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EVALUATION OF THE ACTIVITY OF *OLAX SCANDENS*

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ABSTRACT
The hepatoprotective properties of *Olax scandens*, a medicinal plant traditionally used for various ailments. Liver disease is a significant cause of global morbidity and mortality, and conventional treatments often carry toxic side effects. This has led to a growing interest in herbal medicines as alternative therapies. This study focuses on the phytochemical profile and the hepatoprotective efficacy of an ethanolic extract of *Olax scandens* leaves against paracetamol-induced liver toxicity in Wistar albino rats. Preliminary phytochemical screening revealed the presence of flavonoids, phenols, saponins, and diterpenes. In the animal model, the extract demonstrated a dose-dependent hepatoprotective effect, significantly reducing elevated levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). The findings support the traditional use of *Olax scandens* and suggest that its therapeutic activity may be linked to its rich phytochemical composition.

KEYWORDS: *Olax scandens*, phytochemical screening, liver toxicity, Hepatoprotective, alanine aminotransferase.

1. INTRODUCTION

Herbal medicine, also known as botanical medicine or phytomedicine, involves the use of plant parts such as seeds, roots, leaves, bark, or flowers for medicinal purposes. Used for centuries in traditional healing systems, these natural remedies are gaining popularity in both developing and developed countries due to their natural origin and fewer side effects compared to synthetic drugs. The World Health Organization (WHO) has recognized over 21,000 medicinal plants globally, with India alone housing 2,500 species.[1-12]

The liver is a vital organ susceptible to damage from various chemicals and toxins. This hepatotoxicity is a major health concern, and while many allopathic drugs exist, they can also contribute to liver damage. As a result, there is a growing interest in investigating the hepatoprotective potential of folkloric herbs, which are known for their low toxicity and healing efficacy. This study aims to evaluate the hepatoprotective effect of *Olax scandens*, a plant traditionally used for conditions including cough, cold, fever, and liver-related ailments.[11-15]

2. MATERIALS AND METHODS

An ethanolic extract of *Olax scandens* was prepared, yielding an extractive value of 16.5%. This yield indicates that ethanol was an effective solvent for extracting a significant quantity of bioactive compounds.

2.1. Phytochemical Screening

The ethanolic extract of *Olax scandens* was subjected to preliminary phytochemical screening using standard tests to identify the classes of bioactive compounds present. Tests were conducted to detect the presence of flavonoids, phenols, saponins, diterpenes, proteins, alkaloids, and carbohydrates.[13-28]

Figure 1: Plant of Phyllanthus Olax scandens Roxb.

2.2. Experimental Design for Hepatoprotective Activity

A study was designed to evaluate the hepatoprotective effects of the *Olax scandens* extract against paracetamol-induced liver toxicity in rats. The study involved five groups of six rats each, with treatments administered for eight consecutive days (26-31).

- **Group I:** Normal control, received 0.5% carboxymethyl cellulose (CMC) orally.
- **Group II:** Paracetamol control, received paracetamol subcutaneously at 500 mg/kg body weight to induce hepatotoxicity.
- **Group III:** Received paracetamol (500 mg/kg) and the standard hepatoprotective drug, Silymarin (10 mg/kg), orally.
- **Group IV:** Received paracetamol (500 mg/kg) and *Olax scandens* extract at a dose of 100 mg/kg orally.
- **Group V:** Received paracetamol (500 mg/kg) and *Olax scandens* extract at a dose of 200 mg/kg orally.

The hepatoprotective activity was assessed by measuring the levels of key biochemical markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Profile

The preliminary phytochemical screening of the *Olax scandens* extract showed the presence of several bioactive compounds. Flavonoids were confirmed through positive reactions in both the lead acetate and alkaline tests, indicating their potent antioxidant and therapeutic properties. Phenolic compounds, also contributing to antioxidant potential, were detected with a positive ferric chloride test. The presence of saponins was confirmed by froth and foam tests, suggesting possible antimicrobial or anti-inflammatory activities. Diterpenes were also detected, which may exhibit anti-inflammatory or antimicrobial effects. The study also detected reducing sugars and proteins (specifically, those with aromatic amino acids). Notably, the tests for alkaloids and glycosides were negative. Quantitative analysis found a total phenolic content of 0.965 mg/100 mg and a total flavonoid content of 0.742 mg/100 mg.

