"INVITRO STUDIES OF HEPATOPROTECTIVE ACTIVITY FROM THE LEAF EXTRACT OF SAUSSUREA OBVALLATA"

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ABSTRACT

The present study investigates the hepatoprotective potential of Brahma Kamal (Saussurea obvallata) leaf extracts prepared using chloroform, diethyl ether, and water. Liver diseases pose a significant global health burden, and natural bioactive compounds are increasingly explored for their therapeutic potential. Brahma Kamal, a revered medicinal plant in traditional Ayurvedic and Tibetan medicine, is known for its diverse pharmacological activities. However, its hepatoprotective properties remain underexplored. In this study, phytochemical screening of the different solvent extracts was conducted to identify active constituents such as flavonoids, alkaloids, saponins, and polyphenols, which are known to exhibit hepatoprotective activity. The hepatoprotective efficacy of the extracts was evaluated using an in vivo hepatotoxicity model induced by carbon tetrachloride (CCl_4) in experimental animals. Serum biomarkers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin, were assessed to determine liver function. Histopathological analysis of liver tissues was performed to observe morphological changes and hepatocellular protection. The results demonstrated that all three extracts exhibited significant hepatoprotective activity, with the chloroform and diethyl ether extracts showing superior efficacy in restoring biochemical markers and liver histology. The aqueous extract also showed moderate protective effects, suggesting the presence of bioactive water-soluble compounds. The hepatoprotective mechanism is attributed to the antioxidant and anti-inflammatory properties of the phytoconstituents present in the extracts. These findings suggest that Brahma Kamal leaf extracts, particularly those obtained using non-polar solvents, hold potential as natural hepatoprotective agents. Further research is warranted to isolate the active compounds and elucidate their molecular mechanisms of action. This study highlights the therapeutic significance of Saussurea obvallata in hepatoprotection and its potential application in developing plant-based liver therapeutics.

Keywords: Saussurea obvallata, Brahma Kamal, hepatoprotective activity, liver toxicity, antioxidants, herbal medicine.

INTRODUCTION

Brahma Kamal, also known as Saussurea obvallata, is a sacred plant native to high-altitude regions of the Himalayas, particularly in India, Nepal, Bhutan, and Tibet. It is revered in Hinduism and Buddhism for its purity, spiritual awakening, and blessings of Lord Brahma. The plant's short-lived blooms, a combination of a lotus and a daisy, symbolize auspiciousness and divine grace. Brahma Kamal is also believed to have potent spiritual symbolism, often linked to spiritual enlightenment. In Hindu and Buddhist rituals, the flower is offered in prayer or used in temple decorations, symbolizing purity and divine grace. The plant's rich antioxidant and anti-inflammatory profiles have led to interest in its potential to protect the liver from damage. This study aims to explore the hepatoprotective potential of Brahma Kamal, focusing on its bioactive compounds, traditional applications, and mechanisms of action in liver protection. Understanding its role in mitigating liver damage can provide insights into developing novel therapeutic agents for managing liver diseases.

The plant's wide range of bioactive compounds, such as flavonoids**,** phenolic acids**,** terpenoids**,** alkaloids, and glycosides, holds promise for liver protection and regeneration. Below is an in-depth discussion of its hepatoprotective mechanisms**,** phytochemical constituents**,** and clinical evidence supporting its potential used in liver-related diseases.

PLANT PROFILE



Kingdom	**:** Plantae
Division	**:** Angiosperms
Class	**:** Eudicots
Order	**:** Asterales
Family	**:** Asteraceae (Compositae)
Genus	**:** Saussurea
Botanical name	**:** Saussurea obvallata
Morphological Characteristics	**:** It is a Perennial, Herbaceous plant, erect, stout, and can grow up to 30–60 cm in height.
Stem	Thick, fleshy, and covered with woolly hairs, erect with minimal branching.
Leaves	**:** Large, oblong to lanceolate in shape, Simple, entire margins with a leathery texture,dark green with a woolly underside.
Flower	**:** White or pale pink flowers enclosed in greenish or brownish bracts.
Root	**:** Fibrous roots (not taproots), Thin, hair-like roots that spread out in the soil.
Ethnomedical Uses	**:** Wound Healing, Anti-inflammatory & Pain Relief, Respiratory Health, Digestive Aid, Fever Reduction, Kidney & Urinary Health, antioxidant, antibacterial, hepatoprotective.

