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Nephroprotective activity of *Bilwadi Agada* in Cypermethrin Induced Nephrotoxicity in Wistar Rats

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ABSTRACT

Introduction: One of the most potent and widely used formulations of *Agadatantra* is *Bilwadi Agada* mentioned in the context of management of envenomation from snake in *Ayurveda*. The same formulation is also indicated in multiple ailments like poisoning from other animals or insects and even is general conditions of fever. Nephroprotective activity has been demonstrated by *Bilwadi Agada* and the same was evaluated in this experiment in Wistar rats induced by cypermethrin **Aim:** To evaluate the nephroprotective activity of *Bilwadi Agada* in toxicity induced by cypermethrin in wistar albino rats. **Materials and Methods:** According to OECD guidelines 407, the induction of sub-acute toxicity was done and effect of *Bilwadi Agada* was evaluated in wistar albino rats. **Results:** Degenerative changes in kidneys were found to be increased in lower dose level but moderate reversal was observed in higher dose. **Conclusion:** The thorough analysis of the results shows that the test drug at double the therapeutic dose causes moderate reversal of toxicant induced changes, thereby providing evidence for presence of nephroprotective activity against cypermethrin toxicity. Thus, therapeutic effects of *Bilwadi Agada* along with suitable cytoprotectant will be beneficial in the management of Cypermethrin toxicity.

Key words: *Bilwadi Agada, Nephrotoxicity, Nephroprotective*

INTRODUCTION

Every year, a significant number of individual's worldwide experience the impact of insecticides and pesticides used in our food and beverages, posing a substantial health risk. Despite being present in small quantities, these chemicals contribute to severe health issues such as renal failure, malignancies and many other ailments even affecting the reproductive health.

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The misuse of these substances has emerged as a major health hazard that we currently confront.

The kidneys serve as the primary excretory organs, eliminating metabolic wastes via urine. Individuals are consistently exposed to a range of diverse chemicals that can negatively impact the kidneys. Various substances, including pesticides medicines, industrial substances, ecological pollutants, and more, can cause harm to different organs within the body at different levels.

Cypermethrin, a synthetic pyrethroid, has become one of the most widely used insecticides following the ban on DDT. Its excessive and unrestricted usage leads to accidental poisoning and may have long-term consequences. Within the body, the enzymes responsible for pyrethroid degradation include esterases and oxidases, involving oxidation through the mixed function oxidase (MFO) system in the liver.[1] Subsequently, the byproducts are excreted by the kidneys and liver. The toxic stress imposed by cypermethrin can result in damage to both the kidneys

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and liver, leading to renal and hepatic failures. While data on humans is not available, experimental models[2] have unequivocally demonstrated the nephrotoxic effects of cypermethrin.

The *Bilwadi Agada*[3] discussed in the context of *Ayurvedic* management of snake bites is also recommended for various other conditions, including spider poison, rat bites, scorpion stings, indigestion, artificial poisoning, fever, and possesses antimicrobial and antiviral properties.[4] Multiple components of this formulation have exhibited nephroprotective activity. Therefore, this study aims to assess the nephroprotective effects of *Bilwadi Agada* in Wistar rats with cypermethrin-induced nephrototoxicity.

Aims and Objectives

To evaluate the Nephro-protective role of *Bilwadi Agada* in Cypermethrin toxicity

Materials and Methods

Animals

Healthy, adult, Wistar albino rats belonging to either sex weighing between 175 and 225 g were procured from animal house attached to the Pharmacology Laboratory S.D.M Centre for Research in Ayurveda and Allied Sciences.

Drugs

Cypermethrin was procured from Raman Lab Equipment's and Chemicals, Udupi in Karnataka state and classically prepared *Bilwadi Agada* was procured from Good Manufacturing Practices certified standard pharmacy.

Sub-acute toxicity studies

Sub-acute toxicity studies were carried out in line with OECD guideline for testing of chemicals -407.[5]

Twenty four rats were divided into 4 groups of six animals each.