Table 1: Phytochemical screening of extracts of *Olax scandens.*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids** Mayer's Test Wagner's Test Dragendroff's test Hager's test	 -ve -ve -ve -ve
2.	**Glycosides** Modified Borntrager's Test Legal's test	 -ve -ve
3.	**Flavonoids** Lead acetate	 +ve
	Alkaline test	+ve
4.	**Phenol** Ferric Chloride Test	 +ve
5.	**Proteins and Amino acids** Xanthoproteic test Ninhydrin Test	+ve -ve
6.	**Carbohydrates** Molisch's Test Benedict's Test Fehling's test	-ve +ve +ve
7.	**Saponins** Froth Test Foam test	+ve +ve
8.	**Diterpins** Copper acetate test	 +ve

3.2. Hepatoprotective Activity

The investigation of the hepatoprotective activity demonstrated that the ethanolic extract of *Olax scandens* effectively protects against paracetamol-induced liver damage. Paracetamol overdose is known to cause liver and renal necrosis in experimental animals and humans. The administration of the *Olax scandens* extract in Groups IV and V led to a significant and dose-dependent decrease in the elevated levels of serum enzymes (ALT, AST, and ALP). This reduction indicates a protective effect on the liver cells, preventing the release of these enzymes into the bloodstream. These results were comparable to the effect of Silymarin, the standard hepatoprotective agent used in Group III. The findings suggest that the hepatoprotective effect of the extract is likely due to its antioxidant and reactive oxygen species-

scavenging activities, which can be attributed to the presence of phytochemicals like flavonoids and phenols.

Total Phenolic content estimation (TPC)

The content of total phenolic compounds content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.031X-0.000, R^2= 0.999,** where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Table 2: Preparation of calibration curve of Gallic acid.

S. No.	Concentration (µg/ml)	Mean absorbance
0	0	0
1	5	0.154
2	10	0.318
3	15	0.462
4	20	0.621
5	25	0.784

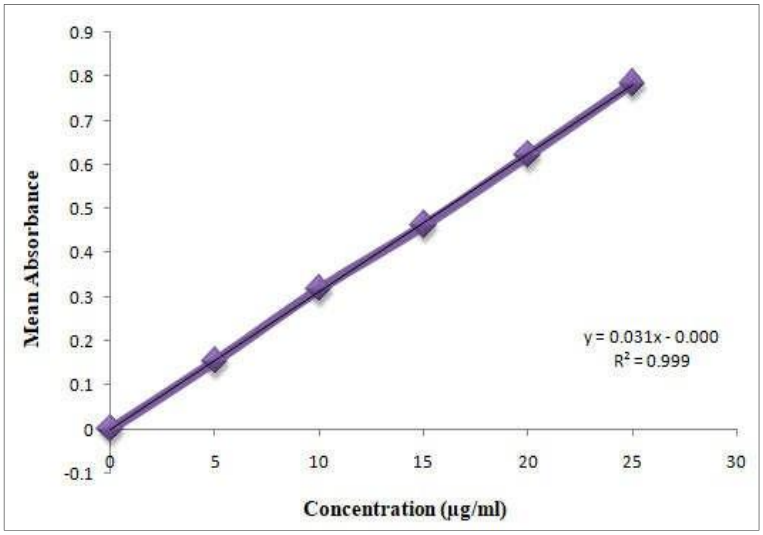

Figure 2: Graph of estimation of total Phenolic content.

Total flavonoid content estimation (TFC)

The content of total flavonoid compounds content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.027X+0.007, R^2= 0.999,** where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 3: Preparation of calibration curve of Quercetin.

