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CHEMICAL EVALUATION AND PREVENTIVE POTENTIAL OF KALANCHOE PINNATA IN CCL_4 INDUCED HEPATOTOXICITY

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ABSTRACT

The present study investigates the chemical composition and hepatoprotective potential of *Kalanchoe pinnata* against carbon tetrachloride (CCl_4)-induced hepatotoxicity in albino rats. A hydro-alcoholic extract of *Kalanchoe pinnata* was prepared, yielding 13.57 ± 1.18%. Phytochemical screening revealed the absence of carbohydrates, alkaloids, triterpenoids, steroids, saponins, tannins, phenolic compounds, flavonoids, and glycosides, while the presence of oils was confirmed. To assess the hepatoprotective effects, serum parameters, including SGOT, SGPT, SALP, and SBLN, were measured in different groups of rats. Results demonstrated a significant increase in these markers in the toxin control group when compared to the normal group, confirming hepatotoxicity. However, treatment with *K. pinnata* extract at both low and high doses resulted in a significant reduction in these markers, indicating its protective effect. The low dose extract group showed the highest percentage recovery in SGOT (51.69%), SGPT (92.47%), SALP (72.50%), and SBLN (105.50%), suggesting a dose-dependent hepatoprotective effect. These findings highlight the potential of *Kalanchoe pinnata* as a natural therapeutic agent for preventing liver damage caused by toxic agents like CCl_4.

Keywords: *Kalanchoe pinnata*, hepatoprotective, CCl_4-induced hepatotoxicity, serum parameters, hydro-alcoholic extract, phytochemical screening.

INTRODUCTION

Hepatotoxicity, or liver toxicity, is a critical health concern caused by various factors, including exposure to chemicals, drugs, and environmental pollutants. Carbon tetrachloride (CCl_4) is a well-known hepatotoxic agent that induces oxidative stress and inflammation, leading to liver damage and dysfunction. The liver plays a vital role in detoxification and metabolism, making it susceptible to toxic insults. The search for effective hepatoprotective agents has gained momentum, especially from natural sources such as medicinal plants.

Kalanchoe pinnata, commonly known as "air plant" or "life plant," is a succulent belonging to the Crassulaceae family. Traditionally used in various cultures for its medicinal properties, K. pinnata is recognized for its potential anti-inflammatory, analgesic, and antioxidant effects. Numerous studies have indicated that *K. pinnata* possesses bioactive compounds, including flavonoids, alkaloids, and phenolic acids, which contribute to its therapeutic efficacy (Arora and Kaur, 2019; Kumar *et al.,* 2020). These compounds exhibit hepatoprotective properties by scavenging

free radicals and modulating inflammatory pathways.

Research has demonstrated that extracts from *K. pinnata* can mitigate liver damage induced by toxins. For instance, its aqueous and ethanolic extracts have shown promising results in reducing liver enzyme levels, enhancing antioxidant defense mechanisms, and restoring histological integrity in liver tissues after exposure to CCl_4 (Muthusamy and Kumar, 2021; Santos *et al.,* 2018). However, the specific chemical constituents responsible for these effects remain to be fully elucidated, warranting a detailed evaluation of both the chemical profile and hepatoprotective potential of *K. pinnata*.

Given the increasing prevalence of liver disorders and the associated morbidity, this study aims to investigate the chemical composition of Kalanchoe pinnata and its preventive potential against CCl_4-induced hepatotoxicity. Understanding the active phytochemicals and their mechanisms of action may pave the way for developing novel, plant-based therapeutic strategies for liver protection.

MATERIALS AND METHODS

Collection of plant material Extraction of *K. pinnata* leaves

The plants have been selected on the basis of its availability and Folk use of the plant. Leaves of *K. pinnata* were collected from the local area of Sagar in the month of June, 2023. The collected plant material were taxonomically identified and authentication was be done by Herbarium IN-charge (Herbarium number- BOT/H/03/60/02) of Department of Botany UGC-DSA/ASIST Sponsored Department, Doctor Hari Singh Gour Vishwavidyalaya, Sagar (M.P.) Drying of fresh plant parts was carried out in the sun but under the shade. Dried leaves of *K pinnata* were preserved in plastic bags and closed tightly and powdered as per the requirements.