MATERIALS AND METHODS

Methods of drying:

Drying is a crucial step in processing medicinal plants, preserving their active compounds and preventing microbial growth. Here are the main drying methods used for medicinal plants:

- Shade Drying (Air Drying)
- Sun light Drying
- Hot air Oven Drying
- Microwave Drying

Steps for drying:

- ✓ Has per Hepatoprotective activity we have taken leaves of Brahma Kamal (*Saussurea obvallata*) plant are harvested.
- ✓ After the process of harvesting the leaves are cutted into small pieces.
- ✓ The cutted leaves were kept on dry place under the shadow for drying.
- ✓ The leaves kept in the shadow for drying will take up to 1-2 weeks.
- ✓ After this above process is done the leaves are dried.

Powdering of plant leaves:

- ✓ The dried are collected, crushed and taken in the mixture (or) grinder.
- ✓ After the process of grinding of plant leaves get passed through the sieve for the fine powder and to remove the unwanted particles.

Extraction:

The Brahma Kamal (*Saussurea obvallata*) plant leaf extraction is done by using Soxhlet extraction method.

Soxhlet Apparatus:

The Soxhlet apparatus is a laboratory device used for extracting lipids or other soluble compounds from solid materials using a continuous solvent extraction process. It was invented by Franz von Soxhlet in 1879 and is widely used in food, pharmaceutical, and environmental analysis.

Components of a Soxhlet Apparatus:

1. Round-bottom Flask – Holds the solvent, which is heated to extract the compound.
2. Soxhlet Extractor – A vertical glass chamber with a siphon mechanism that allows continuous solvent circulation.
3. Thimble (Filter Paper or Porous Material**)** – Holds the solid sample to be extracted.
4. Condenser – Cools the evaporated solvent, allowing it to drip back into the extractor.

Extraction by chloroform:

Weight about 20 g of Brahma Kamal (*Saussurea obvallata*) plant leaf powder. Pack it in the Soxhlet apparatus and add accurately 300 ml of chloroform into clean round bottom flask. Fit the Soxhlet apparatus and run it for 6 hours at 50-60 C.

Extraction by Diethyl Ether:

Weight about 20 g of Brahma Kamal (*Saussurea obvallata*) plant leaf powder. Packed in the Soxhlet apparatus and add accurately 300 ml of Diethyl Ether into clean round bottom flask. Fit the Soxhlet apparatus and run it for 4 hours at 50-60 C.

Extraction by Aqueous phase (water):

Weight about 20 g of Brahma Kamal (*Saussurea obvallata*) plant leaf powder. Packed in the Soxhlet apparatus and add accurately 300 ml of water into clean round bottom flask. Fit the Sechelt apparatus and run it for 6 hours at 50-60 C.



Phytochemical screening of different extract in chloroform and Diethyl Ether and Aqueous prepared after successive extraction method were phytochemically screened by following method. All extract were screened for different types of phytochemical groups such as alkaloids, flavonoids, tannins and phenolic acid, glycosides, anthraquinone glycosides, saponin/steroidal glycosides, amino acid/proteins, carbohydrate. These are the phytochemical components were identified by using extraction of the crude drugs.

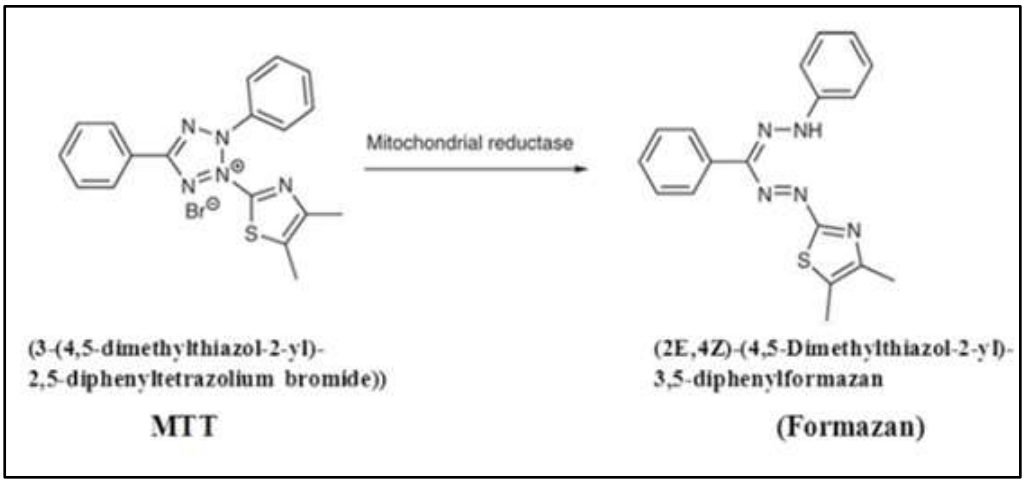
Phytochemical screening of Brahma Kamal (*Saussurea obvallata*) plant leaves