S. No.	Concentration(µg/ml)	Mean absorbance
0	0	0
1	5	0.124
2	10	0.262
3	15	0.394
4	20	0.537
5	25	0.675

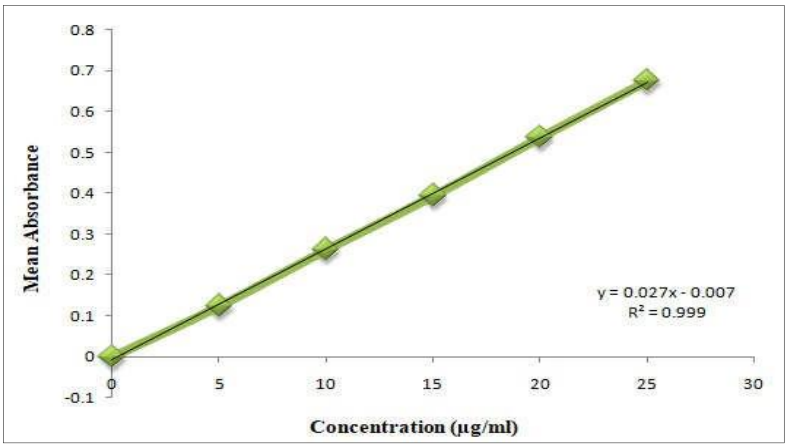

Figure 3: Graph of Estimation of Total flavonoid content.

Table 4: Total phenolic and total flavonoid content of *Olax scandens* extract.

S. No.	Extract	Total Phenol	Total flavonoid
		(mg/100mg)	
1.	Ethanolic extract	0.965	0.742

The quantitative estimation of total phenolic and flavonoid content in the ethanolic extract of *Olax scandens* indicates a modest concentration of these important phytochemicals. The total phenolic content was found to be 0.965 mg per 100 mg of extract, while the total flavonoid content was slightly lower at 0.742 mg per 100 mg. These values suggest that although phenolic compounds are somewhat more abundant than flavonoids, both are present in relatively low to moderate amounts.

Results of hepatoprotective activity of *Olax scandens* extract

Acute toxicity

In an acute toxicity evaluation, the *Olax scandens* extract exhibited no mortality at doses up to 2000 mg/kg body weight. Therefore, 100 mg/kg and 200 mg/kg body weight were chosen to investigate oral activity (p.o.).

Serum levels of ALT and AST were significantly elevated in the paracetamol group (ALT: 95.4 ± 2.8 U/L; AST: 124.3 ± 3.2 U/L) compared to the normal control group (ALT: 28.6 ± 1.2 U/L; AST: 46.7 ± 1.5 U/L), indicating pronounced hepatic injury.

Treatment with the standard hepatoprotective drug silymarin (10 mg/kg) significantly reduced these enzyme levels to 36.8 ± 1.3 U/L (ALT) and 57.7 ± 1.6 U/L (AST). Similarly, administration of *Olax scandens* extract at 100 mg/kg lowered the ALT and AST levels to 52.7 ± 1.6 U/L and 78.3 ± 2.1 U/L, respectively. A higher dose of 200 mg/kg of *Olax scandens* extract further improved the biochemical profile, reducing ALT to 42.2 ± 1.4 U/L and AST to 64.5 ± 1.8 U/L (Table 7.6 and Figure 7.4-7.5).

Table 5: Effect of *Olax scandens* extract on ALT and AST in paracetamol-induced hepatotoxicity in rats.

Group	Drug/Treatment	Dose	ALT (U/L)	AST (U/L)
I	Normal Control	0.5% CMC, 1 ml/kg p.o.	28.6 ± 1.2	46.7 ± 1.5
II	Paracetamol	500 mg/kg	95.4 ± 2.8#	124.3 ± 3.2#
III	Silymarin	10 mg/kg p.o.	36.8 ± 1.3***	57.7 ± 1.6***
IV	*Olax scandens* extract	100 mg/kg p.o.	52.7 ± 1.6*	78.3 ± 2.1*
V	*Olax scandens* extract	200 mg/kg p.o.	42.2 ± 1.4**	64.5 ± 1.8**

