



**EXTRACTION, PHYTOCHEMICAL SCREENING AND NEPHROPROTECTIVE EFFECTS OF HELICTERES ISORA LEAVES EXTRACT**

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**ABSTRACT**

Chronic Kidney Disease (CKD) is a progressive loss of kidney function that poses a significant global health burden. Nephrotoxicity, a major cause of kidney damage, is often induced by therapeutic agents, environmental toxins, and heavy metals. Traditional medicine offers a promising avenue for discovering effective and safer nephroprotective agents. This paper presents a review and an experimental study on the nephroprotective activity of an ethanolic extract of *Helicteres isora*, a medicinal plant traditionally used for various ailments. The study's findings indicate that the plant extract has a rich phytochemical profile with high levels of phenols and flavonoids. *In vivo* studies in rats with gentamicin-induced renal damage showed that the extract significantly improved kidney function markers. These results support the traditional use of *Helicteres isora* and suggest it has significant therapeutic potential in managing renal damage.

**KEYWORDS:** Chronic Kidney Disease, Nephrotoxicity, *Helicteres isora*, renal damage.

**1. INTRODUCTION**

Chronic kidney disease (CKD) is a progressive condition defined as a glomerular filtration rate (eGFR) of less than 60 ml/min/1.73 m<sup>2</sup> *persisting* for at least three months. It can be caused by various factors, including diabetes

mellitus, hypertension, glomerulonephritis, and polycystic kidney disease. The symptoms of CKD include fatigue, swelling (edema), changes in urination, nausea, and itchy skin.<sup>[1-3]</sup>

