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Original Article

Development and Evaluation of Nephroprotective Polyherbal Formulation against Methotrexate Induced Toxicity in Sprague Dawley Rat

Arijit Chaudhuri[1,2], Udichi Kataria[1,*], Subodh Kumar Dubey[2], Neha Chopra[2], Devendra Dhanorya[2], Kamal Shah[3], Nagendra Singh Chauhan[3]

[1]Geetanjali Institute of Pharmacy, Geetanjali University, Udaipur, Rajasthan, INDIA.
[2]School of Pharmacy, ITM University, Gwalior, Madhya Pradesh, INDIA.
[3]Institute of Pharmaceutical Research, GLA University, Mathura, Uttar Pradesh, INDIA.

ABSTRACT

Methotrexate (MTX) is one of the most broadly used anti-cancer derivative of aminopterin and it is a first folic acid antagonist. Adult cancer, various malignancies, multiple sclerosis, dermatomyositis, sarcoidosis, psoriasis, rheumatoid arthritis, and severe inflammatory disorders are all commonly treated with MTX. Medicinal plants and herbs contribute, wide range of treatment and prevention from several diseases. Herbal formulations mean dosage form consisting of one or more plants prepared herbs in selected quantities to cure mitigate diseases, by treatment, prevention and sometimes use as a supplement. *Petrida foetida* is used as traditionally diarrhea, stomach ache and nutritional supplement etc. Vitis vinifera, the fruit is used for dietary and the leaves and seeds are used as several herbal therapy. The current study had carried out to evaluate the nephroprotective activity of leaves and seed extracts of *Petrida foetida* and *Vetis venifera.* Nephroprotective activities of ethanolic and aqueous extracts of *Petrida foetida* and *Vitis vinifera* seeds were examined against methotrexate induced Kidney damage in Sprague Dawley Rats. Biochemical parameter evaluates like Urea, Creatinine, Uric acid, Blood urea nitrogen, total protein like albumin, globulin was analyzed. *Petrida foetida* and *Vitis venifera* seeds extract of exhibited significant ($p<0.05$) nephroprotective activity. Ethanolic seeds extract of *Vitis vinifera* and *Petrida foetida* menifest moderate activity upon Methotrexate induced Rat models. Study outcome shows the traditional-ethno medicinal use of Methotrexate as a potential source of Nephroprotective activity.

Keywords: Methotrexate (MTX), Nephroprotective activity, Polyherbal formulation (PHF), Creatinine, Blood Urea Nitrogen (BUN).

Correspondence:
Prof. Udichi Kataria
Geetanjali Institute of Pharmacy, Geetanjali University, Udaipur, Rajasthan, INDIA.
Email: udichi.gip@gmail.com,

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INTRODUCTION

Methotrexate (MTX) is one of the most broadly used anticancer[1] derivative of aminopterin and it is a first folic acid antagonist. Adult cancer, various malignancies, multiple sclerosis, dermatomyositis, sarcoidosis, psoriasis, rheumatoid arthritis, and severe inflammatory disorders are all commonly treated with MTX. The Methotrexate kill the malignant cancerous cells, during treatment it can also affect the normal body tissues. Hence long-time use of this kind of drug causes several toxicities for different organs in our healthy body.[2] The most severe side effects of Methotrexate intake for any patients are causes nephrotoxicity. One of the notable limitations on its clinical use in the necessary levels is nephrotoxicity. The formation and proliferation of nephrotoxicity is triggered by methotrexate's oxidative stress, which results in reactive oxygen species and decreases antioxidant defence mechanisms. According to numerous studies, inflammatory cytokines including Tumour Necrosis Factor-alpha (TNF-alpha) and inducible Nitric Oxide Formation (iNOS) are what first cause methotrexate-induced nephrotoxicity.[1,2]