S.No	Phytochemical group	Reagents	Chloroform	Diethyl ether	Aqueous phase(water)
01	Alkaloids test	Dragandroffs reagent	+	+	+
02	Flavonoids test	Ferric chloride test	+	+	+
03	Tannins and Phenolic test	Lead acetate test	+	+	+
04	Glycosides test	Keller-killani test	-	-	-

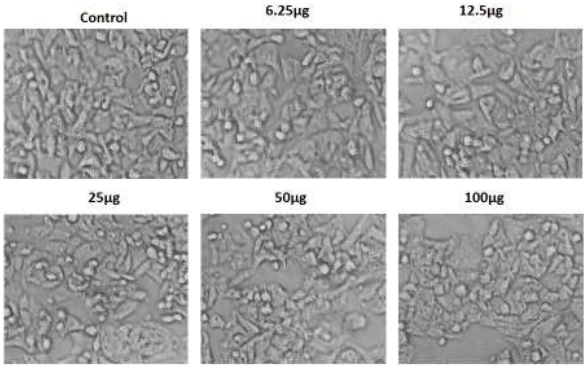
05	Anthraquione glycosides test	Brontrager's test	+	+	-
06	Saponins and steroided test	Libermann's Bernhardz test	+	-	+
07	Amino acid and protein test	Biuret test	+	+	+
08	Carbohydrates test	Bendict's test	+	+	+

1. MTT Assay:

In vitro toxic effects of unknown compounds are determined by counting viable cells and using various methods, such as radioisotope incorporation, automated counters, and dye-based counting. The MTT system measures cell activity via mitochondrial dehydrogenases, yielding simple, accurate, and reproducible results. The key component is MTT, a water-soluble tetrazolium salt, which is converted into an insoluble purple formazan by mitochondrial dehydrogenase enzymes.



Sample: W - 1056(HepG2 cell line)



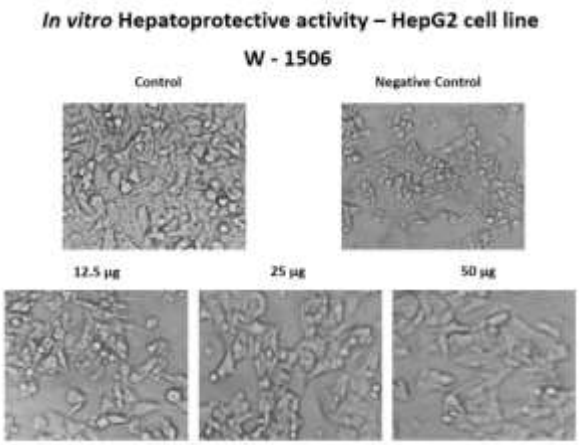
REPORT

The IC_{50} value of the given test sample **W – 1056** were found to be **>100 µg.**

In vitro hepatoprotective activity:

The study involved adding diluted cell suspension to 96 well microtiter plates, incubating for 24 hours, adding Isoniazid, and different test samples. The plate was then incubated with MTT and DMSO, and the absorbance was measured at 570 nm. The percentage of viability was calculated using a formula.