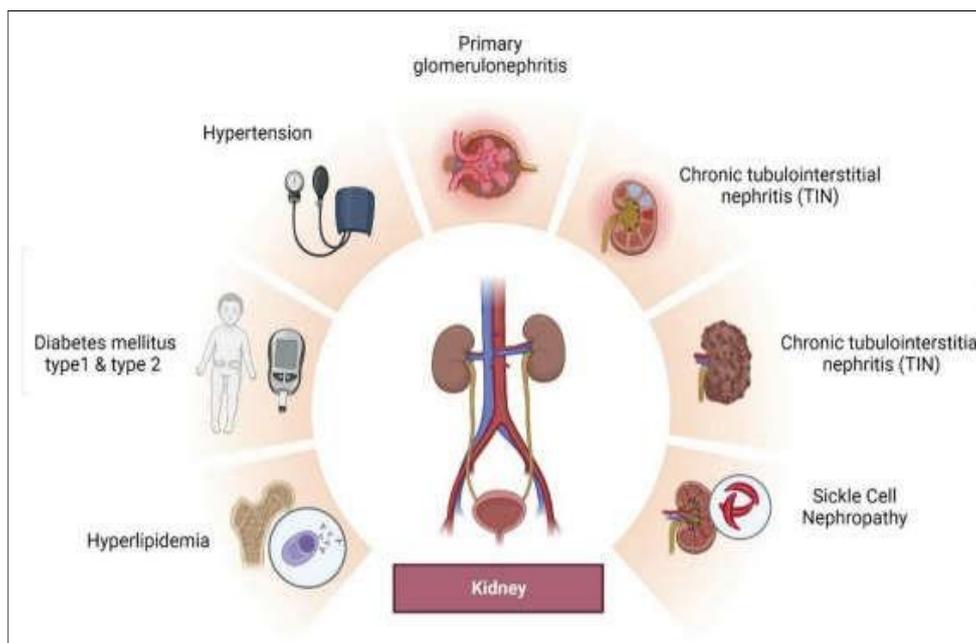


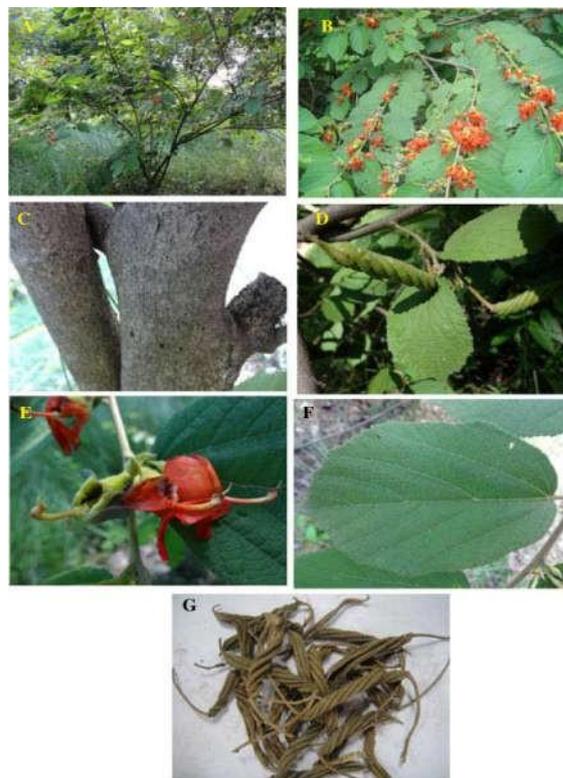
Figure 1: Examples of disease related to CKD.

Nephrotoxicity is the detrimental effect on the kidneys caused by drugs, chemicals, or toxins. It is a frequent cause of kidney disease and can be categorized into three stages: initiation, propagation, and termination. Many therapeutic agents, such as certain antibiotics and anticancer drugs, can lead to renal damage. Traditional medicine, which uses plants and plant extracts, has been used for centuries and is gaining popularity as a source of nephroprotective agents due to the potential for fewer side effects and lower cost compared to synthetic drugs. Plants contain bioactive components with antioxidant properties that can help treat illnesses related to oxidative stress and protect against various renal ailments (2-6). Kidneys play a crucial role in maintaining the body's homeostasis by regulating fluid balance, electrolyte levels, and the removal of metabolic waste products. However, various factors such as exposure to nephrotoxic drugs, environmental toxins, and oxidative stress can compromise renal function, leading to acute kidney injury or chronic kidney disease. Nephrotoxicity, characterized by impaired renal function, structural damage, and inflammation, is a major health concern with limited therapeutic options.

In recent years, there has been growing interest in the use of natural products with nephroprotective properties to prevent or mitigate renal damage. Medicinal plants are rich sources of bioactive compounds, such as flavonoids, phenolic acids, alkaloids, and tannins, which exhibit antioxidant, anti-inflammatory, and cytoprotective activities. These phytochemicals can attenuate oxidative stress, inhibit pro-inflammatory pathways, and enhance renal regeneration, thereby offering a promising alternative for kidney protection.

*Helicteres isora*, commonly known as East Indian Screw Tree, is a traditionally valued medicinal plant used extensively in Ayurvedic, Siddha, and various tribal systems of medicine. It has been employed for the treatment of numerous ailments, including gastrointestinal disorders such as diarrhea, dysentery, and indigestion, owing to its astringent and antidiarrheal properties. Traditionally, the fruits and bark are used for their anthelmintic, wound healing, and anti-inflammatory effects, while the decoction of roots and leaves is administered in respiratory complaints like cough and asthma. Notably, the plant is also reputed for its antidiabetic potential in folk medicine, where fruit extracts are used to regulate blood sugar levels. Additionally, it is employed in the management of reproductive disorders, liver ailments, and oral ulcers. Despite its broad spectrum of traditional applications, the scientific exploration of *Helicteres isora* remains limited. This study seeks to bridge the gap between traditional knowledge and modern scientific evidence, thereby contributing to the development of novel plant-based therapeutics. This research aims to explore the ability of *Helicteres isora* extract to protect against nephrotoxicity and preserve

renal function, thereby contributing to the development of novel, plant-based nephroprotective agents.



**Figure 2: Different parts of *Helicteres isora*.**

## 2. MATERIALS AND METHODS

The study was conducted using an ethanolic extract of the fruits of *Helicteres isora*. The plant parts were collected, dried, and powdered. The extraction was performed using a Soxhlet apparatus.