Vitis vinifera is a commonly known grape species cultivated in Asian countries India, China, Bangladesh and Pakistan that belongs to Vitaceae family. The *Vitis vinifera* fruit is used as nutritional supplement, and the leaves and seed are used as herbal therapy.[3] The most well-known application of black grape, is in red wine manufacturing followed by juices, raisins and several food supplements.[3] *Vitis vinifera* contain various quantities of gallic acid, ferulic acid and caffeic. It is most important flavanols in black grapes seed extract Malvidine-3-glucosite, piceatannol, resveratrol etc.[4] The fruits consist of

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highly antioxidant properties, anti-cancer properties, and used for cure for heart disease, high cholesterol, high blood pressure.[3] The black grape is one of the most common delicious, nourishing and refreshing fruits. *Vitis vinifera* seeds have contain Phenolic compound their pharmacological properties including skin disease protection, anti-cancer, antioxidant properties, and anti-bacterial, anti-fungal, anti-inflammatory and anti-diabetic activities are reported.[5]

Another plant *Paederia foetida* is belongs to Rubiaceae family play most important role in village ancient era. They are used as food dish, fodder, crude oil, and other folk medical practice.[5] *Paederia foetida* is needful in curing widely used in paralysis, rheumatism, gout, abscesses, dysentery, diarrhea, colic and flatulence and infertility.[6,7] These plants roots are used to relieve to discharge gas gastric patients. Plant fruits are relief in tooth pain and cleaning teeth; abdominal visceral pain, abscesses, and rheumatoid arthritis are treated with a decoction of the whole plant. Traditional cure for dysentery are popular in several Asian countries the India, China, Afghanistan, Bangladesh. The Fruit relieved with a compress of leaves. The bark and root of *Paederia foetida* are traditionally used for curing Pepticulcer and vomiting.[8]

MATERIALS AND METHODS[5,8]

Plant material

Vitis vinifera (Black resins) Seeds as shown in Figure 1 are collected from the Local market. Identified by a botanist at Geetanjali University. *Paederia foetida* Plant as shown in Figure 2 is obtained from the outskirts of West Bengal. Identified by a botanist at Geetanjali University, Rajasthan. The leaves were shade-dried and made coarsely powdered for extraction purposes. A voucher specimen of *Vitis vinifera* and *Paederia foetida* was collected in the herbarium of the University for future reference.

Extract preparation

Using the Soxhlet extraction method, ethanol (95%) was extracted from the powdered leaves of *Paederia foetida*. In order to thoroughly eliminate the solvent from the extracted material, it was filtered while still hot, and the resulting extract was then concentrated under reduced pressure in a vacuum. It was then dried in a desiccator. After that, an airtight container was used to store the ethanol extract from the leaves for future research.

Animals

The nephroprotective activity was carried out on Sprague Dawley rats of either sex (150-200 g), SKPCPER, Mehsana by Shree SK Patel College of Pharmacy Education and Research Animal House Facility, Ahmedabad. The rats were maintained in a 12 hr light/dark cycle at 25 ± 2°C. They were allowed free access to a standard hygiene pellet diet (Shree SK Patel College of Pharmacy Education and Research Animal House Facility) and water *ad libitum*. The bedding of animals was changed every 3rd day. The study was approved by the ethics committee CPCSEA and ethical norms were strictly followed during all experimental procedures. (CPCSEA Registration No: SKPCPER/IAEC/2022-01/02).

Polyherbal formulation

Formulations	Plant extracts (gm)	
	A	B
PHF-I	30	30
PHF-II	20	40
PHF-III	40	20

A-Indicate *Vitis vinifera* plant seed extract; B-indicate *Petrida foetida* plant extract.

Methodology[9]

Rats will be randomly divided into five groups with six rats each:

Group 1: Control group is given ordinary saline up until the experiment's conclusion.

Group 2: will receive a single injection of methotrexate (20 mg/kg, i.p.) on the first, seventh, fourteenth, twenty-first, thirty-fifth, and forty-second days.

