

EVALUATION OF NEPHROPROTECTIVE ACTIVITY OF *NEFROWIN* (A POLYHERBAL FORMULATION) ON CISPLATIN INDUCED NEPHROTOXICITY IN MALE WISTAR ALBINO RATS.

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ABSTRACT

Objectives: The present study is mainly focused to experimentally elucidate the nephroprotective potential of Nefrowin (a polyherbal formulation for renal disorders) against cisplatin (5mg/kg, ip) induced nephrotoxicity in albino wistar rats. Cisplatin (Cis-diaminedichloroplatinum II) is one of the most effective chemotherapeutic agents having well documented adverse effect as nephrotoxicity. Cisplatin induced nephrotoxicity depend on several signaling pathways leading to apoptosis in tubular epithelial cells and also results in the depletion of renal antioxidant defense system. **Methods:** Wistar rats (n = 6) were allocated into six groups constituting CMC control, Olive oil control, Toxic control, Standard group (Vitamin E 250mg/kg), Nefrowin I (500 mg/kg), and Nefrowin II (1000 mg/kg) administered orally for a period of 10 days. Intra-peritoneal injection of cisplatin was administered on day 7, to all groups except CMC and Olive-oil Control. At the end of the experiment, 24 hour urine was collected for urinary estimation and blood samples were collected by retro-orbital puncture method for serum estimation (creatinine, urea, uric acid, and total protein). Thereafter all the animals were sacrificed by means of cervical dislocation and kidney were dissected out immediately for both ex-vivo and histopathological examinations. **Results:** In Cisplatin treated group of animals the concentration of serum urea, Creatinine, Uric acid, and Urine creatinine were considerably increased and serum total protein considerably decreased than the normal animals which indicates severe nephrotoxicity. Treatment with Nefrowin significantly ($p < 0.05$) restored the levels of serum creatinine, urea, sodium, protein and potassium. Significantly ($p < 0.05$) increased the antioxidant defense enzyme levels of SOD& GSH on treatment with Nefrowin. **Conclusion:** On evaluating biochemical parameters, antioxidant enzyme and histopathological examinations it was found that the Nefrowin showed a significant nephro-protective activity in cisplatin induced nephrotoxicity.

KEYWORDS: Nefrowin, Cisplatin, Nephrotoxicity.

INTRODUCTION

Nephrotoxicity is one of the leading causes of morbidity and mortality and its prevalence is continuously increasing in industrialized nations. Nephrotoxicity (from Greek: nephrons, "kidney") is a poisonous effect of some substances, both toxic chemicals and medications, on the kidneys. Nephrotoxicity should not be confused with the fact that some medications have a predominantly renal excretion and need their dose adjusted for the decreased renal function. The functional manifestations of nephrotoxicity may occur at several levels like tubular function abnormalities such as potassium, magnesium and sodium wasting, concentrating defects and reduction in glomerular filtration. There are no ideal clues to the occurrence or localization of tubular cell injury. The nephrotoxin

induced changes in the tubule cells may be lethal or sub lethal.

Nephrotoxicity was defined in 1991 by the World Health Organization as a renal disease or dysfunction that arises as a direct or indirect result of exposure to medicines and industrial or environmental chemicals. Chemicals or substances that have nephrotoxic effects are often called nephrotoxins. Nephrotoxic agents are produce damage either by directly reacting with cellular macromolecules and membrane components or from metabolism within the tubular cells to toxic products. These are the agents which cause direct toxicity are, heavy metals like Hg, Pb which interact with sulfhydryl groups, organic cations such as spermine, cationic aminoacids, aminoglycosides, which will interacts with membrane phospholipids, polyene antibiotics like amphotericin B which interacts

with membrane cholesterol. Fluoride and oxalates produced by hepatic metabolism of methoxyflurane, intermediates of cisplatin, cystine conjugates, cephalodrine and acetaminophen induce damage by their metabolites. These toxic metabolites mainly induce free radicals.

Cisplatin is one of the highly effective inorganic platinum based oncologic medications used in the chemotherapy for treating ovarian, bladder, testicular, cervical, and various solid or hematologic tumors. However, its use is limited by tumor cell resistance and various side effects such as suppression of bone marrow, renal toxicity, ototoxicity, emesis and neural toxicity. The development of cisplatin nephrotoxicity is complex and a number of interrelated factors such as transporter mediated cisplatin accumulation, conversion into nephrotoxins, formation of DNA adducts, mitochondrial dysfunction, nitrosative and oxidative stress, inflammation, signal transducers and apoptotic pathway activation are involved.

India has a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. Traditional health care has been flourishing in this country for many centuries. Ayurveda and other Indian systems of medicines may be explored with the modern scientific approaches for better leads in the health care. In spite of tremendous advances in modern medicine, traditional herbal medicines have been practiced with utmost advantage for patients.

Nefrowin, a polyherbal capsule manufactured by Nupal Remedies Pvt Ltd, Cochin, is a time tested formulation for the successful treatment of Nephritis. Nefrowin is a formulation of completely safe herbal ingredients for the successful treatment of kidney disorders especially for Nephritis and ensures optimum level of blood urea. This unique combination is derived from the manuscripts of ancient times. As per the manufacturer's information nefrowin consists of *Achyranthes aspera*, *Asteracantha longifolia*, *Musa paradisiaca*, *Crataeva nurvala*, *Barleria prianitis*, *Asperagus racemosus*, *Plumbago zeylanica*, *Chonemorpha fragrans*, *Aegle marmelos*, *Aristolochia bracteata*, *Solanum indicum*, *Pongamia pinnata*, *Premna serratifolia*, *Terminalia chebula*, *Moringa oleifera* etc. Individually many of these herbs are scientifically screened and reported to possess rejuvenative, nephroprotective, anti-urolithiatic potential along with diuretic, anti-inflammatory and anti-microbial activities. Since large mass of populations used preferable herbal preparation, therefore there is need to evaluate the proper mechanism underlying the nephroprotective activity which can enhance the therapeutic potential of the formulation bringing acceptance among a huge population.

