VOLUME 10 ISSUE 2 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 2, 916-923 (2024)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Pharmacological Investigation of Anti-inflammatory and Hepatoprotective Activity of Leaves Extract of *Aconitum heterophyllum*

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Received: 27th March, 2024; **Accepted:** 5th June, 2024; **Published online:** 7th October, 2024

https://doi.org/10.33745/ijzi.2024.v10i02.090

Abstract: The medicinal plants can be useful for mankind as they are available in abundant. These plants can be used because as they are safe and economic with easy availability. In older days, most of the disease was treated by traditional healers by using of medicinal plants. The effect was very good and the diseases condition was eliminated. Most of the common diseases as well as deadly diseases were treated by using plant as a source of medicine. Present study confirmed that the alcoholic extracts of *Aconitum heterophyllum* show potential anti-inflammatory and hepatoprotective activities. Further the aqueous-alcoholic extract of plant was assessed for isolation and characterization of bioactive constituents for same activities in order to validate for the synthetic approach. The extracts may be further extended for suitable extraction of different phytoconstituents because the selected medicinal plant has potential in treating many diseases. The results obtained in the present study support and extend the rational basis for this plant as hepatoprotective, in traditional and folk-lore medicine. The extract has shown potential anti-inflammatory and hepatoprotective activity.

Keywords: *Aconitum heterophyllum*, Anti-inflammatory, Hepatoprotective, Medicinal plants, Phytoconstituents

Citation: Kumar Nawin, Jain Ritesh, Gupta Naveen and Rajput Dharmendra Singh: Pharmacological investigation of anti-inflammatory and hepatoprotective activity of leaves extract of *aconitum heterophyllum.* Intern. J. Zool. Invest. 10(2): 916-923, 2024.

https://doi.org/10.33745/ijzi.2024.v10i02.090

Introduction

The origin of plant materials was primarily used for the preparation of medicines. In India, around 7000 species are utilized for drug alongside a couple of minerals, metals, and animal stuff. Nature contains a lot of medicinal plant resources and this has typically been transferred from age to age rehearsed by the indigenous collection of individuals from various regions (Farnsworth, 1988). India, having two out of the eighteen Biodiversity Hot Spots of the world, possibly the biggest therapeutic herbs maker on the planet. The huge assets of therapeutic plants have been widely utilized in different conventional frame-works of medication like Ayurveda, Siddha, Unani

and Amchi (Rajasekharan *et al.*, 2005). The total number of plant types reported from all Indian gatherings is 43,000, and 3,000 of them are considered to have restorative properties. Ayurveda, the country's most antiquated and across-the-board social security scheme, employs around 900 plant forms. Among different frameworks, Siddha utilizes 800 types of plants; Unani, 700 species; Amchi or Tibetan framework, around 300 species and Modern drug utilizes 90 types of plants. It displays a broad geological and atmospheric range. Siddha, Unani and Ayurveda have been in the presence for a couple of hundred years in India, the local medication arrangement to be specific. The customary arrangement of the drug along with homeopathy and prescription of old stories continues to take on a huge job in the arrangement of medical services. The requests for therapeutic plants made by the cutting edge pharmaceutical ventures have additionally expanded. In this way therapeutic plants become modernly critical harvests which may convey considerable pay to the nation by method for fare (Ganesan, 2006). Ayurvedic medicines mainly based on plants enjoy a respective position today, especially in the developing countries, where modern health services are limited. Medicines containing plant materials combined with chemically defined active substances, including constituents of plants are considered to be herbal medicines (WHO, 1994). Herbal medicines continue to be a major market in US pharma-ceuticals and constitute a multi-billion dollar business. Approximately 1500 botanicals are sold as dietary supplements; formulations are not subject to Food and Drug Administration (FDA) clinical toxicity testing to assure their safety and efficacy. The Indian herbal drug market size is about $1 billion and the export of plant based crude drug is around $100 million. The current market potential of herbal medicine is estimated about $ 80-250 billion in Europe and USA (El and Karakava, 2004). The valuable medicinal properties of different plants are due to presence of several constituents i.e. saponins, tannins, alkaloids, alkenyl phenols, glycol- alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters (Tiwari and Singh, 2004; Nikahat *et al.*, 2023; Rao *et al.*, 2023, 2024; Srivastava *et al.*, 2023; Yadav *et al.*, 2023, 2024; Chaudhary *et al.*, 2024).