Group 3: received a single injection of methotrexate (20 mg/kg, i.p.) on the first, seventh, fourteenth, twenty-first, twenty-eighth, thirty-fifth, and forty-second days.

Group 4: received a single injection of MTX (20 mg/kg, i.p.) on the first, seventh, fourteenth, twenty-eighth, twenty-eighth, twenty-eighth, and forty-second days.

Groups 5: 225 mg/kg of PHF-III was administered.

After 42 days, all the animals were fasted for 18 hr, and blood samples were collected from the retro-orbital plexus puncture with the last oral administration 1 hr before. The serum samples were obtained and analyzed for renal function.

Biochemical parameters

Kidney function was assessed using blood tests for urea, creatinine, uric acid, and total protein.[1,5,6]

Statistical analysis

The Mean±SEM for each group was used to represent the results. After doing a one-way Analysis of Variance (ANOVA), Dunnett's *t*-test was used to assess statistical differences. At $p<0.05$, the results were deemed statistically significant.[10]

Histopathlogical studies

On the 42nd day of the histopathological examination, the animals were given halothane anaesthesia so that blood samples could be collected from the retro-orbital plexus and allowed to clot. Until needed, serum samples will be collected and stored at -80°C. Then,

rats will be sacrificed by halothane anaesthesia overdose, with all possible measures taken to reduce agony. Dissection and ice-cold saline washing of kidney tissues will take place. The preparation of kidney homogenates involves homogenising the samples in Phosphate-Buffered Saline (PBS, pH 7.4), centrifuging the resulting supernatants, and storing them at -80°C until analysis. For histological analysis, an additional kidney sample will be removed and fixed in 10% neutral buffered formalin solution.

RESULTS

In a model of methotrexate-induced nephrotoxicity, the nephroprotective effectiveness of the relevant Poly herbal formulations 1, 2, and 3 at 225 mg/kg body weight was assessed. The concentration of serum urea, creatinine, uric acid, total protein, and blood urea nitrogen were significantly higher in animal groups treated with methotrexate than in control groups, indicating severe nephrotoxicity. In comparison to Methotrexate-treated groups, treatment with the polyherbal formulation of PHF-I, PHF-II, and PHF-III resulted in a significant decrease ($p<0.05$) in the concentration of serum urea, creatinine, uric acid, total protein, and Blood Urea Nitrogen (BUN), as well as body weight, urine volume, urine pH, and kidney weight. It stated that the renal protective qualities of the polyherbal PHF-I, PHF-II, and PHF-III formulation.

Figure 1: *Vitis vinifera.*

Figure 2: *Paederia foetida*.

Kidney Histopathological Images

In normal control group rat kidney tissue sections, a normal structure of glomeruli, proximal and distal convoluted tubule was shown in given (Figure 3).

In diseased control MTX group, kidney sections of Sprague dawley rats view the signs of Pathological and morphological changes (Figure 4). In the kidney glomerular area, several various glomerulus observed widening of Bowman's capsule space, atrophic changes with obvious necrosis of kidney cells and losing the prominent glomerular structure suggesting apoptosis.

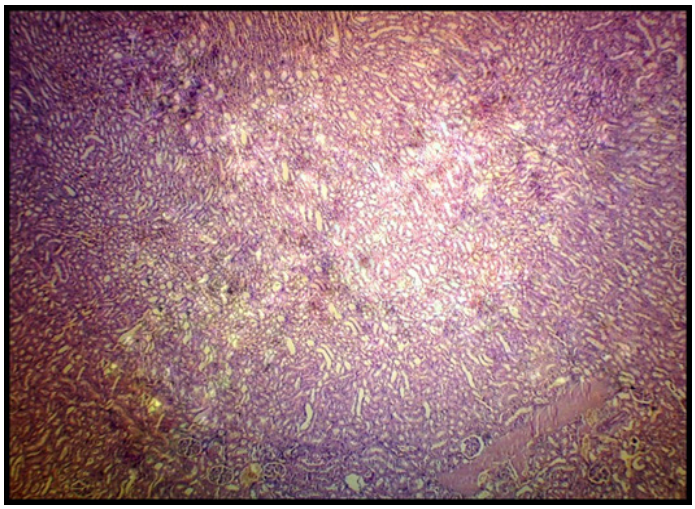


Figure 3: Normal Control group.