1. Group I served as the normal control
2. Group II received Cypermethrin
3. Group III received Cypermethrin and *Bilwadi Agada* in therapeutic dose
4. Group IV received Cypermethrin and *Bilwadi Agada* in double the therapeutic dose.

The animals were dosed daily by gavage for the period of 28 days.

The body weight of all animals treated with drugs/vehicles was documented before treatment initiation and at weekly intervals thereafter, with daily weighing's conducted during the final week. Twenty-four hours after the last dose, animals were anesthetized, and blood samples were obtained from the retro-orbital sinus, using tubes with or without ethylene diamine tetra acetic acid (EDTA). Subsequently, the animals were euthanized with an overdose of anesthetic. Necropsy procedures were performed, and organs were collected for macroscopic and/or microscopic examination.

Biochemical assessment

Clotted blood samples were centrifuged at 3000 ×*g* for 10 min, and the separated serum was subjected to biochemical estimation for AST, ALT, ALP, creatinine, urea, and total protein by using specific kits.

Macroscopic and microscopic evaluation of organs

After necropsy, liver, kidney, and heart were debrided of attached connective tissue and weighed on an analytical balance. Subsequently, the kidney's were preserved in 10% formalin and processed for histopathological evaluation.

Statistical analysis

Comparison of means was carried out with one-way ANOVA followed by Dunnett's Multiple Comparison test as post hoc test (GraphPad InStat; Version 3.05, GraphPad Software Inc.). *P* ≤0.05 was statistically significant.

Results and Discussion

Sub-acute toxicity

Table 1: Biochemical Parameters

Group	ALP (IU/L)	Serum Total Protein (g/dl)	Serum Albumin (g/dl)	Serum Globulin (g/dl)	Serum Urea (mg/dl)	Creatinine (mg/dl)	Serum Uric Acid (mg/dl)
Control	505.33 ±103.68	7.04±0.11	3.51±0.16	3.52± 0.23	34.71 ±1.10	0.37±0.01	1.35±0.08
Cypermethrin	1053.33±203.29	6.76±0.15	4.15±0.08**	2.61± 0.19*	22.00 ±2.28 **	0.48±0.04	1.63±0.06

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Bilwadi Agada (Ted)	792.16 ±215.2 4	6.91±0. 23	4.00±0. 10	2.91± 0.24	27.00 ±5.00	0.77±0. 06**	1.18±0. 21*
Bilwadi Agada (Tedx2)	2088.7 1±187. 40**	6.57±0. 27	3.48±0. 09**	3.08± 0.23	17.85 ±1.33	0.37±0. 03	1.45±0. 05

Data: MEAN ± SEM, *P<0.05, **p<0.01

Table 1 shows effect of *Bilwadi Agada* on biochemical parameters. Blood urea level was found to be decreased in the Cypermethrin group which is statistically significant compared to the normal control group. Blood Urea level was found to be mildly increased in Bilwadi (TED) group which is statistically non-significant compared to the Cypermethrin control group. Blood Urea level was found to be increased in Bilwadi (TEDx2) group which is statistically non-significant compared to the Cypermethrin control group. Decreased blood urea level may be due to the dysfunction of liver resulting in less protein synthesis.

Serum creatinine was found to be increased in the Cypermethrin group which is statistically non-significant compared to the normal control group. Serum creatinine was found to be increased in Bilwadi (TED) group which is statistically significant compared to the Cypermethrin control group. Serum creatinine was found to be mildly increased in Bilwadi (TEDx2) group which is statistically non-significant compared to the Cypermethrin control group.

Serum creatinine is an important indicator of renal health because creatinine is a non-protein waste product of creatine phosphate metabolism by skeletal muscle tissue. Creatinine production is continuous and is always proportional to muscle mass. Hence the respective significant increase & decrease in the blood urea & creatinine level might be because of the catabolism of the muscle tissue. As a result of this, a decrease might have been observed in the increasing gradient of the body weight among the test group animals which is discussed in food & faecal matter analysis part. It may be indicative of decreased muscle activity also.