The present study is focused to evaluate the Nefrowin for claimed nephroprotective potential by using cisplatin induced nephrotoxicity model in rats.

MATERIALS AND METHODS

Experimental Animals

Thirty three healthy adult Wistar rats of either sex, age between 4-5 months, weighing about 150-200g were procured from Small animals breeding station, Kerala Veterinary and Animal Sciences University, Trissur and housed in polypropylene cages. They were kept under standard laboratory conditions; fed with standard pellet diet and water ad libitum. Prior to experimental work, all the animals were acclimatized to experimental laboratory conditions for at least seven days. The study protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) [Protocol No. IAEC/M.Pharm/DPS/2018-03] and all the experiments were performed as per the guideline of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Herbal Formulation

The herbal formulation Nefrowin is an innovative polyherbal formulation especially developed, for improving renal functions and treating various renal disorders, by Nupal Remedies Pvt Ltd, Cochin and is obtained as gift sample for conducting this research.

Induction of nephrotoxicity

Nephrotoxicity was induced in wistar albino rats by single dose of Cisplatin (5mg/kg, i.p.).

Acute toxicity study

The preliminary pharmacological studies were conducted to assess the acute toxic effects of nefrowin. The acute toxicity study was carried out in albino rats by up and down method (OECD guideline 425). The animals were fasted overnight and Nefrowin were administered starting at 5000mg/kg, food was withheld for next 3-4 hrs. The animals were observed continuously for changes in physical appearance, the gross behavioral changes like sign of tremors, convulsion, salivation, diarrhea, lethargy, sleep and coma was closely monitored for every 30 min for next 3hr and till 24hr. The animals were kept at close observation further 14 days for any signs of long term toxic effects including mortality.

Experimental protocol: Cisplatin-induced nephrotoxicity

Male Wistar albino rats weighing between 150-200 g were used for the study. Before and during the experiment, animals were fed with normal laboratory diet and water ad libitum. The animals were acclimatized for a period of 7 days in the new environment before initiation of experiment. Healthy adult male wistar strain albino rats that had been starved overnight were divided into six experimental groups and each group consisted of six rats.

Group 1 (Normal): 0.5% CMC solution (1 ml/kg/day; p.o.) was administered to rats for a period of 10 days.

Group 2 (Olive oil -control): Olive oil (1 ml/kg/day; p.o.) Was administered to rats for a period of 10 days

Group 3 (Toxic-control): 0.5% CMC solution was administered to rats for a period of 10 days and on 7th day, a single injection of cisplatin (5 mg/kg; i.p.) was given.

Group 4 (Standard group): Vitamin E 250 mg/kg/day; p.o. was administered to rats for a period of 10 days and on 7th day, a single injection of cisplatin (5 mg/kg; i.p.) was given.

Group 5 (Nefrowin group I): Nefrowin (500 mg/kg/day; p.o.) was administered to rats for a period of 10 days and on 7th day, a single injection of cisplatin (5 mg/kg; i.p.) was given.

Group 6 (Nefrowin group II): Nefrowin (1000 mg/kg/day; p.o.) was administered to rats for a period of 10 days and on 7th day, a single injection of cisplatin (5 mg/kg; i.p.) was given.

Sample Collection

At the end of the experiment, Urine was collected over 24 h on 10th day by keeping the test animals in individual metabolic cages. The volume of collected urine samples was measured followed by estimation of biochemical parameter, namely urine creatinine. After 24hrs animals were taken out and blood samples were collected by retro-orbital puncture method in plain plastic tubes, and serum parameters including creatinine, urea, uric acid, and total protein were estimated. Thereafter all the animals were sacrificed by means of cervical dislocation and kidney were dissected out immediately for both ex-vivo and histopathological examinations.

Histopathological Analysis

For histopathological evaluation of kidneys, rats were sacrificed by cervical dislocation at the end of experiment. The kidneys were removed, washed with cold saline and preserved in 10% formalin in buffered form. After embedding in paraffin, the sections were cut, stained with hematoxylin and eosin. Now the slides were then examined for renal architecture at the magnification of 450X using a laboratory light microscope attached with camera.

Statistical Analysis

All the data were expressed as mean \pm SEM (standard error of mean) and analyzed by one-way Analysis of Variance (One-way ANOVA) followed by "Tukey's multiple comparison test" by using Graph Pad Prism-5. The P<0.05 was considered to be significant.

RESULTS

Cisplatin-induced nephrotoxicity is one of the important and validated models used to elucidate the nephroprotective potential. The different mechanisms involved in Cisplatin nephrotoxicity consist of oxidative stress, apoptosis, necrosis, up regulation of transforming growth factors, elevation of endothelin level, an increase in the infiltration of monocyte/macrophages, etc.