Extraction of *K. pinnata* leaves

The dried leaf powder has been weighed about 25 gm and is subjected to extraction by using hydro-alcoholic solvents at temperature 60C for 48 hours by using soxlet apparatus. The solvent was then recovered using distillation apparatus and the concentrated extract was further evaporated to get dry powder. The dried powder was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation of phytochemical screening and quantification of phytochemicals.

Phytochemical screening

Extracts were subjected to qualitative secondary metabolites screening to identify the presence of saponins, flavonoids, terpenoids, steroids, coumarins, alkaloids, phenolics, and tannins. The analysis was carried out as described by Trease and Evans (Trease; 1989).

Test for Saponins

One mL of extract was diluted with 3 mL of distilled water. Then, it was shaken for 15 min. The formation of a 1 cm layer of foam indicates the presence of saponin.

Test for Flavonoids

One mL of extract was diluted with 1 mL of sodium hydroxide and hydrochloric acid. The development of yellow solution indicates the presence of flavonoids.

Test for Terpenoids

One mL of extract was mixed with 2 mL of chloroform and 2 mL of concentrated sulfuric acid. The formation of a reddish-brown color

at the interface indicates the presence of terpenoids.

Test for Steroids

One mL of extract was mixed with 10 mL of chloroform followed by the addition of 10 mL of concentrated sulfuric acid. The formation of a red color on the upper layer indicates the presence of steroids while the addition of sulfuric acid showed yellow with green fluorescence.

Test for Coumarins

One mL of extract was mixed with 1 mL of sodium hydroxide (10%). The yellow color indicates the presence of coumarins.

Test for Alkaloids

An equal volume of extracts was mixed with concentrated hydrochloric acid. Then, a few drops of Mayer's reagent were added into a mixture. The presence of a green color or white precipitate indicates the presence of alkaloids.

Test for Phenolics and Tannins

One mL of leaves extract was mixed with 2 mL of distilled water followed by a few drops of ferric chloride (10%). A blue or green color indicates the presence of phenols.

***In vivo* hepatoprotective activity**

Preparation of extracts suspension:

The extract of *K. pinnata* was suspended in 2% gum acacia prior to administration.

Oral administration:

For administration of vehicle/ toxicant/ suspensions of extracts an oral feeding needle attached to a syringe was used. The needle was curved and round tipped. The animals were grasped by the nope of neck with left hand thus making the animal to open its mouth and the oral feeding needle was inserted through intra dental space into the esophagus and the substance were administered to the respective groups by gently pushing the plunger of the syringe. Then the oral feeding needle was withdrawn slowly and smoothly (Ganapaty *et al.,* 2007).

Experimental animals:

Albino Wistar rats (150-200g) were procured from the College of Veterinary Sciences and Animal Husbandry Mhow, Indore (M.P.). All experimental animals were kept in polypropylene cages (6 rats per cage) at 24±2°C temperature with relative humidity 40-55% under 12 h light and dark cycles. All the animals were acclimatized for laboratory conditions as per CPCSEA guidelines for a week before the start of the experiment. The animals were fed with standard pellet diet and fresh water as often as necessary. The experimental protocol was as per the ethics committee on research in animals, as well as nationally accepted principles for use and care of the laboratory animals. Animal experiment protocol was approved from Institutional Animal Ethical Committee of Adina Institute of Pharmaceutical Sciences, Sagar (M.P).

On 22^{nd} day, that is 24 h after the last dose, under proper anesthesia (2% ether) all animals were sacrificed by cervical dislocation. Blood was allowed to clot for 60 min at room temperature (20C). Then, serum was separated by centrifuging at 1000 rpm for 5 min from the clotted blood. The straw coloured serum was used to study in-vivo liver marker enzymes. Liver was separated from diaphragm by cutting the falciparum and coronary ligaments. The liver was washed with phosphate buffer saline to remove blood. Liver tissue was chopped and preserved in 10% formaldehyde solution for histological study (Setty *et al.,* 2007).