Normally elevated blood or serum urea level is considered to reflect renal insufficiency when considered in association with serum creatinine level. In this study only moderate elevation was observed in serum creatinine level. Decreased level is indicative of lower turnover of nitrogenous matter as indicated by decreased serum globulin level. However, histological examination revealed moderate inflammatory changes in the kidney in the form of cell infiltration indicating that kidney structural integrity is affected to moderate extent. In *Bilwadi Agada* TED dose treated Cypermethrin receiving rats- elevation of serum creatinine level along with moderate to severe pathological changes in the kidney in the histological examination was observed. It is perplexing to note *Bilwadi Agada* TED dose increases the toxicological effects of the toxicant. The reason is not known and needs to be ascertained. Surprisingly this activity profile was not seen at higher dose group of the test formulation. This group showed attenuation of toxicant induced degenerative changes. Lower dose of the test formulation decreased serum uric acid level.

Uric acid is the metabolic product of nucleic acid metabolism. Altered serum uric acid concentrations have been linked to number of disease states. High uric acid levels have been correlated with gout, hypertension, cardiovascular diseases, and renal disease. Marked lowering has been found to be associated with neurodegenerative disorders. In the present study a moderate and statistically non-significant elevation in serum uric acid level was observed in the toxicant control group. This reversed to significant extent in lower dose *Bilwadi Agada* administered cypermethrin receiving group. This may have protective effect.

Alkaline Phosphatase was found to be moderately increased in Cypermethrin group which is statistically non-significant compared to normal control group. Alkaline Phosphatase was found to be mildly decreased in Bilwadi (TED) which is statistically non-significant compared to Cypermethrin control group. Alkaline Phosphatase was found to be increased in Bilwadi (TEDx2) which is statistically significant compared to Cypermethrin control group. This increased ALP levels might be because of interference in the activities of the liver and skeletal muscles and might be a consequence of cell membrane damage induced by cypermethrin.[6]

Total proteins were found to be mildly decreased in the Cypermethrin group which is statistically non-significant compared to the normal control group. Total proteins were found to be mildly increased in

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Bilwadi (TED) group which is statistically non-significant compared to the Cypermethrin control group. Total proteins were found to be mildly decreased in Bilwadi (TEDx2) group which is statistically non-significant compared to the Cypermethrin control group. There were no statistically significant alterations in any of the groups.

Serum Albumin was found to be increased in the Cypermethrin group which is statistically significant compared to the normal control group. Serum Albumin was found to be mildly decreased in Bilwadi (TED) group which is statistically non-significant compared to the Cypermethrin control group. Serum Albumin was found to be decreased in Bilwadi (TEDx2) group which is statistically significant compared to the Cypermethrin control group. Increase of serum albumin in Cypermethrin group may be to meet the energy level required to meet the toxic stress induced by the toxin and serum albumin is the major plasma protein targeted in oxidative stress.[8] And reduction of serum albumin in the test drug group may be due to the reduced toxic stress in the system created by *Bilwadi Agada*.[7]

Serum Globulin was found to be decreased in the Cypermethrin group which is statistically significant compared to the normal control group. Serum Globulin was found to be mildly increased in Bilwadi (TED) and Bilwadi (TEDx2) groups which are statistically non-significant compared to the Cypermethrin control group. Decrease in Serum Globulin may be due to the dysfunction of kidney and liver.[9] And increase in comparison may be due to the nephro or hepato protective ability of *Bilwadi Agada*.[7]