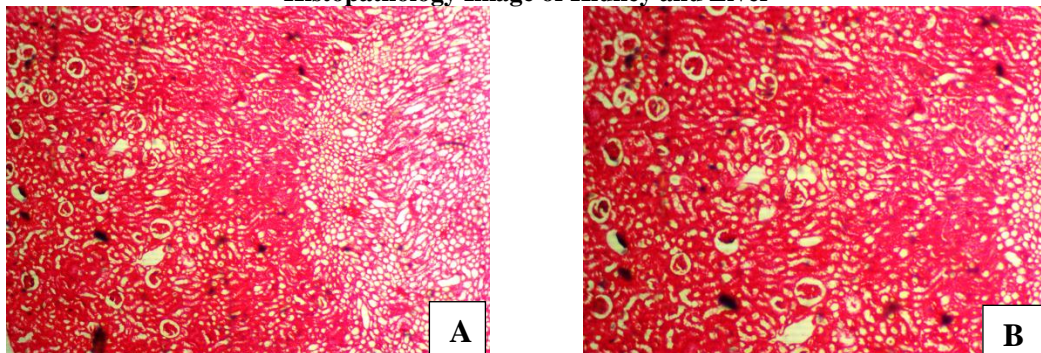
The results showed that cisplatin caused a marked increase in serum creatinine, serum urea, serum uric acid and kidney weight. It also caused decrease in serum total protein, urine volume, urine creatinine, creatinine clearance and body weight. All these factors indicate the renal stress. The efficacy of Nefrowin in reversing the above parameters were studied both nefrowin groups.

Acute Toxicity Studies

The Nefrowin was found to be safe up to 5000 mg/kg body weight by oral route. After 24 hr animals were found well tolerated, there was no mortality and no signs of toxicity. There was no significant change in body weight and food consumption of the animals. The Nefrowin was found to be safe, so the dose of 1000 mg/kg and 500 mg/kg were selected for the present study.

The weight of the organ like liver, kidney did not show any significant change. In acute toxicity study, no mortality was found at a dose of 5000 mg/kg which indicates that oral LD50 of Nefrowin is more than 5000 mg/kg. All animals survived until the scheduled necropsy and their physical and behavioral examinations did not reveal any treatment-related adverse effects. No pathological changes were observed in histological section of kidney and liver of Nefrowin treated rats as compared to normal control animals.

Histopathology Image of Kidney and Liver



Histopathology micrographs of kidney section, A- Normal control-treated, B- Nefrowin-treated, and both kidneys show regular morphology with well-preserved glomerulus and tubular epithelial cells.

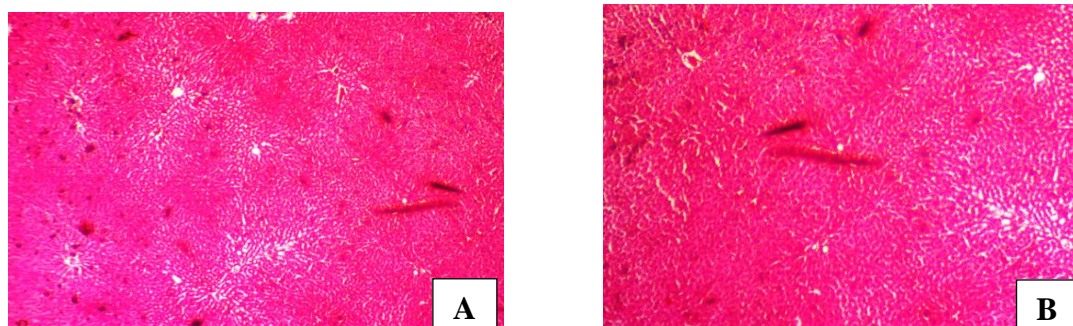


Figure 1: Histopathology micrographs of liver section, A- Normal control-treated, B- Nefrowin-treated group, and both liver show regular morphology.

Effect of Nefrowin on change in body weight

Before Cisplatin treatment, the body weight were evaluated statistically and compared each group with every other groups, it showed there was no significant

difference in body weight. Each groups showed a relatively constant increase in the body weight up to 7 days. The results are tabulated in table no.1.

Table 1.

SL. No.	Groups	Periodical weight changes after Nefrowin treatment		
		Day 1	Day 7	Day 10
1	CMC- Control	147.50 ± 4.8476	158.16 ± 2.1369	165.8 ± 3.4880***
2	Olive oil- Control	150.33 ± 4.1793	155.33 ± 3.8297	159.00 ± 3.3862***
3	Toxic- Control	153.50 ± 2.5884	164.83 ± 3.5449	156.33 ± 2.5819
4	Standard group	155.0 ± 3.1622	161.33 ± 3.0767	163.66 ± 2.8047**
5	Nefrowin group-I (Nefrowin 500mg/kg)	167.16 ± 5.8452	179.66 ± 8.7101	177.0 ± 8.6717**
6	Nefrowin group-II (Nefrowin 1000mg/kg)	153.50 ± 3.2093	166.83 ± 2.7141	171.16 ± 1.9407***

Values are statistically evaluated by Two way ANOVA by Bonferonni's multiple comparison test and are expressed as mean ± SEM, n=6, *p< 0.05, **p< 0.001, ***p< 0.0001 as compared to toxic control.

On 7th day, the rats were treated with single dose of Cisplatin in all groups except CMC and Olive oil control, it shown marked reduction of body weight as compared to control groups (P< 0.001) indicating nephrotoxicity. There was significant decrease in body weight of animals treated with 500 mg/kg and 1000 mg/kg of Nefrowin when compared to Cisplatin treated (Toxic- control)

group. When compared to standard group there was no significant difference in body weight. The control groups alone showed relatively constant increase in body weight throughout the experimental period. There was no significant difference between olive oil control and CMC control group in body weights.