### 2.1. Preliminary Phytochemical Screening

The ethanolic extract was screened for the presence of various phytochemicals, including alkaloids, glycosides, saponins, flavonoids, phenolic compounds, tannins, proteins, carbohydrates, steroids, and triterpenoids.<sup>[7-12]</sup>

### 2.2. Quantitative Analysis

Total phenol content and total flavonoid content were estimated using standard methods with a calibration curve of Quercetin.

### 2.3. Evaluation of Nephroprotective Activity

The *in vivo* nephroprotective activity of the extract was evaluated using a gentamicin (GM)-induced renal damage model in Wistar albino rats. The study assessed the effect of the extract on several biochemical parameters, including serum creatinine, blood urea nitrogen (BUN), total proteins, albumin, and urea levels. The study used a standard drug for comparison.<sup>[12-16]</sup>

## 3. RESULTS AND DISCUSSION

The phytochemical screening revealed the presence of a variety of secondary metabolites. Quantitative analysis

showed that the ethanolic extract of *Helicteres isora* contained significant amounts of total phenols and flavonoids. The percentage yield of the extract was also measured.

The in vivo results demonstrated that the *Helicteres isora* extract had a nephroprotective effect against gentamicin-induced renal damage in rats. The extract effectively reversed the elevated levels of serum creatinine and blood urea nitrogen (BUN), which are markers of impaired kidney function. It also helped restore total

protein, albumin, and urea levels to near-normal ranges. These findings indicate that the extract mitigated the renal damage caused by gentamicin, likely due to the antioxidant properties of its phytochemical constituents.

The provided document mentions tables and figures displaying these results, such as "Effect of *Helicteres isora* extract on serum creatinine levels" and "Effect of *Helicteres isora* extract on blood urea nitrogen levels". However, the actual numerical data to generate these graphs and tables is not included in the file.

**Table 1: Result of phytochemical screening of extract of *Helicteres isora*.**

S. No.	Constituents	Ethanolic extract
1.	Alkaloids A) Hager's Test:	-Ve
2.	Glycosides A) Legal's Test:	-Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	+Ve +Ve
4.	Saponins A) Froth Test:	-Ve
5.	Phenol A) Ferric Chloride Test:	-Ve
6.	Proteins A) Xanthoproteic Test:	-Ve
7.	Carbohydrate A) Fehling's Test:	+Ve
8.	Diterpenes A) Copper acetate Test:	-Ve
9.	Tannins A) Gelatin Test	-Ve
10.	Sterols A) Salkowski Test	-Ve



**Figure 3: Phytochemical screening of ethanolic extract of *Helicteres isora*.**

The phytochemical screening of the ethanolic extract of *Helicteres isora* revealed the presence of certain key constituents while others were absent. Notably, flavonoids and carbohydrates were detected, as evidenced by the positive results in the Lead acetate,

Alkaline reagent, and Fehling's tests. The presence of flavonoids is particularly significant due to their well-documented antioxidant, and nephroprotective properties, which may contribute to the plant's therapeutic potential. On the other hand, tests for

alkaloids, glycosides, saponins, phenols, proteins, diterpenes, tannins, and sterols yielded negative results, suggesting their absence or presence in concentrations below detectable limits. The selective presence of