Liver damage is a metabolic disorder which is the most common cause of mortality and morbidity globally. Liver injury treatments have gained global attention in recent times because of many allopathic drugs and their toxic influence which leads to liver damage. Hence, folkloric herbs with hepatoprotective potentials to treat liver injury or disease have received considerable attention from researchers due mainly to their low toxicity and healing efficacy. Recently, several folkloric herbs have been investigated for their hepatoprotective role in experiments on animals. Traditional medicines have been utilized for the benefit of the beneficial outcomes of herbal medicine for human health. On the other hand, the shelf life of herbal medicines should be evaluated to monitor their stability during the period of use. Degradation reactions are enhanced by temperature, humidity, pH, oxygen, and light. Herbal medicines are complex mixtures of different classes of chemical compounds, such as carbohydrates, proteins, lipids, and secondary metabolites. Inflammation is a part of the complicated biological reaction of vascular tissues to harmful stimuli, including pathogens, damaged cells or irritants. It is characterized via redness, swollen joints, joint pain, its stiffness and lack of joint characteristic (Oluseyi and Moshood, 2011; Shivananda *et al*., 2011). Inflammation is presently treated via NSAIDs. Unfortunately these capsules motive elevated danger of blood clot ensuing in heart assaults and strokes. Inflammation is a normal, protective reaction to tissue damage caused by physical trauma, noxious chemical compounds or microbiological marketers. Inflam-mation is a commonplace scientific situations and rheumatoid arthiritis (RA) is a persistent debili-tating autoimmune disease that affects approximately 1% of the population in developed nations. The classic symptoms of inflammation are redness, swelling, pain, warmness and loss of

characteristic. Inflammation is a stereotyped reaction, inherent to vascularized tissues, which has the goal of reestablishing tissues homeostasis. The inflammatory process has cell and humoral additives, such as leucocytes (neutrophils, macrophages, eosinophils, mast cells and lymphocytes) and the humoral proteolytic structures (complement, kinins and coagulation), respectively. These components paintings synergistically and concurrently, inflicting vascular changes and leukocyte recruitment to the lesion. According to World Health Organization, about 80% population of the developing countries relies on traditional medicines, mostly plant originated drugs (Dominiczak, 2005; Pattanayak *et al*., 2011). Today a substantial number of drugs are developed from plants which are active against a number of diseases. The majority of these involve the isolation of the active ingredient (chemical compound) found in a particular medicinal plant and its subsequent modification. *Aconitum heterophyllum* is a herbal medicinal plant. *Aconitum* species is used as the major component in the Indian Ayurvedic formulation as well as Chinese and Bhutanese herbal medicines. This species contains many phytoconstituents having a lot of pharmacological activities like anti-inflammatory, hepatoprotective activity, digestive property. *A. heterophyllum* possesses some phytochemical constituents which have medicinal values (Ekaidem *et al*., 2007). The composites of plant *A. heterophyllum* i.e. alkaloids, amide alkaloids, flavonoids, flavonol glycosides, diterpenoid and norditerpenoid compounds are being safer, economical available herbs. The phytochemicals and herbal products can affect the course of inflammatory diseases and may provide an amalgamation of nutritional substances, which help in re-establishing and maintaining wear and tear of tissues. Therefore, it would be rational to logically evaluate the traditional medicines used for their potential use in inflammatory diseases. *A. hetrophyllum* plant has been reported to hold antifungal cytotoxic, antiviral and immune-stimulant properties. Other compounds extracted from *A. heterophyllum* include flavonoids, tannins, saponins and sugars (Ekaidem *et al*., 2007).

Present study evaluated the alcoholic leaves extract of *Aconitum heterophyllum* for anti-inflammatory and hepatoprotective activities.

Materials and Methods

Ethnobotanical surveys were conducted in different tribal localities of Madhya Pradesh. The method adopted for collection of data was interview with tribals, local medicine men and one to one discussion about therapeutic use of local plants in the treatment of various diseases. Present work was carried out on plant species *Aconitum heterophyllum.* Fresh leaves of *Aconitum heterophyllum* were collected from area adjoining forests of Bhopal in the month of March. Leaves of *Aconitum heterophyllum* were dried under the shade. Leaves of *Aconitum heterophyllum* were kept in plastic bags and closed tightly and powdered as per the requirements.