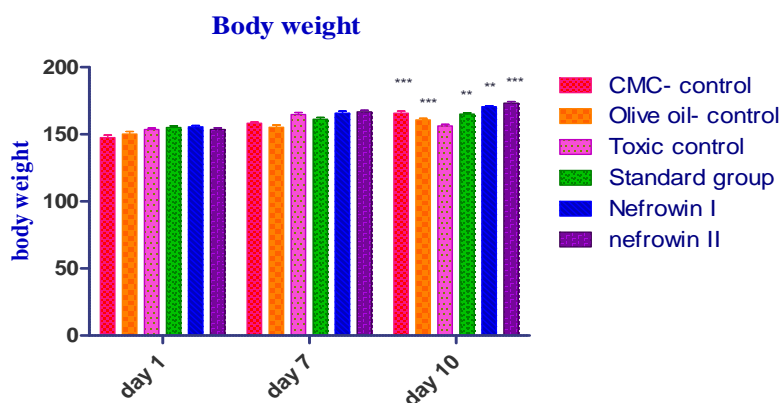


Figure: 2.

Effect Nefrowin on Serum Parameters

In the current study, cisplatin resulted in acute renal failure as indicated by significant increase in serum

creatinine, serum urea and uric acid and decrease in serum total protein compared with control rats. The results are tabulated in Table No: 2.

Administration of cisplatin (ip) was found to increase the serum creatinine, serum urea and uric acid level in toxic group indicating nephrotoxicity. Serum creatinine, serum urea and uric acid was significantly ($p < 0.0001$) increased in Cisplatin treated group (group -III) when compared to control (group I & II) groups. Serum creatinine level, serum urea and uric acid were decreased significantly in Nefrowin group- I (group- V). Similarly serum creatinine, serum urea and uric acid level were significantly decreased more in Nefrowin group- II (group- VI). Vitamin E treated group (group- IV) showed more significant decrease in serum creatinine serum urea and uric acid when compared to cisplatin treated group, when compared to Nefrowin-II there was no significant difference in serum creatinine level. There was no significant difference between olive oil control and CMC control group in serum creatinine level, serum urea and uric acid.

Serum total protein was significantly ($p < 0.0001$) decreased in Cisplatin treated group (group -III) respectively when compared to control (group I & II) groups. Serum total protein level was increased significantly in Nefrowin group- I (group-V) compared to cisplatin group. Similarly serum total protein level was significantly increased more in Nefrowin group- II (group- VI) when compared to cisplatin group. Vitamin E treated group (group- IV) showed more significant increase in serum total protein when compared to cisplatin treated group, when compared to Nefrowin-II there was no significant difference in serum total protein level. There was no significant difference between olive oil control and CMC control group in serum total protein level.

Table 2:

SL. No.	Groups	Serum Cr ($\mu\text{g/dL}$) Mean & SEM	Serum Urea($\mu\text{g/dL}$) Mean & SEM	Serum Uric acid ($\mu\text{g/dL}$) Mean & SEM	Serum Total Protein (g/dL) Mean & SEM
1.	CMC- Control	$0.73 \pm 0.0384^{***}$	$47.26 \pm 1.863^{***}$	$1.91 \pm 0.1267^{***}$	$6.51 \pm 0.2014^{***}$
2.	Olive oil- Control	$0.72 \pm 0.0408^{***}$	$46.23 \pm 1.775^{***}$	$1.93 \pm 0.1106^{***}$	$6.55 \pm 0.1430^{***}$
3.	Toxic- Control	1.73 ± 0.0399	116.30 ± 3.197	3.57 ± 0.1213	4.25 ± 0.2169
4.	Standard group	$1.00 \pm 0.0266^{***}$	$73.18 \pm 1.039^{***}$	$2.18 \pm 0.0165^{***}$	$6.26 \pm 0.1597^{***}$
5.	Nefrowin group-I (Nefrowin 500mg/kg)	$1.22 \pm 0.0159^*$	$88.22 \pm 2.891^{**}$	$2.85 \pm 0.1536^{**}$	$5.35 \pm 0.1469^{**}$
6.	Nefrowin group-II (Nefrowin 1000mg/kg)	$1.04 \pm 0.0344^{**}$	$73.07 \pm 1.079^{***}$	$2.21 \pm 0.0149^{***}$	$6.26 \pm 0.0965^{***}$

Values are statistically evaluated by One way ANOVA by Tukey's multiple comparison test and are expressed as mean \pm SEM, $n=6$, $*p < 0.05$, $**p < 0.001$, $***p < 0.0001$ as compared to toxic control.