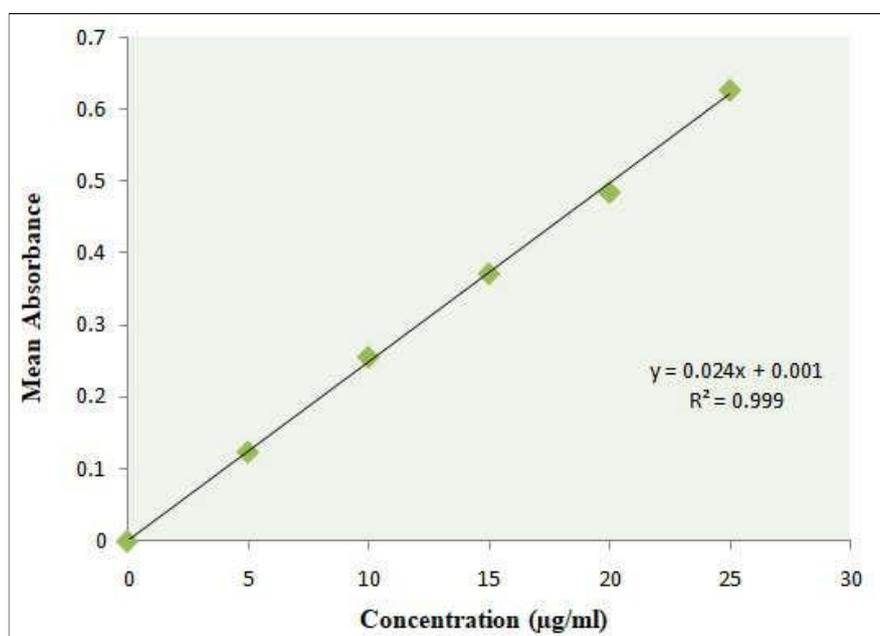
phytochemicals may reflect the solvent's specificity and the plant's intrinsic chemical profile, highlighting the importance of solvent choice in phytochemical extraction.

### 7.1 Results of estimation of total flavonoids content (TFC)

**Table 2: Preparation of calibration curve of Quercetin.**

S. No.	Concentration ( $\mu\text{g/ml}$ )	Mean Absorbance
1	5	0.124
2	10	0.256
3	15	0.372
4	20	0.485
5	25	0.627

N=3 Average of three determination



**Figure 4: Graph of calibration curve of Quercetin.**

**Table 3: Estimation of total phenol and flavonoids content of *Helicteres isora*.**

S. No.	Ethanollic extract	Total flavonoids content
1.	<i>Helicteres Isora</i>	1.62 mg/100mg

The total flavonoid content of the ethanolic extract of *Helicteres isora* was found to be 1.62 mg per 100 mg of extract, indicating a moderate concentration of flavonoids. The moderate flavonoid content also supports the traditional medicinal use of *Helicteres isora* and provides a quantitative basis for further pharmacological evaluation.

#### Results of *in vivo* nephroprotective activity of *Helicteres isora* extract

Gentamicin administration (Group II) markedly elevated serum creatinine ( $2.14 \pm 0.08$  mg/dL), blood urea nitrogen ( $49.8 \pm 1.35$  mg/dL), and urea ( $65.3 \pm 1.12$  mg/dL) levels compared to the normal control group (Group I), which showed  $0.65 \pm 0.03$  mg/dL,  $18.4 \pm 0.72$  mg/dL, and  $29.6 \pm 0.81$  mg/dL respectively. Concurrently, serum albumin and total protein levels significantly dropped in

the gentamicin group ( $2.3 \pm 0.09$  g/dL and  $4.2 \pm 0.15$  g/dL) relative to the control group ( $4.5 \pm 0.12$  g/dL and  $6.9 \pm 0.18$  g/dL).

Treatment with *Helicteres isora* extract at 100 mg/kg (Group III) showed a moderate nephroprotective effect, evidenced by reduced serum creatinine ( $1.32 \pm 0.06$  mg/dL), blood urea nitrogen ( $34.6 \pm 1.11$  mg/dL), and urea levels ( $48.7 \pm 1.05$  mg/dL), along with increased albumin ( $3.5 \pm 0.10$  g/dL) and total protein ( $5.6 \pm 0.14$  g/dL).