Table 2: Ponderal Changes

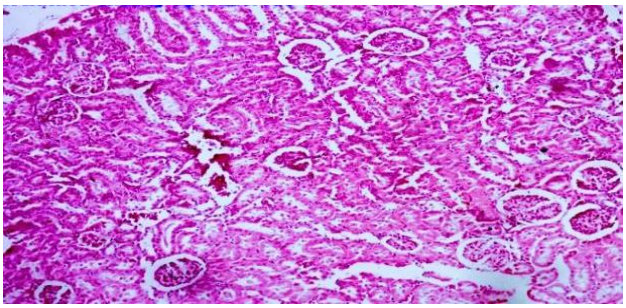
Group	Heart Weight (g)	Liver Weight (g)	Kidney Weight (g)
Control	0.93±0.01	9.66±0.36	1.96±0.07
Cypermethrin	0.76±0.05*	8.36±0.57	1.45±0.10**
Bilwadi Agada (TED)	0.68±0.04	7.76±0.56	1.19±0.06*
Bilwadi Agada (TEDx2)	0.76±0.02	7.20±0.31	1.36±0.01

Data: MEAN ± SEM, *P<0.05, **P<0.01

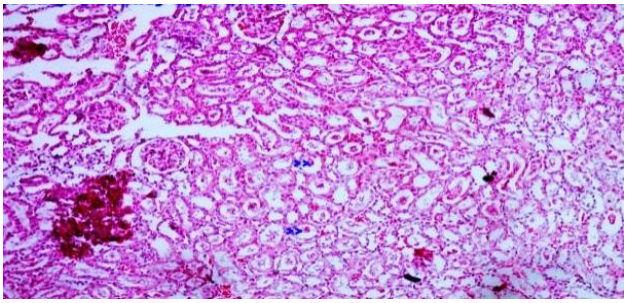
Table 2 shows affect *Bilwadi Agada* on liver, kidney and heart weights. Analysis of the Pondaral changes related to organs shows that all the 3 organ weight recorded has shown decrease in weight. Heart and Kidney was found to be decreased significantly in Cypermethrin group. Bilwadi (TED) also shows decrease significantly. Liver shows mild decrease in their value which is statistically not significant. Conceptually speaking decrease in weight may be due to tissue degenerative changes in the important organs. To confirm these results were considered with reference to histopathological changes. In consonance with the histological examination no such decrease was observed in TED x 2 dose level. This may be due to the protective effect of *Bilwadi Agada*.

Report of histopathological examination

Figure 1a and 1b – Section of Kidneys of control rats showing normal cytoarchitecture