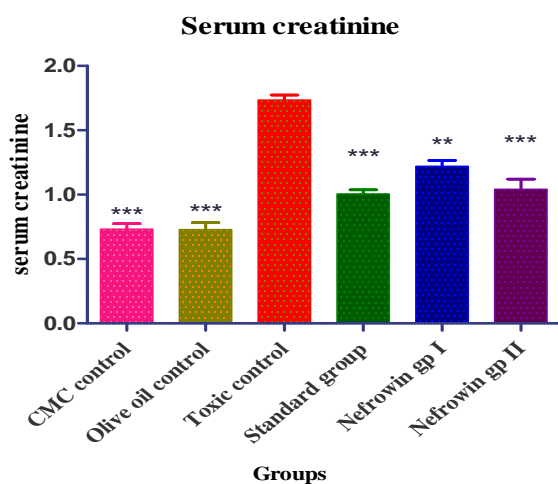


Figure: 3

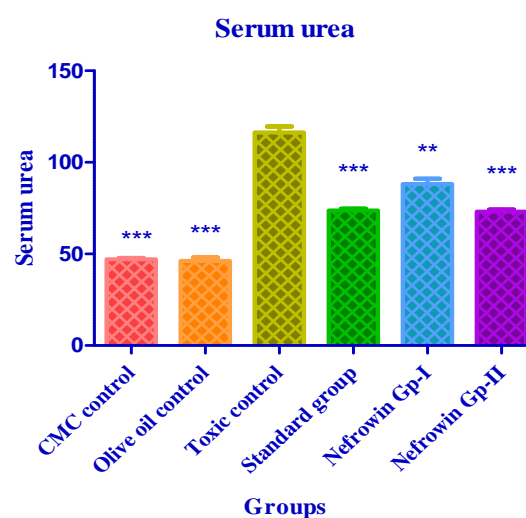


Figure: 4.

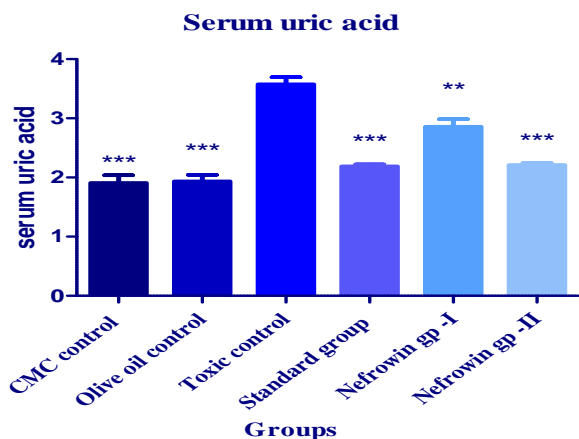


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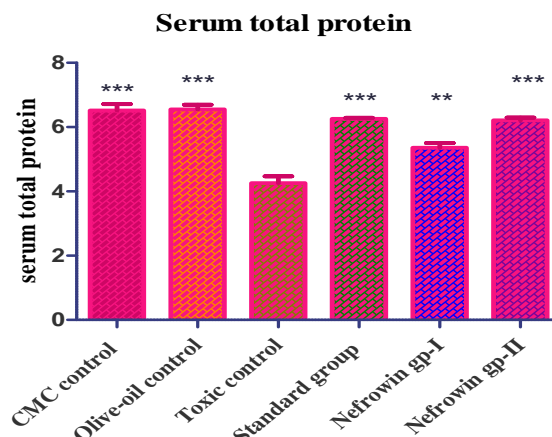


Figure: 6

Effect of Nefrowin on Urine Parameters

Urine output and urine creatinine level of normal control, toxic control, standard and Nefrowin treated rats on last day of experiment are shown in the table no: 3.

Groups received Cisplatin alone showed marked decrease in urine volume when compared to toxic ($p < 0.0001$) control indicating nephrotoxicity. There was significant increase in urine volume in Nefrowin group-I (group-V) when compared toxic control (group-III). Similarly urine volume was significantly increased more

in Nefrowin group- II (group- VI) when compared to cisplatin group. Vitamin E treated group (group- IV) showed more significant increase in urine volume when compared to cisplatin treated group

Nephrotoxicity is also revealed from the increased level of urine creatinine after the administration of cisplatin in the toxic control group when compared to both CMC and olive oil groups. Administration of Nefrowin produce a significant decrease in urine creatinine level when compared to cisplatin treated group.

Table 3:

SL. No	Groups	Urine volume (mL) Mean & SEM	Urine Creatinine (µg/dL) Mean & SEM
1	CMC- Control	10.45 ± 0.2998***	0.9050 ± 0.0189***
2	Olive oil- Control	10.18 ± 0.3410***	0.8950 ± 0.0284***
3	Toxic- Control	4.30 ± 0.1549	2.2530 ± 0.03756
4	Standard group	9.20 ± 0.1673***	1.2080 ± 0.0205***
5	Nefrowin group-I (Nefrowin 500mg/kg)	7.80 ± 0.1432**	1.7080 ± 0.1041 **
6	Nefrowin group-II (Nefrowin 1000mg/kg)	9.03 ± 0.2813***	1.2030 ± 0.0182***

Values are statistically evaluated by One way ANOVA by Tukey’s multiple comparison test and are expressed as mean ± SEM, n=6, * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$ as compared to toxic control.