Values are expressed as the mean ± SEM of six observations. * P<0.05, ** P< 0.001, *** P< 0.001 vs. control treatment (One-way ANOVA followed by Tukey's Post hoc test)

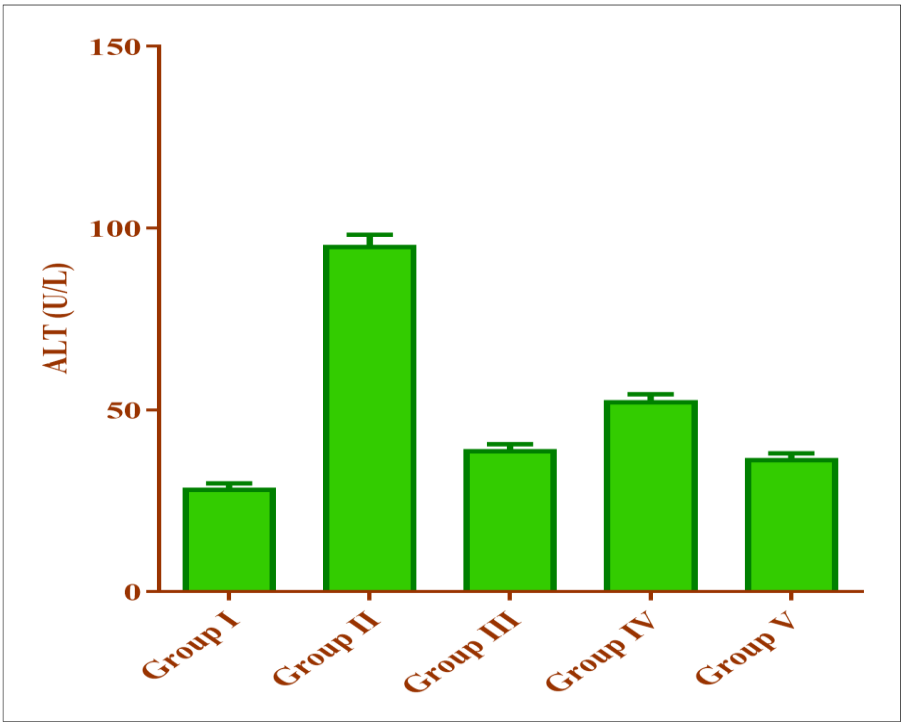

Figure 4: Effect of *Olax scandens* extract on ALT in paracetamol-induced hepatotoxicity in rats.

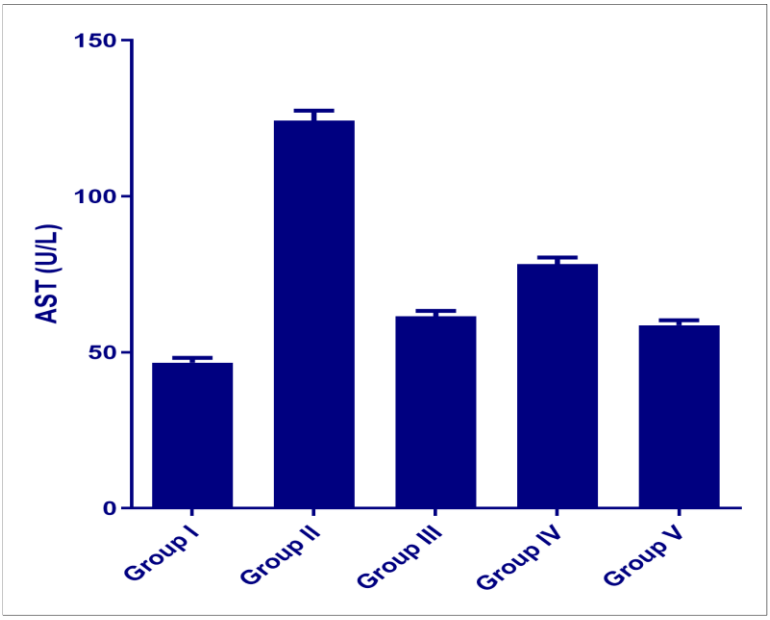

Figure 5: Effect of *Olax scandens* extract on AST in paracetamol-induced hepatotoxicity in rats.

