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Toxicity assessment of single dose of leaf aqueous extract of *Angelica archangelica* L.

Pooja Sharma and Era Upadhyay*

Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur-3030022 (India)
*era.upadhyay@gmail.com; 91-9772815346

Abstract

Angelica archangelica L. is an extreme valued medicinal herb, cultivated in Norden, and exported to different parts of Europian countries. Treditionally, it is used as a vitalizing bronchial tonic. It is also used for digestion problem, basic debility, and persistent bronchitis. The fresh crushed leaves of the plant are applicable in poultices in lung and chronic chest diseases. It is basically a potent therapeutic drug so every part of this plant is a matter of concern. Thus, our aim is to establish safety of useful parts of this plant. We have assessed the oral acute toxicity as per OECD guideline 423 for 2000 mg kg^{-1} dose and observed behavioural, haematological and biochemical parameters on 14^{th} days. After administration of single 2000 mg kg^{-1} dose of leaf aqueous extract of *Angelica archangelica* to albino wistar rat, not a single adverse symptom noticed related to behavioural, haematological and biochemical parameters. It is concluded that the dose of leaf aqueous extract of *Angelica archangelica* was found safe up to 2000 mg kg^{-1} in single dose in albino wistar rat.

Ancient medical system recognized the significance of herbs specially in developing countries where majority of the people depend on herbal medicines. They rely on natural herbs to cope up with their routine health requirements[12]. Though different allopethic and contemporary medicines are existing now, but herbal medical system has become the reality of life for its antique, ethnic and economic reasons. Currently, the herbal medicines are being used at large scale, but on the other hande their quality, safety, and efficacy is the major concern[13]. These concerns towards the herbal medicines are need to evaluate scientifically and screen various long-established arguments to provide a scientifically proven health system before using traditional medications[2]. In present scenario, both public and healthcare specialists covet for rationalized, alterative data, toward the safety and effectiveness with least toxicity effect of any advocated medicinal plant as a medicine, before its use[8].

The *Angelica archangelica* L. plant belongs to Umbelliferae, Apiaceae family[3] having rich medicinal values since decades. *Angelica archangelica* is cultivated in Nordic nations and used and transported as medicinal

herbs to different European countries[7]. This is a towering perennial herb having compact hollow trunk, huge bipinnate leaves, and green-white blossoms umbels. It is also found in some Himalayan regions of India at altitudes 3200-4200 meter such as Kashmir, Chamba, Kullu, Lahaul, and Kinnaur[5,10]. *Angelica archangelica* is used to elude constipation and hypersensitivities, stimulate digestion, inhibit cancer and lung tissue diseases. A resin named Angelicin is found in the plant which has property of invigorating to the lungs and skin. This is also contraindicated in bleeding disorders, gastric ulcers and pregnancy condition[4].

Angelica archangelica is being used extensively world wide so we were concerned to comprehend whether this drug has any toxicity after oral consumption. Thus we have conducted theoral acute toxicity experiment of the plant substances to determine its toxic consequences after oral intake by living being. The guidelines 423 of Organisation for Economic Co-operation and Development (OECD) define the acute oral toxicity as the adverse effects occurred after oral administration of a single dose of a substance or chemical, or multiple doses given within 24 hours.OECD recommendations for the testing of elements and substances are regularly reviewed on the basis of scientific advancement or altering evaluation practices. The section 423 of the guideline was embraced in March 1996 as the additional substitute to the conventional acute toxicity test, designated in Test Guideline 401. According to that regulation, testing in only one sex (generally females) can also be considered as sufficient. The acute toxic class method established in this guideline is a stepwise practice on three distinct and single sex animals per step. The use of animals per step is subject to the mortality and morbidity of the animals. On an average 2-4 steps might be crucial to decide the acute toxicity of the test sample. This procedure is optimum for judicious use of the limited number of animals. The acute toxic class method is focused on uniform doses. We found this most suitable test method for present study on Acute Oral Toxicity Testing. The document of Guideline[4] also comprises further direction about the conduct and interpretation of Test Guideline 423.

Principle of the Test :

According to the principles of OECD guideline 423 for oral acute toxicity, least number of animals should be tested for the procedure in each step along with the generation of sufficient data to facilitate its categorization. Each step of the test should be proceeded with three animals of a single sex (conventionally females). Apart from these two priciples, dosing of three additional animals, should be treated with the similar dose[9].

Procedure :

The Oral Acute toxicity test was carried out as per the OECD guideline 423 at maximum test dose of 2000 mg kg^{-1} of aqueous extract of *Angelica archangelica*.

A group of three healthy albino wistar rats was orally administered with single dose of 2000 mg kg^{-1}/PO aqueous leaf extract of *Angelica archangelica* as test substance with the help of oral feeding needle. The wistar rats were fasted prior to dosing (food and water had been restricted for 12 hours) and withheld

for 3-4 hours. During the fasting period, the animals were weighed and administered with the test substance. After that procedure of weighing and dose administration, feeding was withdrawn for additional 3-4 hours in rats. Each animal was examined independently during first 30 minutes after administered with dose followed by periodical observation during initial twenty-four hours. The subjects were under the crucial observation with special recognition given to each animal during the initial four hours and on daily basis thereafter, for total fourteen days. All the examined parameters of each subject were systematically recorded. The results were obtained for two simultaneous studies: Oral Acute Toxicity study and Haematology & Biochemistry study.