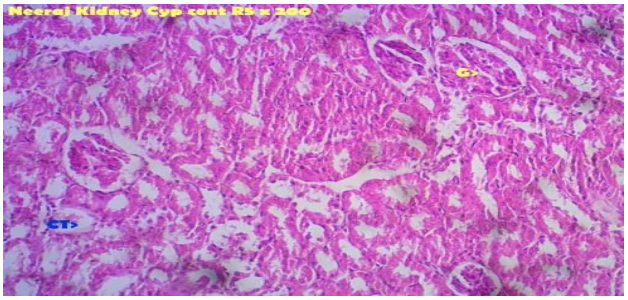


Normal Control: 1a



Normal Control: 1b

Figure 2a and 2b – Section of Kidneys of positive control



Control: 2a

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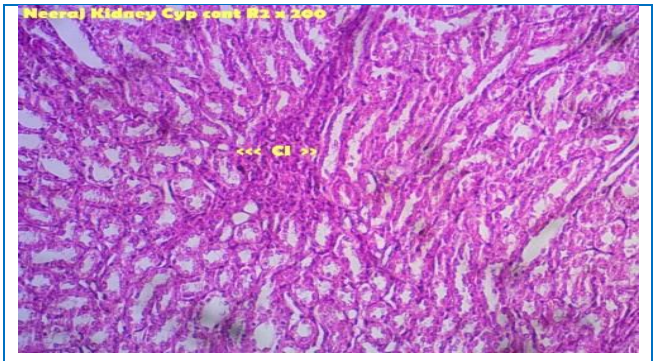
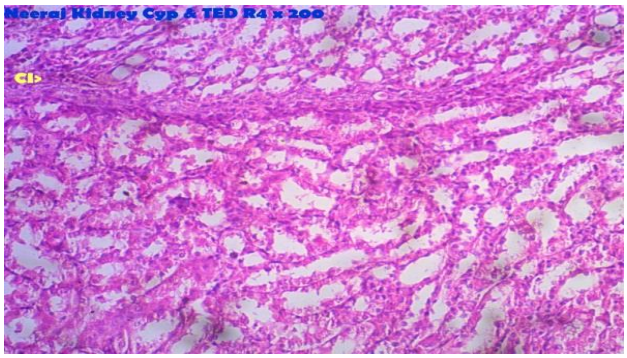
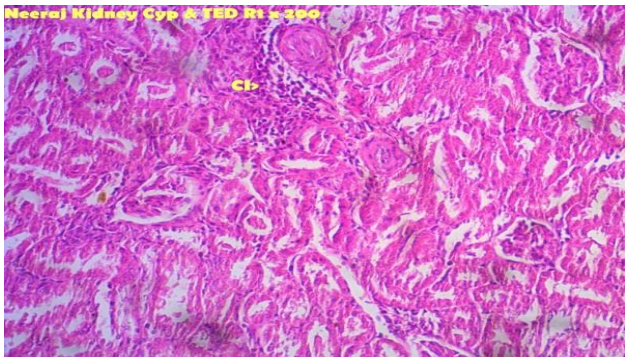


Control: 2b

Figure 3a and 3b – Section of Kidneys of test 1 *Bilwadi Agada* (TED)

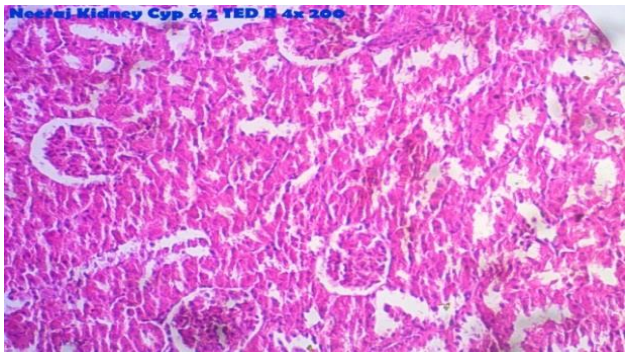


TED: 3a

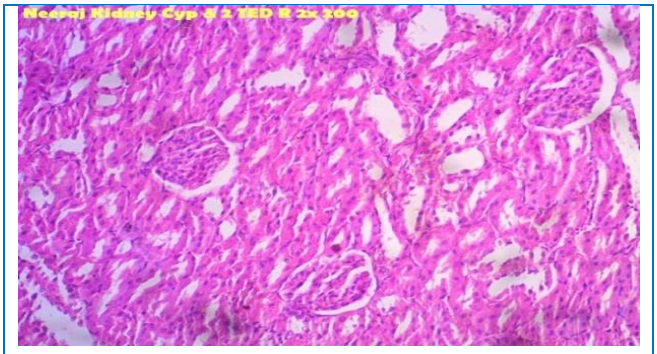


TED: 3b

Figure 4a and 4b – Section of Kidneys of test 2 *Bilwadi Agada* (TEDx2)



TEDx2: 4a



TEDx2: 4b

Conclusion

The objective of this study was to assess the nephroprotective effects of *Bilwadi Agada* in Cypermethrin-induced toxicity. Results indicate moderate reversal of toxicity at twice the therapeutic dosage, suggesting the presence of nephroprotective properties against cypermethrin toxicity. Analysis of the study data confirms the harmful impact of the insecticide cypermethrin, with the majority of effects mitigated by the test formulation at double the standard dose. This underscores the efficacy of *Bilwadi Agada* in managing Cypermethrin toxicity.

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