In the normal control group, ALP and bilirubin levels were measured at 85.6 ± 2.1 IU/L and 0.42 ± 0.02 g/dL, respectively. Paracetamol significantly increased these levels to 176.3 ± 3.5 IU/L and 1.25 ± 0.04 g/dL, indicating liver damage. Treatment with the standard hepatoprotective drug silymarin at 10 mg/kg notably decreased ALP to 90.6 ± 2.2 IU/L and bilirubin to 0.51 ± 0.02 g/dl. Similarly, *Olax scandens extract* at 100 mg/kg lowered ALP and bilirubin to 113.8 ± 2.8 IU/L and 0.78 ± 0.03 g/dL, respectively, while the higher dose of 200 mg/kg achieved even greater reductions, bringing levels down to 94.2 ± 2.4 IU/L and 0.59 ± 0.03 g/dl. These findings indicate that *Olax scandens extract* exhibits a dose- dependent hepatoprotective effect.

Table 5: Effect of *Olax scandens* extract on ALP and bilirubin in paracetamol- induced hepatotoxicity in rats.

Group	Drug/Treatment	Dose	ALP (U/L)	Bilirubin (g/dL)
I	Normal Control	0.5% CMC, 1 ml/kg p.o.	85.6 ± 2.1	0.42 ± 0.02
II	Paracetamol	500 mg/kg	176.3 ± 3.5#	1.25 ± 0.04#
III	Silymarin	10 mg/kg p.o.	90.6 ± 2.2***	0.51 ± 0.02***
IV	*Olax scandens* extract	100 mg/kg p.o.	113.8 ± 2.8*	0.78 ± 0.03*
V	*Olax scandens* extract	200 mg/kg p.o.	94.2 ± 2.4**	0.59 ± 0.03**

Values are expressed as the mean ± SEM of six observations. * P<0.05, ** P< 0.001, *** P< 0.001 vs. control treatment (One-way ANOVA followed by Tukey's Post Hoc test)

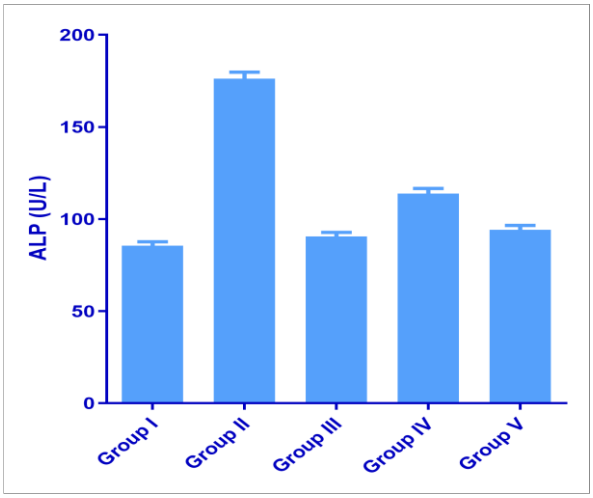

Figure 6: Effect of *Olax scandens* extract on ALP in paracetamol-induced hepatotoxicity in rats.

4. SUMMARY AND CONCLUSION

This study successfully evaluated the hepatoprotective activity of an ethanolic extract of *Olax scandens* leaves. The plant extract demonstrated a promising phytochemical profile, rich in phenols, flavonoids, and saponins. The in vivo study on paracetamol-induced hepatotoxicity in rats showed that the extract significantly reduced liver enzyme levels, indicating a strong protective effect. This research justifies the traditional use of *Olax scandens* for liver ailments and provides scientific evidence for its potential as a natural hepatoprotective agent. Further research is warranted to isolate and characterize the specific bioactive compounds responsible for this therapeutic effect.

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