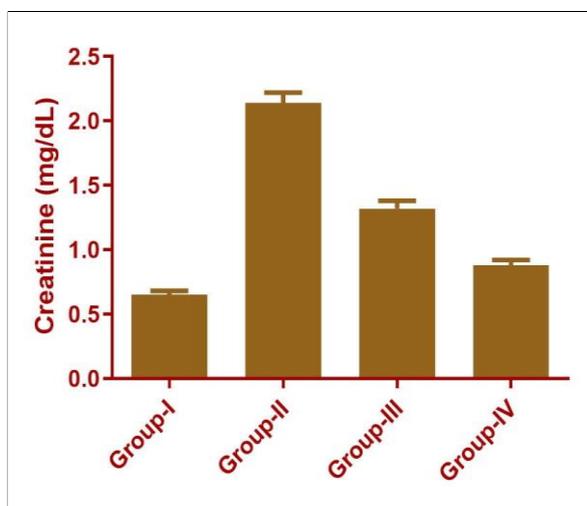
Notably, *H. isora* extract at 200 mg/kg (Group IV) exhibited more pronounced protective effects, with near-normal levels of serum creatinine ( $0.88 \pm 0.04$  mg/dL), blood urea nitrogen ( $25.7 \pm 0.89$  mg/dL), and urea ( $36.2 \pm 0.93$  mg/dL), and improvement in albumin ( $4.1 \pm 0.08$  g/dL) and total protein ( $6.4 \pm 0.13$  g/dL).

suggesting dose-dependent renal protection.

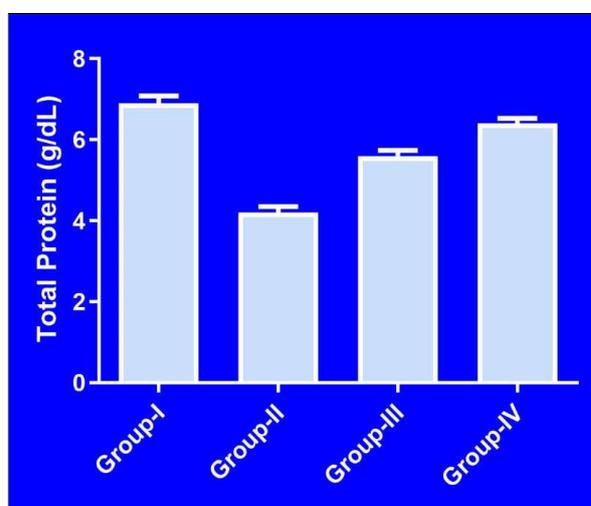
**Table 4: Effect of *Helicteres isora* extract on serum creatinine, blood urea nitrogen, total proteins, albumin and urea levels in GM-induced renal damage in rats.**

Group	Treatment	Serum Creatinine (mg/dL)	Blood Urea Nitrogen (mg/dL)	Serum Albumin (g/dL)	Total Protein (g/dL)	Urea (mg/dL)
Group I	Normal Control (0.5% CMC)	0.65 ± 0.03	18.4 ± 0.72	4.5 ± 0.12	6.9 ± 0.18	29.6 ± 0.81
Group II	Gentamicin (GM) (100 mg/kg)	2.14 ± 0.08#	49.8 ± 1.35#	2.3 ± 0.09#	4.2 ± 0.15#	65.3 ± 1.12#
Group III	H. isora Extract (100 mg/kg) + GM	1.32 ± 0.06*	34.6 ± 1.11**	3.5 ± 0.10*	5.6 ± 0.14*	48.7 ± 1.05**
Group IV	H. isora Extract (200 mg/kg) + GM	0.88 ± 0.04***	25.7 ± 0.89***	4.1 ± 0.08**	6.4 ± 0.13**	36.2 ± 0.93***

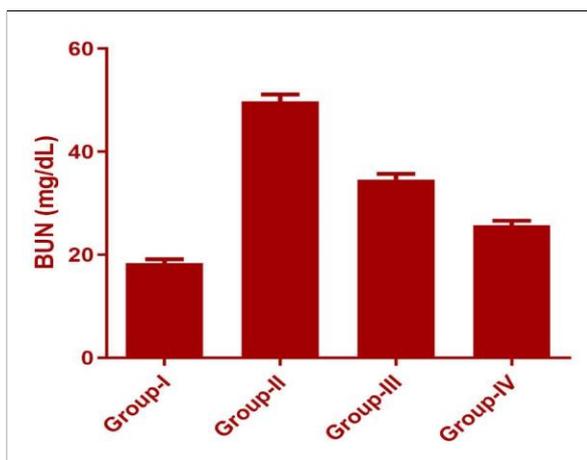
Results are expressed as Mean ± SEM (n = 6). #p < 0.001 as compared to Normal Control rats and \*p < 0.01, \*\*\*p < 0.001 compared to Gentamicin rats