RESULTS AND DISCUSSION

The present study aimed to evaluate the chemical composition and hepatoprotective potential of *Kalanchoe pinnata* against carbon tetrachloride (CCl_4)-induced hepatotoxicity in albino rats. The extraction yield of the hydro-alcoholic extract of *K. pinnata* was determined to be 13.57 ± 1.18%, indicating a substantial amount of extractable compounds, which is consistent with findings from previous studies highlighting the richness of bioactive components in *K. pinnata* (Kumar *et al.,* 2020).

The phytochemical screening results revealed that the hydro-alcoholic extract of *K. pinnata* was devoid of carbohydrates, alkaloids, triterpenoids, saponins, tannins, flavonoids, glycosides, and proteins. The presence of oils, however, suggests the potential for certain lipid-soluble compounds that could play a role in its hepatoprotective effects. These findings align with previous reports emphasizing the complexity of phytochemical profiles in medicinal plants, where some bioactive constituents may be present in varying amounts depending on the extraction method used (Arora and Kaur, 2019).

The significant alterations in serum parameters in the toxin control group (Group B) indicated severe hepatocellular damage due to CCl_4 exposure, reflected by elevated levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (SALP), along with increased serum bilirubin levels (SBLN). These findings are consistent with the established understanding that CCl_4 induces oxidative stress and cellular injury in liver tissues, leading to the release of these enzymes into the bloodstream (Muthusamy and Kumar, 2021).

In contrast, groups treated with *K. pinnata* extracts (Groups C and D) showed significant reductions in SGOT, SGPT, SALP, and SBLN levels compared to the toxin control group. The high dose extract notably improved these parameters, suggesting a dose-dependent hepatoprotective effect. Specifically, the percentage recovery values indicate that the hydro-alcoholic extract of *K. pinnata* effectively mitigated liver damage, which is in agreement with earlier studies reporting similar protective outcomes in various experimental models of liver toxicity (Santos *et al.,* 2018).

Histopathological examination of liver tissues further corroborated the biochemical results. Normal liver architecture was observed in the control group, while CCl_4-intoxicated rats displayed extensive necrosis and fatty infiltration. Conversely, treatment with K. pinnata extracts led to a significant restoration of hepatic structure, highlighting the protective mechanism of the extract against oxidative stress-induced damage.

CCl4 induced hepatotoxicity in Wistar rats:

Following treatment schedule was adopted:

S. No	Group	Treatment	Dose	Duration of treatment	No of animals
1	G-I	Normal Saline			6
2	G-II	Toxic Group: CCl4 Induced hepato-toxicity	1:1 (v/v) CCl4 in olive oil	Daily administration for 21 days	6
3	G-III	CCl4 + Low dose Extract of *K. pinnata*	1:1 (v/v) CCl4 in olive oil + 100 mg/kg Extract of *K. pinnata*	Daily administration for 21 days	6
4	G-IV	CCl4 + High dose Extract of *K. pinnata*	1:1 (v/v) CCl4 in olive oil + 200 mg/kg Extract of *K. pinnata*	Daily administration for 21 days	6

Table 1: Extraction yield of *Kalanchoe pinnata*

S. No.	Plant	Extraction Yields (%)
		Hydro-alcoholic
1.	*Kalanchoe pinnata*	13.57 ± 1.18

Table 2: Result of phytochemical screening of extracts of *Kalanchoe pinnata*

S. No.	Experiment	Result
		Hydro-alcoholic Extract
Test for Carbohydrates		
1.	Molisch's Test	-ve
2.	Fehling's Test	-ve
3.	Benedict's Test	-ve
4.	Barfoed's Test	-ve
Test for Alkaloids		
1.	Dragendorff's Test	-ve
2.	Wagner's Test	-ve
3.	Mayer's Test	-ve
4.	Hager's Test	-ve
Test for Triterpenoids and Steroids		
1.	Libermann-Burchard Test	-ve