The parameters which were observed for Oral Acute Toxicity study are- changes in skin and mucous membranes, salivation, lethargy, sleep, coma, convulsions, tremors, fur, eyes, diarrhoea, morbidity, mortality has been observed on daily basis (once in 24 hours). On 14th day blood was withdrawn from orbital puncture and these test Haemoglobin, WBC, Lymphocytes, Eosinophils, Monocytes, Basopnils, RBC, Neutrophils, SGOT (Serum Glutamic-Oxaloacetic Transaminase), SGTP (Serum Glutamic Pyruvic Transaminase), Total Bilirubin by using biochemistry (model Micro lab ARX-199) and haematology analyser (model KT6400 veterinary).

Approval for this research activity on animal received from Institutional Animal Ethical Committee (IAEC), BilwalMedchem and Research Laboratory Pvt. Ltd. and the IACE approval no. is BMRL/AD/CPCSEA/ IAEC/2022/1/3.

Oral acute toxicity study :

Oral acute toxicity test involves the behavioral parameters such as changes in skin and mucous membranes, salivation, lethargy, sleep, coma, convulsions, tremors, fur, eyes, diarrhoea, morbidity, mortality. All the parameters were examined on daily basis (once in 24 hours). In behavioural observation loss of fur, any aczima on skin have not been found. Stool consistency found as prior to the experiment. Animal was still active physically after the dose administration and not any kind of coma, tremors and loss of sleep have been observerd. Morbidity and mortality during and after the experiment has not taken place (Table-1).

Haematology and Biochemistry study :

The blood sample was taken from orbital sinus on14th day for conducting the haematological test. The tests for Haemoglobin, WBC,RBC, Neutrophils, Lymphocytes, Eosinophils, Monocytes, Basopnils, SGOT, SGTP, Total Bilirubin were performed with help of biochemistry and haematology analyser.

According to the hematological analysis, all test result found in normal range and Basopnil formation was not taken place (Table-2). The biochemistry test of SGOT, SGTP were found in normal range (Table-3). Although total bilirubin was found near the last range but still normal. The SGOT and SGTP (normal range, 143 and 175 IU/l respectively) are responsible for liver functioning. In this study, we observed SGOT and SGTP as 123.45 and 132.45 respectively on 14th dayof dose administration.

Table-1. Oral acute toxicity study: Behavioral observation in wistar rat during the experiment

Observation	30min.	4hr.	24hr.	48hr.	1w	2w
Skin and fur	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Absent	Absent	Absent	Absent	Absent	Absent
Lethargy	Absent	Absent	Absent	Absent	Absent	Absent
Sleep	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Absent	Absent	Absent	Absent	Absent	Absent
Convulsions	Absent	Absent	Absent	Absent	Absent	Absent
Tremors	Absent	Absent	Absent	Absent	Absent	Absent
Diarrhoea	Absent	Absent	Absent	Absent	Absent	Absent
Morbidity	Absent	Absent	Absent	Absent	Absent	Absent
Mortality	Absent	Absent	Absent	Absent	Absent	Absent
Weight (gm)	145.54	145.67	146.54	146.98	149.56	153.45

Table-2. Hematological test: hematological analysis on 14^{th} day of experiment

S. No.	Tests	Normal range	Observed Value (Mean)
1.	Haemoglobin	11.5-16.1gm/dl	14.34
2.	WBC	6.6-12.6 $X10^3/mm^3$	7.12
3.	RBC	6.76-9.75 X $10^6/ mm^3$	8.34
4.	Neutrophils	1.77-3.38 $X10^3/mm^3$	2.24
5.	Lymphocytes	4.78-9.12 $X10^3/mm^3$	7.54
6.	Eosinophils	0.03-0.08 $X10^3/mm^3$	0.04
7.	Monocytes	0.01-0.04 $X10^3/mm^3$	0.03
8.	Basopnils	0.00-0.03 $X10^3/mm^3$	0.00

Table-3. Biochemistry test: Biochemistry test on 14^{th} day of experiment.

S. No.	Tests	Normal range	Observed Value (Mean)
1	SGOT (IU/ML)	74-143 IU/l	123.45
2	SGTP (IU/ML)	63-175 IU/l	132.45
3	Total bilirubin (mg/dl)	0.54-0.80 mg/dl	0.78

We have observed that a group of three wistar rat which was administered with single 2000 mg kg^{-1} dose of leaf aqueous extract of *Angelica archangelica*, was found with the absence of even single adverse symptom related to behavioural, haematological, and biochemical parameters. Behaviour observation during 14^{th} days was recorded with no abnormality, morbidity, mortality. Haematology and Liver functions were noticed in normal range. Thus, it is concluded that the dose of leaf aqueous extract of *Angelica archangelica* was found safe up to 2000 mg kg^{-1} in single dose in albino wistar rat.

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Conflicts of Interest :

The authors declare no conflict of interest regarding the publication of this work.

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