A., Capasso, F. Castor oil induced diarrhea:involvement of nitric oxide. In Natural Drugs and the Digestive Tract, ed Capasso,F., Mascolo,N.p121-128. Rome: EMSI. (1992).

[20] Capasso, F., Mascolo,N. Izzo,A.A., Gaginella,T.S. Dissociation of castor oil-induced diarrhea and intestinal mucosal injury in rat: effect of N^G-nitro-L-arginine methyl ester. Br. J. Pharmacol. 113, 1127-1130. (1994).

AUTHOR'S BIOGRAPHY

Dr. R. K. Singh, M.Sc. Ph.D. [Zoology - Pharmacology] from Dept. of Pharmacology, Institute of Medical Sciences, Banaras Hindu University of Varanasi, India. He has previously worked as a Senior Research Scientist at Department of Pharmacology, National Research Institute of Ayurvedic Drug Development, CCRAS, Department of AYUSH, Ministry of Health & Family Welfare, New-Delhi, and Government of India. Presently working as Associate - Professor of Zoology, faculty of Life –Sciences, Dr. C.V. Raman University, Kota, Bilaspur (C.G.). He has published sixty seven papers in international and national journals on Ethno pharmacology, Cardio-Vascular Pharmacology, Neuro psycho pharmacology and Toxicology.