Preparation of extract:

The leaves of *Aconitum heterophyllum* were thoroughly washed in tap water and rinsed in distilled water and cut into small pieces and dried under shade for 3 to 4 weeks. The procedure of Ozougwu and Eyo (2014) was followed for preparing extract from the dried leaves.

100 g of dried leaves of *Aconitum heterophyllum* was extracted with solvent mixure of methanol and water by maceration method. Over their boiling points the extract was evaporated. Finally, the percentage yields for the dried extracts were determined.

Plant leaves were subjected to hot continuous extraction with (500 ml) 80% methanol (30-40^{o}C) in a Soxhlet apparatus for 24 h. The extraction procedure was ensured by pouring a few drops of extract from thimble left no residue on evaporation. After complete extraction the solvent was evaporated and concentrated to dry residue. % yield was calculated for each extract after drying under vacuum.

Table 1: Drugs treatment on various animal groups for anti-inflammatory activity

Group	Treatment
Group I (Negative control)	Carrageenan (0.1 ml of 1% *w/v*)
Group II (Standard control)	Carrageenan (0.1 ml of 1% *w/v*) + Diclofenac sodium (10mg/kg, p.o.) as standard reference
Group III (Treatment Control)	Carrageenan (0.1 ml of 1% *w/v*) + Hydro-alcoholic extract (250 mg/kg, p.o.) of *Aconitum heterophyllum* leaves
Group IV (Treatment Control)	Carrageenan (0.1 ml of 1% *w/v*) + Hydro-alcoholic extract (500 mg/kg, p.o.) of *Aconitum heterophyllum* leaves

Table 2: Drugs treatment on various animal groups for hepatoprotective activity on isoniazid induced hepatotoxicity

Group	Treatment
Group I	Sterile distilled water ml/kg, p.o.
Group II	INH (Isoniazid) solutions were prepared in sterile distilled water (100 mg/kg, p.o.)
Group III	Aconitum heterophyllum leaves Extract (250 mg/kg, p.o.) + INH (100 mg/kg, p.o.)
Group IV	Aconitum heterophyllum leaves Extract (500 mg/kg, p.o.) + INH (100 mg/kg, p.o.)
Group V	Silymarin (2.5 mg/kg, p.o.) + INH (100 mg/kg, p.o.)

Acute oral toxicity:

In the present investigation the Swiss albino rats of either sex, weighing between 150- 200 g were used. Animals were allowed to acclimatize for two weeks before commencing the study and maintained under standard laboratory conditions (25±2ºC temperature, 45-65% Relative Humidity and 12 h light and 12 h dark cycle). The animals were fed with standard laboratory animal feed and water *ad libitum* throughout the study.

Acute oral toxicity was performed according to Organization for Economic Co-operation and Development (OECD) guideline No. 420. Swiss albino rats fasted overnight, accessing water *ad libitum* were used in this study. The extract was administered orally at a dose of 2000 mg/kg b wt. and the animals were observed for mortality or any abnormal behavior for first 24 h, then for next 14 days. Further behavioral responses, neurological responses as well as autonomic responses were observed.

Evaluation of in vivo anti-inflammatory activity by using Carrageenan-induced paw edema model:

Rats were divided into four groups; each group consisting of six animals (Table 1). Paw edema was induced by injecting 0.1 ml of 1% *w/v* carrageenan suspended in 1% CMC into sub-plantar tissues of the left hind paw of each rat. After 180 min using vernier caliper paw thickness was measured. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the carrageenan control group. The percentage (%) inhibition of edema is calculated using the formula:

$$\% \text{ Inhibition} = \frac{To - Tt}{To} \times 100$$

Where, Tt is the thickness of paw of rats given test extract at corresponding time and To is the paw thickness of rats of control group at the same time.

The data is expressed as mean ± Standard Deviation (SD). Results were analyzed using one-way ANOVA followed by Dunnet's test. Differences were considered as statistically significant at $P < 0.05$, when compared with control.