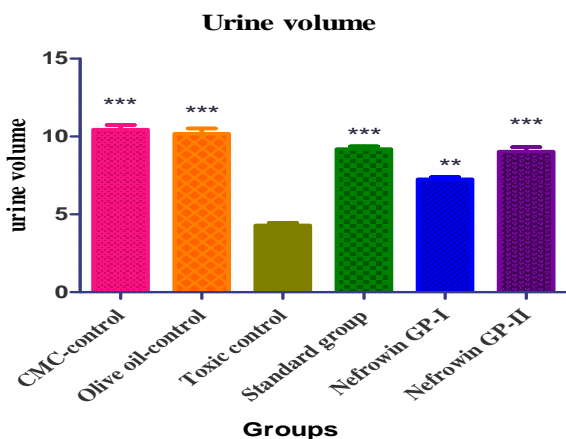


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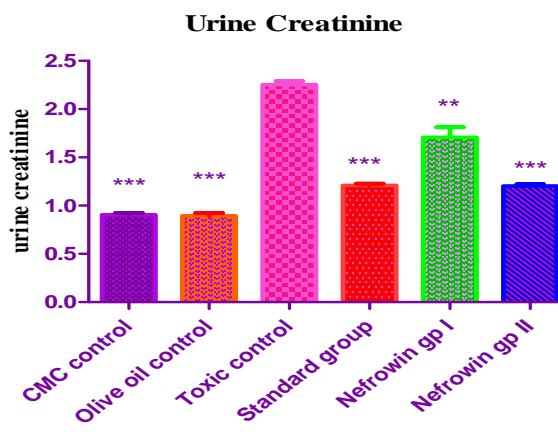


Figure: 8

Effect of Nefrowin on kidney weight and Ex-vivo Antioxidant activity

Groups received Cisplatin alone showed a significant increase in kidney weight when compared to toxic control (Group-III) group indicating nephrotoxicity. Animals that received Nefrowin showed decrease in kidney weight as compared to toxic control group. There was more significant decrease in kidney weight in animal treated with high dose of nefrowin when compared to toxic control group. Vitamin E treated group (group- IV) showed more significant decrease in kidney weight when compared to cisplatin treated group, when compared to Nefrowin-II there was no significant difference in kidney weight.

Cisplatin treated groups (group-III) showed marked decrease in superoxide dismutase concentration when

compared to CMC and Olive oil control. Superoxide dismutase level was increased significantly in Nefrowin group-I when compared to cisplatin treated group. Similarly superoxide dismutase level was significantly increased more in Nefrowin group-II and standard group when compared to cisplatin treated group. The results are tabulated in table no: 4

Reduced glutathione were measured in all groups of animals and it was found that there were significantly decreased enzyme activities in toxic control group compared to the CMC and Olive oil controls. Reduced glutathione level was increased significantly in Nefrowin group-I when compared to cisplatin treated group. Similarly reduced glutathione level was significantly increased more in Nefrowin group-II and standard group when compared to cisplatin treated group.

Table 4.

SL. No	Groups	Kidney weight (g) Mean & SEM	Superoxide dismutase activity Mean & SEM	Reduced glutathione activity Mean & SEM
1.	CMC- Control	0.4917 ± 0.0112***	9.17 ± 0.2067***	15.50 ± 0.2513***
2.	Olive oil- Control	0.5017 ± 0.0102***	9.00 ± 0.2170***	14.78 ± 0.3310***
3.	Toxic- Control	0.7693 ± 0.0074	4.43 ± 0.1642	4.52 ± 0.2153
4.	Standard group	0.5747 ± 0.0078***	7.91 ± 0.2419***	12.75 ± 0.2118***
5.	Nefrowin group-I (Nefrowin 500mg/kg)	0.6797 ± 0.0297*	5.76 ± 0.2039**	10.10 ± 0.1725***
6.	Nefrowin group-II (Nefrowin 1000mg/kg)	0.5403 ± 0.0038***	7.90 ± 0.2249***	12.90 ± 0.3238***

Values are statistically evaluated by One way ANOVA by Tukey's multiple comparison test and are expressed as mean ± SEM, n=6, *p< 0.05, **p< 0.001, ***p< 0.0001 as compared to toxic control

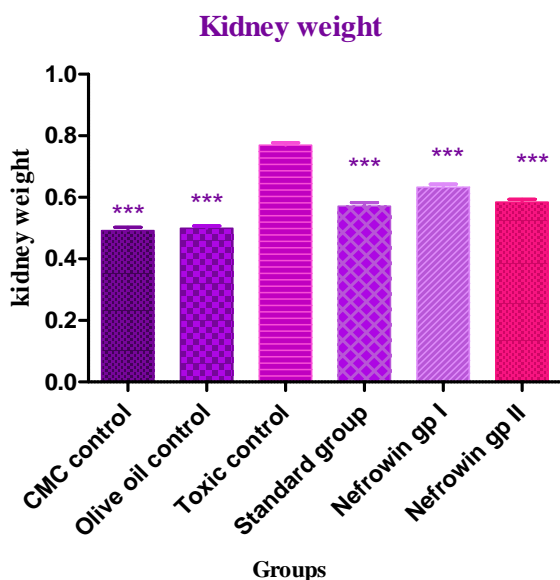


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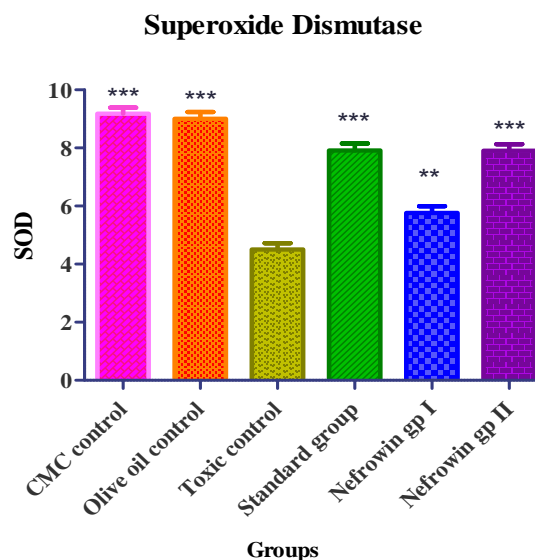