% of viability = Sample abs/Control abs x 100



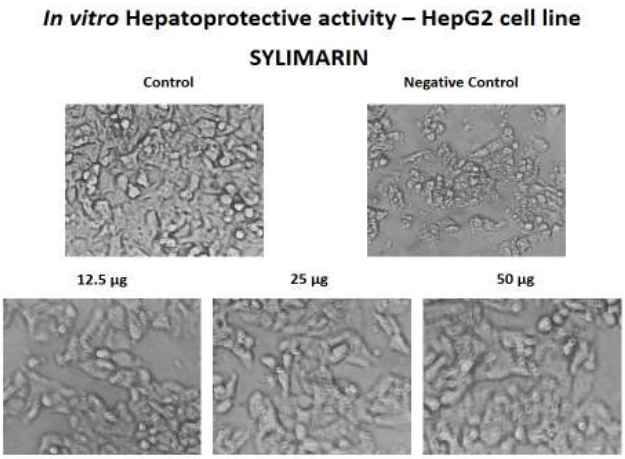
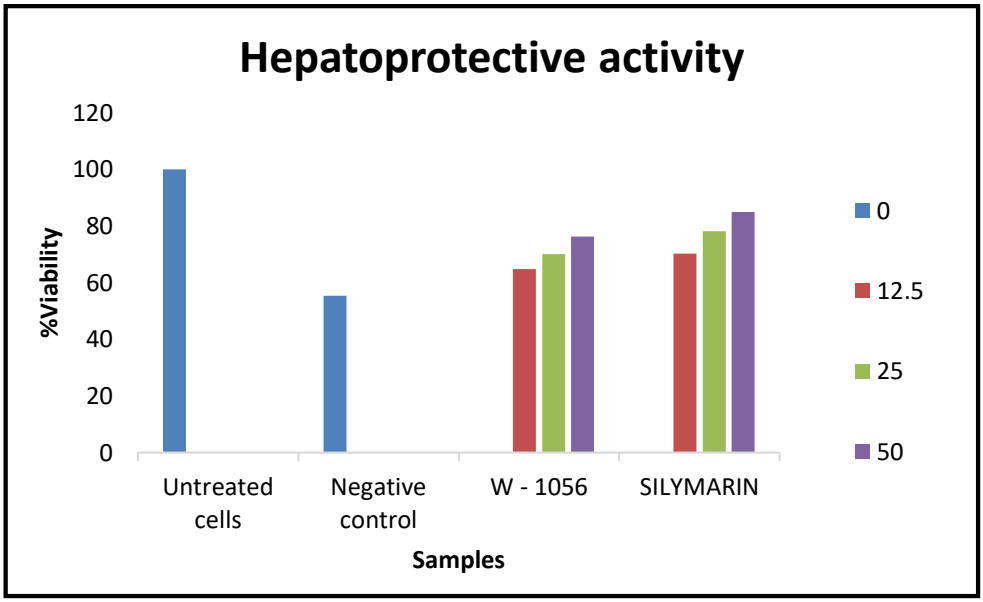


Table for Hepatoprotective Activity - HepG2 Cell Line

		OD at 570 nm		
Untreated cells	0	0.686	0.671	0.664
Negative control	70 mM	0.389	0.36	0.371
W - 1056	12.5	0.447	0.438	0.426
	25	0.486	0.461	0.47
	50	0.525	0.516	0.502
SILYMARIN	12.5	0.481	0.475	0.463
	25	0.534	0.526	0.519
	50	0.58	0.571	0.567

	% viability				
Untreated cells	100	100	100	100	
Negative control	56.70553936	53.65126677	55.87349398	55.41010003	1.578985664
W-1056	65.16034985	65.2757079	64.15662651	64.86422809	0.615509437
	70.84548105	68.70342772	70.78313253	70.11068043	1.219115246
	76.53061224	76.90014903	75.60240964	76.3443903	0.668611128
SILYMARIN	70.11661808	70.78986587	69.72891566	70.21179987	0.53684124
	77.8425656	78.390462	78.1626506	78.13189273	0.27524017
	84.54810496	85.09687034	85.39156627	85.01218052	0.428060766

	Untreated cells	**Negative control**	**W - 1056**	**SILYMARIN**
0	100	55.41010003		
12.5			64.86423	70.21179987
25			70.11068	78.13189273
50			76.34439	85.01218052



CONCLUSION

The present in vitro study demonstrates that the leaf extract of *Saussurea obvallata* exhibits significant hepatoprotective activity. The extract showed promising antioxidant properties, contributing to the stabilization of hepatic cells against induced oxidative stress and cytotoxicity. The observed hepatoprotective effects may be attributed to the presence of bioactive phytochemicals such as flavonoids, phenolic, and terpenoids, known for their antioxidant and anti-inflammatory properties. These findings suggest the potential of *Saussurea obvallata* as a natural source for hepatoprotective agents. However, further in vivo studies and clinical trials are necessary to confirm its efficacy, elucidate the underlying mechanisms, and evaluate its safety profile for therapeutic applications. The in vitro study reveals that the leaf extract of *Saussurea obvallata* has notable hepatoprotective activity, likely due to its antioxidant and bioactive compounds. These results suggest its potential as a natural liver-protective agent, warranting further in vivo studies and clinical trials to confirm its efficacy and safety.

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