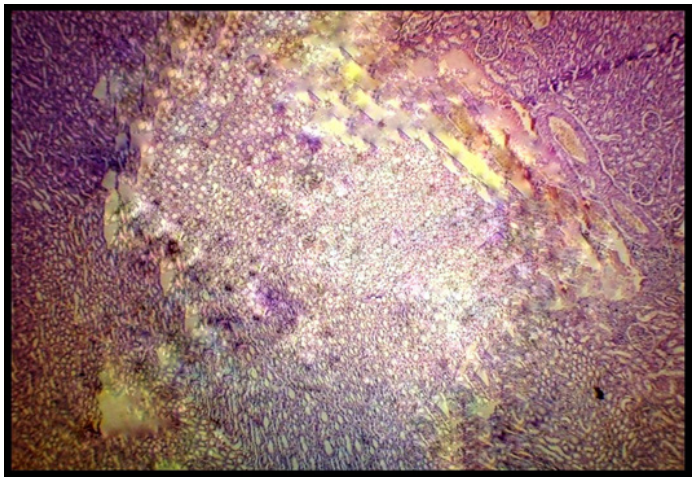


Figure 4: Group 2 Methotrexate group MTX.

Table 1: Effect of polyherbal formulation on Kidney function test in animals treated with Methotrexate.

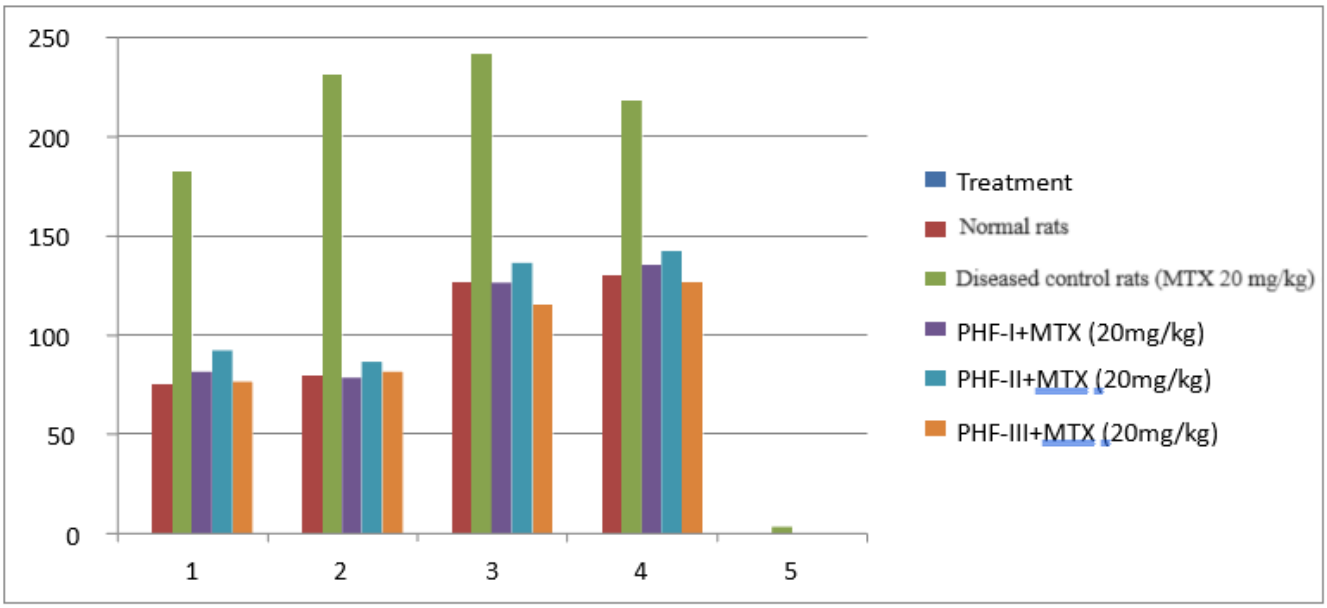
Treatment	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)	BUN (mg/dL)	Total protein (gm/dL)
Normal rats	41.53±0.27	1.13±0.92	0.62±0.74	20.72±0.32	4.19±0.41
Diseased control rat (Methotrexate 20 mg/kg)	163.74±0.58*	8.43±0.63*	2.89±0.42*	110.38±0.59*	17.21±0.83*
PHF-I + MTX (20 mg/kg)	44.28±0.47[a]	1.41±0.51[a]	0.66±0.29[a]	31.48±0.72[a]	4.71±0.79[a]
PHF-II + MTX (20 mg/kg)	50.31±0.35[a]	1.73±0.29[a]	0.72±0.18[a]	36.84±0.48[a]	5.02±0.28[a]
PHF-III + MTX (20 mg/kg)	38.57±0.71[a]	1.23±0.46[a]	0.59±0.67[a]	28.17±0.63[a]	4.23±0.34[a]