Hepatoprotective activity of Aconitum heterophyllum leaves extract on isoniazid induced hepatotoxicity:

INH (Isoniazid), Silymarin (Micro labs, India) were used in present study. All other chemicals and other biochemical used in the experiments were of analytical grade from different firms. Animals were divided into five groups of 6 animals each. The first group received Sterile distilled water 1 ml/kg p.o. The group II received 100 mg/kg, p.o. INH (Isoniazid) solutions (Table 2). The groups III, IV and V received 250 mg/kg and 500 mg/kg of Hydroalcoholic leaves extract of *Aconitum heterophyllum* and silymarin (2.5 mg/kg p.o.) respectively once a day for 21 days. After 21st days animals was anaesthetized with ether for collection of blood from retro orbital plexus, and then sacrificed under ether anesthesia for the removal of liver. Various biochemical analysis were carried out (Jiang et al., 2004; Saleem et al., 2008).

Biochemical Evaluation in Serum:

Serum glutamic pyruvate transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), Alkaline phosphatase (ALP) and total bilirubin was estimated by using commercial kits as per the manufacturer instructions.

Results and Discussion

In vivo anti-inflammatory activity of Aconitum heterophyllum:

Acute oral toxicity: No adverse changes and mortality were observed in animals, which orally received hydoralcoholic extract (2000 mg/kg) *Aconitum heterophyllum*. This indicates that 2000 mg/kg is maximum safe dose. So $1/10^{th}$ and $1/5^{th}$ *i.e.* 250 and 500 mg/kg of b wt. of the maximum safe doses were selected for studying *in vivo* anti-inflammatory effects.

Evaluation of in vivo anti-inflammatory activity by using Carrageenan- induced paw edema model:

Carrageenan-induced acute inflammation is one of the most suitable test procedure to screen anti-inflammatory agents. The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve. The first phase of inflammation occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also due to histamine and serotonin component. Table 3 and Figure 1 show the effect of hydro-alcoholic extract of *Aconitum heterophyllum* leaves and diclofenac sodium (standard drug) as compared to carrageenan control at different hours in carrageenan-induced paw edema model using vernier caliper. Hydro-alcoholic extract administered at a dose of 250 mg/kg p.o and 500 mg/kg p.o. prevented carrageenan-induced paw edema. with a percentage inhibition at 1, 2, 3 and 4 h. Diclofenac sodium at a dose of 10 mg/kg p.o. prevented carrageenan-induced paw edema at 1, 2, 3 and 4 hour (Table 3; Fig. 1).

In vivo hepatoprotective activity of extract of Aconitum heterophyllum:

Aconitum heterophyllum leaves are an important medicinal plant which is used in traditional medicine to treat many diseases. The liver may be considered as the most important organ in drug toxicity for two reasons: on the one hand it is functionally interposed between the site of absorption and the systemic circulation and is a major site of metabolism and elimination of foreign substances; but on the other hand these features also render it a preferred target for drug toxicity. Drug- induced liver injury (DILI) therefore poses a major clinical problem. Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury or impairment of its function may lead to several implications on one's health. Management of liver diseases is still a challenge to modern medicine. Increase in the level of activities

Table 3: In vivo anti-inflammatory activity of *Aconitum heterophyllum* leaves extract by using Carrageenan-induced paw edema model

Group	Dose of extract (mg/kg, p.o.)	Change in paw thickness (mm)±SD (% inhibition)			
		1 h	2 h	3 h	4 h
Group I (Negative control)	Carrageenan (0.1 ml of 1% *w/v*)	3.50±0.17	4.55±0.12	5.40±0.16	6.02±0.1
Group II (Standard control)	Carrageenan (0.1 ml of 1% *w/v*) + Diclofenac sodium (10 mg/kg, p.o.)	1.58±0.18**	1.81±0.12**	1.95±0.14***	1.60±0.18***
Group III (Treatment Control)	Carrageenan (0.1 ml of 1% *w/v*) + Hydro- alcoholic extract (250 mg/kg, p.o.)	2.70±0.11*	2.89±0.12*	3.38±0.11**	2.61±0.17***
Group IV (Treatment Control)	Carrageenan (0.1 ml of 1% *w/v*) + Hydro- alcoholic extract (500mg/kg, p.o.)	2.41±0.07*	2.49±0.19**	2.05±0.17***	1.65±0.09***

n=6; mean ± SD; Values are statistically significant at *p<0.05, **p<0.01 and ***p<0.005

