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Acute-Toxicity and in Vitro Rat Mast Cell Studies on Terephthalic Acid Dimethyl Ester(TADE)from Abies pindrow leaves

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Abstract: *Monoterpenoid, terephthali cacid dimethyl ester (TADE) isolated from Abies pindrow leaves inhibited spontaneous as well as Compound 48/80 challenge mast cell on in vitro (0.5-5.0 mg/ml) direct treatment. Acute toxicity study of TADEwas performed in mice (Swiss)given single dose 1.0, 2.5 ,5.0 and 10.0 mg/kg, po showed normal behavior and no mortality upto 14 days.In agreement with the traditional remedially use in respiratory ailments.*

Keywords: *Abies pindrow: Acute- toxicity :Terephthalic acid dimethyl ester (TADE): Mast Cell degranulation*

1. INTRODUCTION

AbiespindrowSpach (Pinaceae) leaves, described as "talisapatra" tree in Sanskrit and popularly called "morinda" in Hindi, is found in the deciduous forests of Himalayas [1].Leaves have been used as Ayurvedic remedy for fever, respiratory and inflammatory ailments [2].Anti-inflammatory,analgesic, hypnotic and anti-ulcerogenic activities in rats; hypotension in dogs and endurance enhancing in swim stress tests in mice have been observed with extracts and fractions of theAbies pindrow leaves [3].Terephthalic acid dimethyl ester (TADE), a leaf-isolate exhibits anti-inflammatory activity and inhibition of histamine induced bronchospasm in experimental studies [4].The compound isolated from the leaves of A. pindrow include flavonoids[5,6] lactones[7] and terephthalic acid dimethyl ester (TADE) [8].

The present investigation was conducted to study acute toxicity and in vitro rat mesenteric mast cell in view of its medicinal importance in folklore medicine .

2. MATERIALS AND METHODS

2.1. Plant Material

A. pindrowSpach (Pinaceae)leaves, were harvested from Kumaon hills, Himalayas and specimen authenticated at the Regional Research Centre for Ayurveda, Jammu-Tawi, Jammu & Kashmir, India ,with deposit of voucher specimens.

2.2. Extraction

The dried leaves powder (500 g)A. pindrow were extracted with ethanol by soxhlet extractor.The compound was isolated by silica gel column chromatograph of extract of the leaves of A. pindrow.The final yield of TADE was 40 mg (0.008%).

2.3. Ethical Clearance

All the experiments were conducted following the CPCSEA after the approval of Institutional Animal Ethics Committee, Institute of Medical Sciences ,Banaras Hindu University,Varanasi,India.

2.4. Animals

Albino mice (Swiss) 20-25g and Albino-rats (Wistar)100-150 g of either sex, were obtained from the Central Animal House ofIMS, BHU, Varanasi. They were housed in colony cages and fed standard Hind Lever pellet chow and kept at an ambient room temperature of $25^{o}\pm 2^{o}C$ and relative humidity 45-55% with 12 h light/12 h dark cycle.

2.5. Acute –Toxicity studies on Mice

Albino mice (Swiss: 3 M) weighing 20-25 g were given graded doses of TADE 1.0, 2.5 ,5.0 and 10.0 mg/kg, po and 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) [9] .The animals were kept in observation for 96 h 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 number 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.

2.6. Effect of TADE on In-vitro mesenteric mast cell of rats

Wistar rats were sacrificed by cervical dislocation. The abdomen was opened and mesentery of the jejunam and ileam were carefully exposed.The mesentery along with small pieces of jejunam or ileam were removed andplaced in a petri dish containing oxygenated Ringer Locke`s solution (NaCl 9.0, KCl 0.42, $CaCl_2$ 0.24, $NaHCO_3$ 0.5 and glucose 1.0 g/L of double distilled water ph 7.4) at $37.0^o \pm 0.5^oC$.Tissue transferred to different dose (0.5, 1.0 2.5 and5.0 mg/ml) for 30 min and then challenged by Comp 48/80(2.5 µg/ml) for 10 min. The tissue was then stained 0.1% Toludine blue in 4% Formaldelyde in saline for 15-20 min[10]. The tissue was next transferred and kept in acetone (two changes) and then mounted on slides. Before mounting, excess pieces of fats were trimmed and the mesentery was stretched from the edges with the help of a needle.

Each cell was considered either disrupted or not disrupted. The term disrupted was selected instead of fragmented because granules were found around many cells which did not appear to be in fragments. The sole criterion for calling a cell disrupted was the presence of granules outside the cell. Many cell did not show extrusion of granules but appear swollen at low concentration of Comp. 48/80. For each dose concentration 100 to150 mast cell were examined and average percentage of disruption was calculated.

2.7. Data analysis

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

3. RESULTS

3.1. Acute –Toxicity studies on Mice

All animals treated with different doses of TADE showed normal behavior and No mortality was recorded up to 14 days. Individual groups of animals were sacrificed under CO_2 following the animal ethical guidelines. After postmortem, histopathological examination was performed.Organs viz.liver, kidney ,heart, lung, spleen ,ovaries and testis were examined. The histological report of TADE treated group of animal tissues was compared with control.All vital organs showed normal architectures and no specific pathological changes have been detected.

3.2. Effect of TADE on In-vitro mesenteric mast cell of rats

TADE pretreatment with three doses reduced mast cell degranulation significantly(Table 1).

Table1. *Effect of TADE (0.5, 1.0 and 2.5 and 5.0 mg/ml) and Comp.48/80(2.5µg/ml) induced histamine release from the rat mesenteric mast cells.*

Treatment (mg/ml+2.5 µg/ml *Comp. 48/80*)	% *degranulation*	%inhibition
CONTROL	17.67± 0.13	-
Comp. 48/80	91.02± 0.26*	-
TADE 0.5	8.49± 1.16*	-
1.0	9.21± 1.27*	-
2.5	10.82± 2.06*	-
5.0	11.63± 2.90	-
0.5 TADE + *Comp. 48/80*	35.27± 1.97*	**61.25****
1.0 TADE + Comp. 48/80	41.67± 1.23*	**54.26****
2.5 TADE + Comp. 48/80	45.67± 1.03*	**49.82****
5.0 TADE + *Comp. 48/80*	53.12± 2.07*	**41.64****

[a]*Values are **mean± SE (N=6)** *P<0.001 Vs Control, **P<0.001 Vs Comp.48/80; Student's t test.*

4. CONCLUSION

Acute toxicity of terephthalic acid dimethyl ester (*TADE*) from *A. pindrow* leaves has safe upto the doses of 10mg/kgand caused no mortality and normal behavior.Inhibition of mediator release from rat

mesenteric mast cells by direct pre- treatment with *TADE*, in addition to earlier demonstrated protective systemic effect against histamine induced bronchospasm in Guinea –pigs provide mechanistic basis for traditional use of *A. pindrow* leaves in respiratory ailments.

The present study thus suggests possible use of *TADE* inbronchial asthma in light of present finding and earlier reported safety margin[4].

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