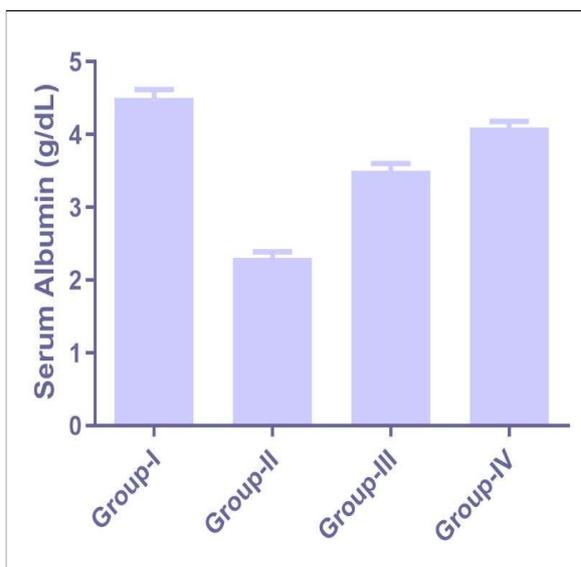
**Figure 5: Effect of *Helicteres isora* extract on serum creatinine levels in GM-induced renal damage in rats.**



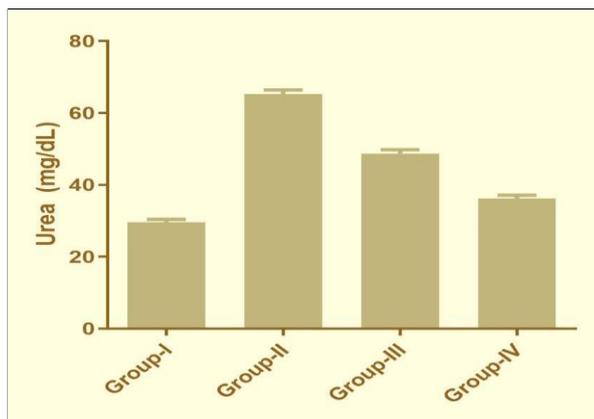
**Figure 7: Effect of *Helicteres isora* extract on total protein levels in GM-induced renal damage in rats.**



**Figure 6: Effect of *Helicteres isora* extract on blood urea nitrogen levels in GM-induced renal damage in rats.**



**Figure 8: Effect of *Helicteres isora* extract on albumin levels in GM-induced renal damage in rats.**



**Figure 9: Effect of *Helicteres isora* extract on urea levels in GM-induced renal damage in rats.**

#### 4. SUMMARY AND CONCLUSION

This study confirms the nephroprotective potential of an ethanolic extract of *Helicteres isora* against gentamicin-induced renal damage in rats. The plant extract, rich in phenols and flavonoids, demonstrated a significant ability to protect kidney function, as evidenced by its effect on serum creatinine, BUN, and other biochemical markers.

In conclusion, GM at 100 mg/kg, administered intraperitoneally, causes notable kidney injury. The potential protective effect of *Helicteres isora* may stem from its ability to restore serum biochemical parameters. As a strong free radical scavenger, *Helicteres isora* offers protection against GM-induced kidney damage. Therefore, this research highlights *Helicteres isora* as a promising nephroprotective agent, with 100 and 200 mg/kg doses being more effective than 200 mg/kg, suggesting a dose-dependent response in preventing GM-related renal injury.

These findings provide scientific validation for the traditional use of *Helicteres isora* for kidney-related ailments and support its potential as a source for a new nephroprotective drug. Further research is needed to isolate the specific compounds responsible for this activity.

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