n = 6 in each group; values are expressed as mean SEM; *p<0.05 compared to control group; [a]p< 0.05 compared to MTX-treated group considered statistically significant.

Table 2: Effect of polyherbal formulation on Body weight, Urine volume, Urine pH and Kidney weight treated in rats

Treatment	Body weight (gm)	Urine volume (mL)	Urine pH	Kidney weight (gm)
Normal rats	179.32±1.05	25.04±0.47	5.72±0.62	1.35±0.41
Diseased control rat (MTX 20 mg/kg)	128.61±0.85*	10.72±0.24[a]	9.32±0.41*	3.47±0.58*
HF1 + MTX (20 mg/kg)	174.65±1.21[a]	25.17±0.68[a]	5.84±0.38[a]	1.38±0.67[a]
HF2 + MTX (20 mg/kg)	172.39±1.36[a]	23.67±0.25[a]	5.91±0.57[a]	1.43±0.97[a]
HF3 + MTX (20 mg/kg)	175.27±0.49[a]	26.58±0.53[a]	5.82±0.73[a]	1.39±0.38[a]

n = 6 in each group; values are expressed as mean SEM; *p<0.05 compared to control group; [a]p<0.05 compared to MTX-treated group considered statistically significant.



Graph 1: Effect of Polyherbal Formulation (PHF) on kidney function test for different parameters in animals treated with Methotrexate (MTX).

In group 3, "treatment 1" Sprague Dawley Rat kidney tissue sections, showing a minimal damage of glomeruli, proximal and distal convoluted tubule was shown, as compared to MTX group as well as normal control group (Figure 5).

Above histopathological (Figure 6) representation of kidney sections of group-4 PHF-II Treatment in Sprague Dawley Rat. PHF-II gave well nephrotoxic protection against MTX, as compare to group 1, control group and group 2, MTX only. Rat's kidney with histopathological minor changes in atrophic glomeruli.

Above histopathological (Figure 7) of represent kidney sections of group 5 PHF-III Treatment in Sprague Dawley Rat. PHF-III

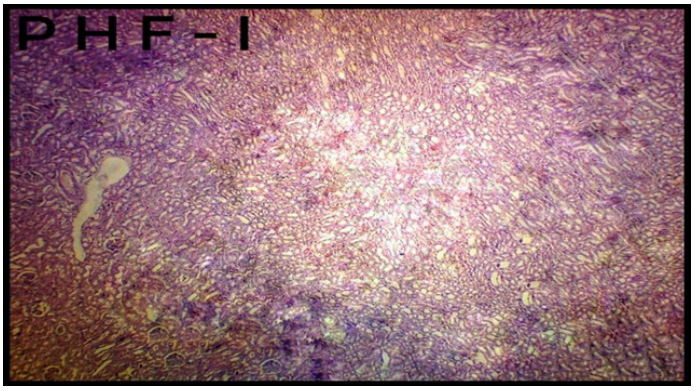


Figure 5: Group 3 PHF-I.