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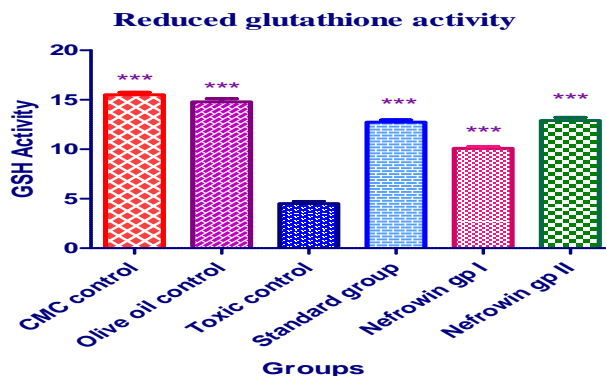


Figure: 11

Histopathological Examination

The histopathological examination of kidney sections from normal rats showed regular architecture of cortex without any evidence of inflammation. The kidneys of Cisplatin-treated rats showed diffuse acute proximal tubular necrosis, glomerular atrophy and degeneration of renal tubular cells. Nefrowin treatment with low dose

(500 mg/kg) shows mild proximal tubule degeneration and inflammation. Kidney sections from Cisplatin plus Nefrowin at 1000 mg/kg, tubules appeared nearly normal and there was no inflammation. The histopathological changes of normal and treated rat kidneys are shown in figure: 12.

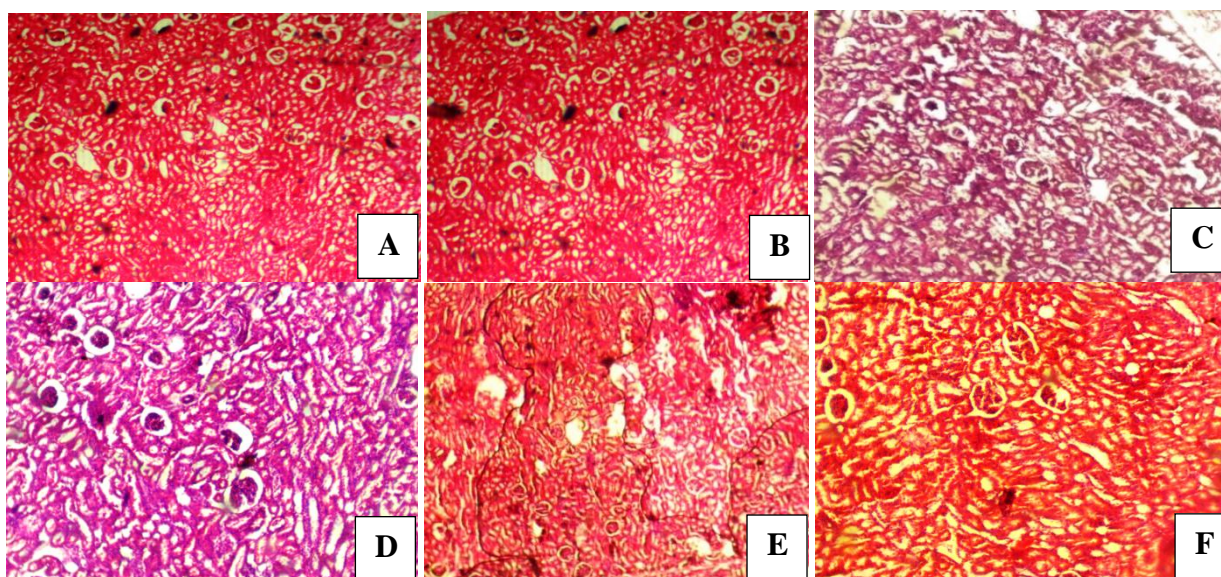


Figure 12: Histopathology micrographs of kidney section of different groups, A- CMC control, B- Olive oil Control, C- Toxic control, D- Nefrowin group-I, E- Standard group, F- Nefrowin group-II.

DISCUSSION

Kidneys endowed with million units are termed as nephrons that act as a natural sieve. Unfortunately, kidney diseases may be silent for long time. Early detection is the key to preventing kidney diseases, thereby significantly reducing the associated morbidity and mortality. The kidneys provide the final common pathway for the excretion of many drugs and their metabolites and therefore are frequently subjected to high concentrations of potentially toxic substances.

Drugs and their metabolites are taken up selectively and concentrated into the urine, so high intracellular concentrations are attained, particularly in the renal medulla which has relatively little vasculature compared

with the cortex. As a result, direct toxic damage occurs, generally affecting the renal tubular cells and renal papillae. Nephrotoxicity of this type tends to be dose dependent. Many groups of drugs can cause renal damage including allopurinol, carbamazepine, cimetidine, NSAIDs, erythromycin, getamincin, cisplatin, amikacin, and heavy metals. In recent years herbal medicines are used to prevent nephrotoxicity in both animal model and human subjects. Therefore in the present study, evaluate the nephroprotective activity of Nefrowin on Cisplatin-induced rats.

Cisplatin is one of the highly effective inorganic platinum based oncologic medications used in the chemotherapy for treating ovarian, bladder, testicular, cervical, and various solid or hematologic tumors.