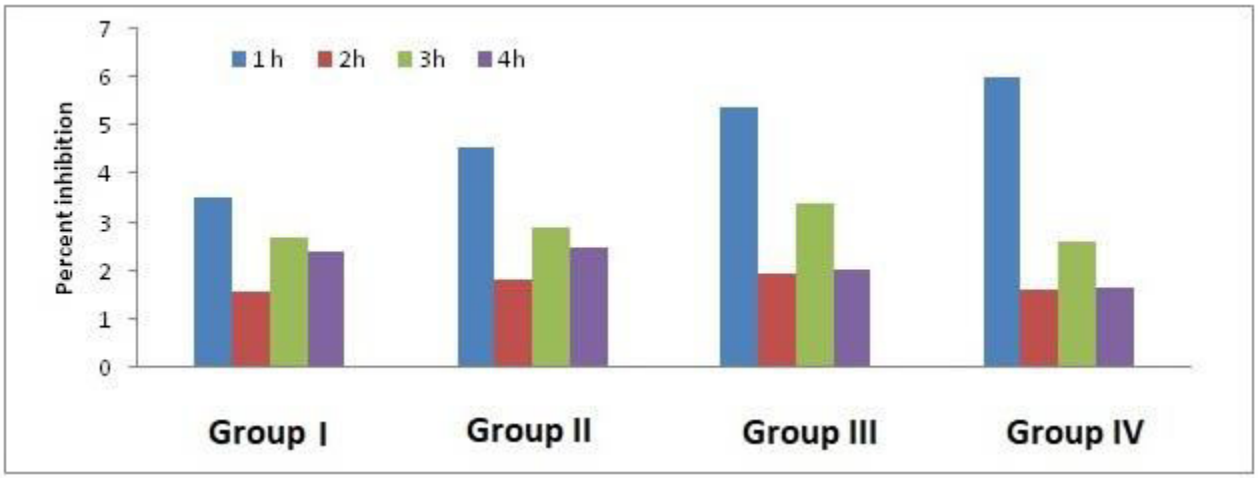

Fig. 1: In vivo anti-inflammatory activity of *Aconitum heterophyllum* leaves extract by using Carrageenan-induced paw edema model.

Table 4: Effect of hydroalcoholic extract of Aconitum heterophyllum leaves in isoniazid induced hepatotoxicity in rats

Treatment	Dose	SGOT (%)	SGPT (%)	Serum Bilirubin (%)
Normal	1 ml/kg, p.o.	172 ± 3.2	150.0 ± 2.60	125.0 ± 3.50
INH	100 mg/kg, p.o.	335.45 ± 5.2	295.0 ± 3.20	255.0 ± 5.50
Aconitum heterophyllum Extract	250 mg/kg p.o.	231.0 ± 4.5***	182.0 ± 4.70***	177.0 ± 5.51***
Aconitum heterophyllum Extract	500 mg/kg p.o.	203.0 ± 3.5***	169.0 ± 3.50***	145.0 ± 4.60***
Silymarin	2.5 mg/kg p.o.	151.0 ± 2.5***	133.0 ± 4.80***	128.0 ± 4.50***

n=6; mean ± SD; Values are statistically significant at *p<0.05, **p<0.01 and ***p<0.005