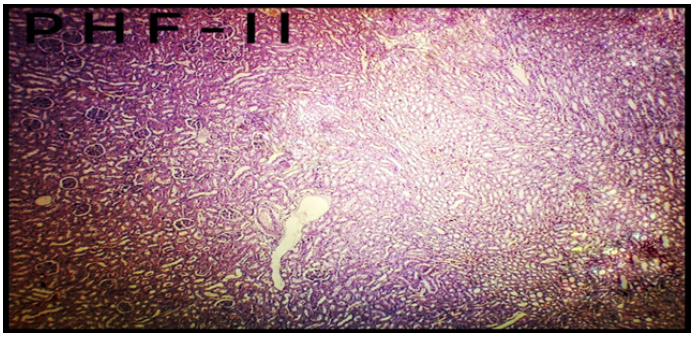


Figure 6: Group-4 PHF-II.

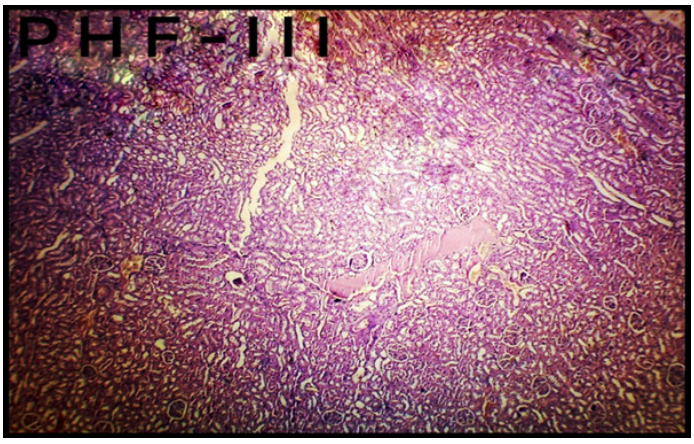


Figure 7: Group-5 PHF-III.

gave better nephrotoxic protection against MTX, as compare to group 1, control group and group 2, MTX only. rat kidney with histopathological minor changes in atrophic glomeruli.