2.	Salkowski Test	-ve
Test for Saponins		
1.	Froth Test	-ve
Test for Tannin and Phenolic Compounds		
1.	Ferric Chloride Test	-ve
2.	Gelatin Test	-ve
3.	Lead Acetate Test	-ve
Test for Flavonoids		
1.	Shinoda's Test	-ve
Test for Glycosides		
1.	Borntragers Test	-ve
2.	Keller Killiani Test	-ve
Test for Protein		
1.	Biuret's Test	-ve
2.	Ninhydrin Test	-ve
3.	Millon's Test	-ve
Test for Oil		+ve

Table 3: Effect of *K. pinnataon* serum parameters of CCl4-treated albino rats

Group	SGOT (U/l)	SGPT (U/l)	SALP (KA unit)	SBLN (mg/l)
A. Normal	120.66±5.44	45.00±6.16	37.16±3.76	6.16±1.94
B. Toxin control	233.83±5.76	175.66±7.29	77.16±3.06	12.16±2.0
C. Low dose extract	175.33±8.04 (51.69)	54.83±9.58 (92.47)	48.10±2.97 (72.50)	5.83±1.18 (105.50)
D. High dose extract	200.50±7.52 (29.45)	69.33±10.19 (81.37)	58.83±4.95 (45.82)	9.22±0.64 (49.00)
F-ratio[a]	295.52	305.99	122.55	21.83
Significant difference between various groups	A and B	A and B	A and B	A and B
P<0.001	B and C, D	B and C, D	B and C, D	B and C, D

Values are mean ± S.D. No. of rats in each group=6. Values in parenthesis indicate percentage recovery.

[a]Critical value = 8.10.

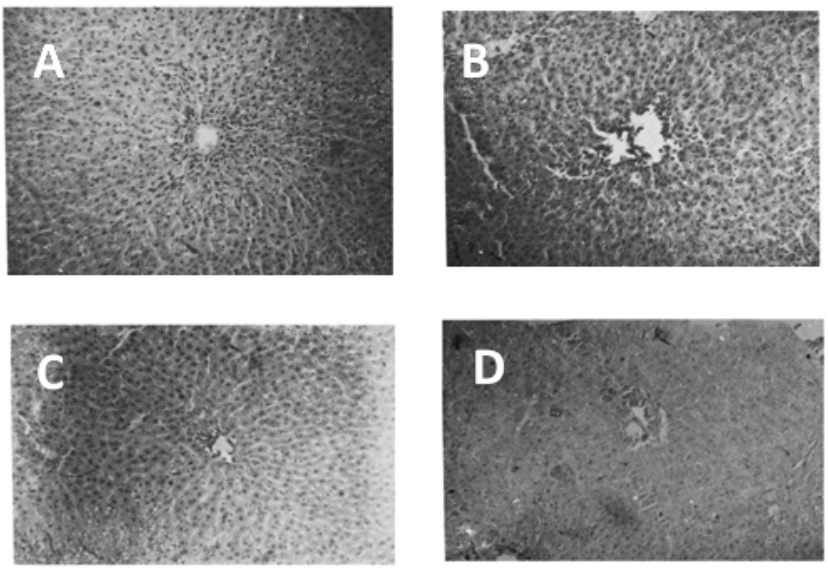

Figure 1: (A) Liver cells of normal rats; **(B)** Liver cells of rats intoxicated with CCl4; **(C)**Liver cells of rats intoxicated with CCl4+Low dose extract; **(D)**Liver cells of rats treated with CCl4+High dose extract of *K. pinnataon*

CONCLUSION

The findings from this study demonstrate that *Kalanchoe pinnata* possesses notable hepatoprotective properties, primarily attributed to its bioactive constituents, which warrant further investigation. Understanding the specific mechanisms by which these compounds exert their effects could pave the way for developing effective herbal therapies for liver protection.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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