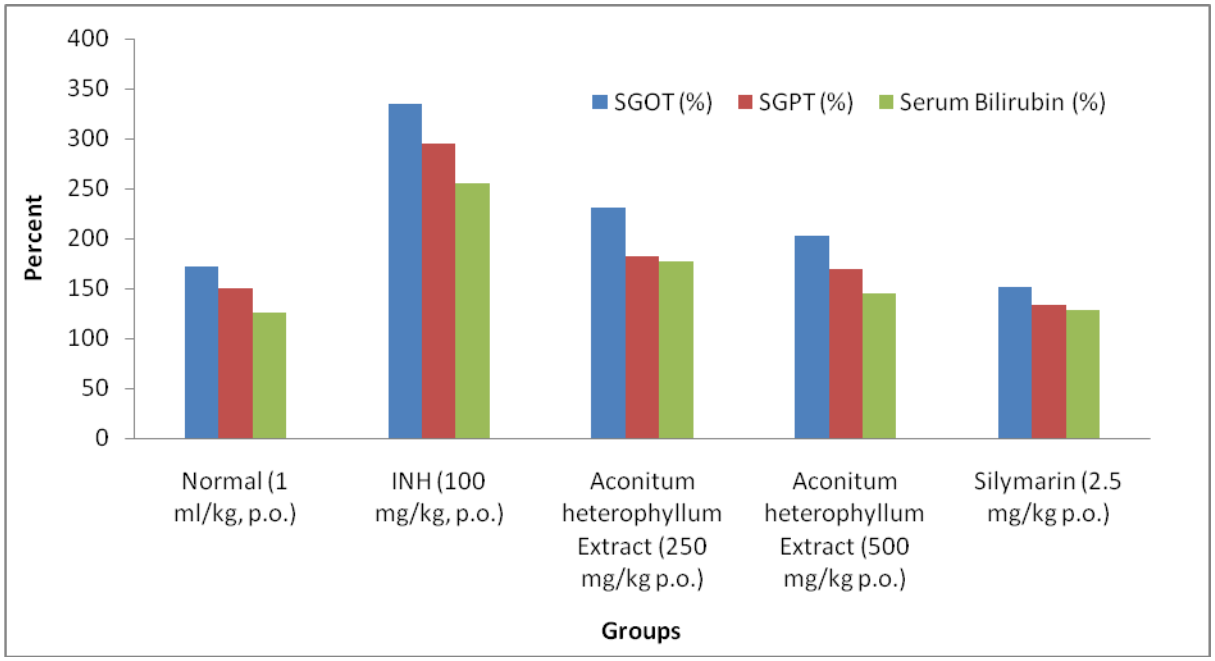

Fig. 2: Effect of hydroalcoholic extract of *A. heterophyllum* leaves in isoniazid induced hepatotoxicity in rats.

of SGPT, SGOT and ALP in the blood reflect the damage of liver hepatocytes and indirectly impairment of liver functions Isoniazid induced hepatotoxicity. SGPT, SGOT and ALP activities were significantly elevated ($p<0.05$) after administration of isoniazid. Treatments with 250 and 500 mg/ kg of *Aconitum heterophyllum* leaves extract significantly reduced the elevation of these enzymes ($p<0.05$) (Table 4; Fig. 2). The reduction of liver enzymes was seen to be to the level of the control group and it was also similar to the level of group pretreated with silymarin. One of the hallmark signs of hepatic injury or damage is apparent leakage of cellular enzymes into plasma. In addition, the extent and type of liver injury or damage can be accessed based on the presence or absence of specific enzymes in the blood stream. In general measurement of alanine amino-transferase, aspartate aminotransferase and alkaline phosphatase are commonly used as marker enzymes in accessing isoniazid induced hepatotoxicity. In this study, hepatoprotective effect of *Aconitum heterophyllum* leaves is evidenced by the improvement SGPT, SGOT, ALP and serum bilirubin levels. Treatment with *Aconitum heterophyllum* leaves extract suppresses Isoniazid induced SGPT, SGOT, ALP and serum bilirubin elevations. Previous studies have reported elevations of transaminases after Isoniazid treatment (Lei *et al*., 2021). The increase is time dependent with significant elevation noted after 48 h ($p<0.05$) suggesting severe hepatocellular damage caused by leakage of these enzymes into circulation that is normally cytoplasmic in location. Both the test groups i.e. low dose and high dose treated groups showed dose dependent hepatoprotective activity. The test groups containing the plant extract alone showed an improvement in the liver activity. It clearly indicates that the plant *Aconitum heterophyllum* has the hepatoprotective activity. This study showed that *Aconitum heterophyllum* leaves have a significant protective action against the hepatotoxicity induced by the drugs used in the treatment of tuberculosis. The hepato-protective role of *Aconitum heterophyllum* might be due to its antioxidant potential mechanism suggesting that the extract of plant may be useful to prevent the oxidative stress induced liver damage.

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