DISCUSSION

In our present work, an attempt was made to evaluate the renal protective activity of *Petrida foitida* and *Vitis vinifera*, against Methotrexate-induced renal toxicity in Sprague Dawley rats. The ethanol fraction of the plant was subjected to preliminary phytochemical investigations and further *in vitro* screenings. The results from a phytochemical investigation of the herbal extract revealed the presence of wide classes of phytoconstituents including Phenolic compounds, glycosides, flavonoids, tannins, and proteins.in it. Finally, we isolated 2 active components *Petrida foitida* and *Vitis vinifera* Experimental rats treated with Methotrexate developed significant renal damage as well as an increase in oxidative stress. The renal damages were evident from a substantial increase in the levels of serum Creatinine, and Blood Urea Nitrogen (BUN). The changes in the levels of these biochemical were indicative of cellular leakage and loss of functional integrity of cell membrane in the kidney as shown in Tables 1 and 2. Kidney damage was assessed by biochemical studies and by histopathological and gross necropsy examinations of the respective organs. Methotrexate produced substantial damage that histologically resembles viral nephritis. Toxicity begins with changes in the endoplasmic reticulum resulting in the loss of metabolic enzymes located intracellularly. The cytotoxic drug Methotrexate results in the production of free radicals which further react with oxygen to produce trichloromethyl peroxy radical. Cytochrome P450 is the group of enzymes responsible for these conversions. These radicals bind covalently to the macromolecules and cause oxidative degradation of lipid membranes of the adipose tissues. In this view, reduction in the levels of creatinine, blood urea nitrogen, sodium, potassium, and serum protein treated with *Petrida foitida* and *Vitis vinifera* exhibited stabilization of plasma membrane as well as repair of renal tissue damage caused by Methotrexate. Histological reports revealed that administration of Methotrexate caused degeneration of fatty acids cysts, infiltration of lymphocytes, proliferation of JG cells, and congestion of the kidney. Administration of *Petrida foitida* and *Vitis vinifera* decreased the elevated levels of biochemical markers including blood Creatinine and Blood Urea Nitrogen levels, and sodium potassium level. Pharmacological observations showed that renal bowmen's capsule architecture was normalized, fewer GFR infiltrations were seen and JG cell proliferation appeared normal. These observations suggest that the treatment drugs possessed renal protective activity against Methotrexate-induced renal toxicity. Moreover, the preliminary phytochemical analysis of the herbal drugs showed the presence of flavonoids and phenolic compounds having reported renal protective effects. It has been hypothesized that one of the principal causes of Methotrexate-induced kidney injury is the formation of lipid peroxidase by free radicals derivative Lt4. The body possesses defense mechanisms to prevent and neutralize free radical-induced damages. Weights of excised kidneys of all animals were recorded and a slight increase in the kidney weights was observed in the Methotrexate treated group. Renal toxicants such as Methotrexate require metabolic activation particularly by the kidney cytochrome P450 enzymes to form reactive, toxic metabolites which in turn cause kidney injury in experimental animals. It is well known that free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation with the associated tissues. All treatment drugs showed appreciable antioxidant property and thereby protected against oxidative damaged of cells. The treatment with *Petrida foitida* and

Vitis vinifera normalized the methotrexate induced biochemical and tissue abrasions. In line with the results and conclusions drawn, it may be suggested that renal protective activity against challenge is probably due to its free radical scavenging activity and prevention of lipid peroxidation.

CONCLUSION

According to the results of the current investigation, pretreatment with ethanol extracts of *Vitis vinifera* and *Petrida foetida* reduced the kidney damage caused by MTX. As a result, administering PHF-I, PHF-II, or PHF-III may lessen methotrexate's negative effects without sacrificing its effectiveness. Future research is needed to determine whether *Vitis vinifera* and *Petrida foetida* can be used to treat methotrexate-induced toxicities in other organs and organ systems. This research may help to increase the therapeutic efficacy of these two plants.

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This work is an end of my wonderful journey leading to Ph.D. in Pharmacology. During this period, I have been accompanied and supported by many people, without whom it would have been impossible for me to accomplish the task. Now it's time for me to acknowledge the efforts of all those people who have contributed to my work, described in this report.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MTX: Methotrexate; **BUN:** Blood Urea Nitrogen; **SOD:** Superoxide Dismutase; **GSH:** Reduced Glutathione; **MDA:** Malondialdehyde.

AUTHORS' CONTRIBUTIONS

AC, UK, HS, performed whole experimental procedures. All authors read and approved the final manuscript.

SUMMARY

This study developed a polyherbal formulation to counteract nephrotoxicity induced by Methotrexate (MTX) in Sprague Dawley rats. The formulation, derived from traditional medicinal herbs, demonstrated significant improvement in renal function compared to the MTX-alone group. Biochemical analysis revealed lowered serum creatinine and blood urea nitrogen levels, while histopathology showed reduced tubular damage and inflammation. The formulation also exhibited potent antioxidant activity, suggesting its role in mitigating oxidative stress. These findings highlight the potential of the polyherbal formulation as a nephroprotective agent in MTX therapy. Further research is needed to validate its clinical applicability.

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