However, its use is limited by tumor cell resistance and various side effects such as suppression of bone marrow, renal toxicity, ototoxicity, emesis and neural toxicity. The mechanism of the anticancer activity of cisplatin is not completely understood, but a widely held view is that cisplatin binds to DNA, leading to the formation of inter- and intra-strand cross-links. Cross-linking results in defective DNA templates and arrest of DNA synthesis and replication. In rapidly dividing cells, such as those in cancers, cross-linking can further induce DNA damage. Mildly damaged DNA can be repaired, whereas extensive DNA damage leads to irreversible injury and cell death.

In the view of the above findings the present study includes the pharmacological investigation of polyherbal formulation (Nefrowin) for nephroprotective activity. Cisplatin dose (5mg/kg, ip) was used to induce the nephrotoxicity in wistar albino rats. Administration of cisplatin exerted significant increase in serum creatinine, serum urea, serum uric acid and kidney weight. Body weight, urine creatinine, serum total protein, urine volume, SOD and GSH levels were significantly decreased in cisplatin treated group.

Creatinine is an end product of muscle catabolism, which is removed at a constant rate by the kidneys. The concentration of creatinine in serum is the most widely used and commonly accepted measure of renal function in clinical medicine. The clinical utility of the serum creatinine concentration centers on its relation to the glomerular filtration rate (GFR). The serum creatinine concentration is the most commonly used index of the kidney function. The level of creatinine in the blood rises if the kidney does not function properly. Cisplatin have been reported to increase creatinine measurement. Administration of Nefrowin restored the level of creatinine in Cisplatin treated rats.

Urea is an end product of protein catabolism. It is freely filtered by the glomerulus, passively reabsorbed in the both the proximal and distal nephron and excreted in high concentration in urine. The excretion of urea was recognized as an estimate of kidney function. In renal diseases, the serum urea accumulates because of the rate of serum urea production exceeds the rate of clearance. Elevation of urea and creatinine levels in serum was taken as the index of nephrotoxicity. Creatinine derives from endogenous sources by tissue creatinine breakdown. Thus serum urea concentration is often considered a more reliable renal function prediction than serum creatinine. In the present study also observed the increased level of urea in cisplatin intoxicated rats. Supplementation of Nefrowin restored the increased level of urea in cisplatin-induced groups.

Uric acid is the final oxidation product of purine metabolism and is renally excreted. Therefore, elevated serum uric acid levels are seen in patients with reduced glomerular filtration rate (GFR). Anyhow the level of

uric acid is non-significantly increased in the toxicant group when compared to control. Oral administration of Nefrowin significantly decreases the uric acid level in both treatment group compare to toxicant group. The level of serum total protein is decreased It was established that cisplatin is actively transported into proximal tubules after glomerular filtration in a small proportion where it causes proximal tubular injury and abnormalities in renal circulation that leads to a reduction of GFR.

Nephrotoxicity is also revealed from the increased level of urine creatinine after the administration of cisplatin in the toxic control group when compared to both CMC and olive oil control groups. Administration of Nefrowin produce a significant decrease in urine creatinine level when compared to cisplatin treated group. Physiological parameters body weight, urine volume was reduced in toxic control group. The reduction may be due to tubular injury which affects water reabsorption leading to dehydration and loss of body weight or due to cytotoxic effects of cisplatin on the GIT, which affects eating behavior in rats. The reduction in urine volume may be either due to significant reduction in water or due to severe kidney injury and increased in treatment groups compared to cisplatin control which might be due to diuretic effect of Nefrowin and kidney weight is increased in toxic control group and decreased in treatment groups compared to toxic groups.

The histopathological examination of kidney sections from normal rats showed regular architecture of cortex without any evidence of inflammation. The kidneys of Cisplatin-treated rats showed diffuse acute proximal tubular necrosis, glomerular atrophy and degeneration of renal tubular cells. Nefrowin treatment with low dose (500 mg/kg) shows mild proximal tubule degeneration and inflammation. Kidney sections from Cisplatin plus Nefrowin at 1000 mg/kg, tubules appeared nearly normal and there was no inflammation. The free radical-scavenging enzymes are important to protect against the cell oxidative stress caused by cisplatin. However, these antioxidant enzymes are easily inactivated by reactive oxygen species, which result in decreasing in the activities of these enzymes in cisplatin toxicity. In the present study, the result point that the activity of SOD and GSH were significantly upgraded by the treatment Nefrowin to cisplatin-intoxicated rat, implying that Nefrowin has the capacity to maintain/restore the level of enzymatic antioxidants in cisplatin-injured kidneys.

In light of the above text, the test drug formulation Nefrowin exhibits significant nephroprotective and antioxidant potential. This might be due to presence of multiple active constituents as the said formulation is a polyherbal formulation. These constituents are claimed to be potent diuretic, anti-inflammatory and antimicrobial, thus protecting nephrons from conditions like nephritis and probability of infections. Further since the beginning of time, treatment using herbal formulations

has been a favorite tool of naturopathically inspired practitioners and approximately 75% of the global population, including that from most of the developing world, depends on herbal medicines for their basic healthcare needs as herbal medicines are not only inexpensive but are better compatible with human body and have fewer side effects.

This study is the first scientific report that scrutinizes nephroprotective potential of Nefrowin and also validates its use against dysuria and urinary disorders.

CONCLUSION

On evaluating biochemical parameters, antioxidant enzyme and histopathological examinations it was found that the polyherbal formulation Nefrowin showed a significant nephroprotective activity in cisplatin induced nephrotoxicity. It concluded that Nefrowin is not merely antioxidant, but nephroprotective also and it might be due to additive/synergistic interaction of different herbal drug components present in Nefrowin having multiple nephroprotective, diuretic and antioxidant potential.

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