Nephroprotective study of polyherbal formulation in drug induced nephrotoxic rats

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Abstract

The nephroprotective effect of polyherbal formulation (HM) in Albino wistar rats was studied. Rats were divided into six groups. Vehicle was administered to the group I (control). Nephrotoxicity was induced in the rats of group II by administering gentamicin (80mg/kg, i.p.) for 8 days. The HM (250,500 and 1000mg/k.g.p.o) was administered to the rats of group II and VI for 15 days together with gentamicin (8days). Kidney function was assessed by measuring urine urea and creatinine and serum urea and creatinine. Kidney superoxide dismutase, catalase were also measured in control and treated groups. Histopathological examinations were conducted to confirm the nephroprotective effect. The sign of nephrotoxicity in rats was significantly alleviated by herbal medicine. The results of histopathological examinations also confirm the nephroprotective effect of herbal medicine**.**

Keywords: Nephroprotective, Polyherbal formulation, Nephrotoxicity

Introduction

Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or toxin. Aminoglycoside antibiotics, especially gentamicin. Despite rigorous monitoring, nephrotoxicity appears in 10-25% of therapeutic courses. Gentamicin mainly causes tubular toxicity; it is an aminoglycoside antibiotics used for the treatment of Gram negative bacterial infections. Overdose of gentamicin causes renal damage. It may give serious side effects while continuous consuming at higher concentrations. When kidney damage occurs, body unable to rid of excess urine and wastes from the body and blood electrolytes (such as potassium and magnesium) will all become elevated [1]. The use of herbs as nephroprotective is a major avenue in Indian perspectives particularly for treating kidneys damage, which require to be explored more successfully as there are many literatures available on these aspects. Ayurveda, an indigenous system of medicine, offers a vast scope of renal treatment for renal failure. Plants and other natural substances have been used as the rich source of medicine. Also, herbal drugs are more easily accessible. Medicinal plants are having curative properties and therapeutic values due to the presence of various complex phytochemical compounds. This traditional medicines are assuming greater important because of very effective, safer, locally available, and no side effects. In present study we will try to evaluate the neproprotective activity of polyherbal formulation.

Materials and Methods

Collection of plants: Following listed plants were collected from North Maharashtra region during June 2009 to September 2009. The plants were identified by the taxonomists of Department of Botany, Moolji Jaitha College, Jalgaon, Maharashtra (India).

Table 1: List of selected plants for nephroprotective activity

Sr. No.	Botanical Name	Family	Vernacular name	Part used
1.	*Bauhinia recemosa* (Lam)	Caesalpiniaceae	Aapata	Stem bark
2.	*Dolichos biflorus* (Linn.)	Fabaceae	Kulitha	Seed
3.	*Sphaeranthus indicus* (Linn)	Asteraceae	Gorakhmundi	Flower
4.	*Tectona grandis* (Linn)	Verbenaceae	Sag	Seed
5.	*Tephrosia purpurea* (Linn)	Fabaceae	Sharpunkha	Leaves
6.	*Tribulus terrestris* (Linn)	Zygophyllaceae	Gokhru	Fruit

Poly herbal preparation (Herbal Mixture – HM)

Shade dried plant material pulverized using an electrically operated grinder. Equal quantities of the respective herbal powders were blended and then mixed with 1% Carboxy Methyl Cellulose for preparing the doses. The doses were prepared fresh daily, be for administration, the rats were dosed at approximately the same time each day.

Animals (Wistar rat)

Animals for the study were obtained from the National Biosciences, Pune. The animals were acclimatized to laboratory conditions (temperature: 22 ^{0}C–24 ^{0}C, humidity: 65 - 70% and 12 h light and 12 h dark rhythm) prior to the start of the experiment in the CPCSEA registered (1062/cpcsea/2007) Animal House of the Department of Zoology, Moolji Jaitha College, Jalgaon.

Methodology

Study plan: Animal experimentation

Eight to twelve week old, either sex (weighing between 200-300 gm.) Wistar rats were divided into following 6 groups.

Sr. No	Group	Types of group
1	Control	Treated with Saline
2	CMC	Treated with 1 % Carboxy methyl cellulose
3	GM induced nephrotoxicity (GAG)	Introduced Nephrotoxicity, with gentamicin at 80 mg/kg b.w.
4	Herbal mixture I	Treated with Hm250 mg/kg b.w.
5	Herbal mixture II	Treated with Hm 500 mg/kg b.w.
6	Herbal mixture III	Treated with Hm1000 mg/kg b.w.

On 9th day, blood samples from each group of animals were collected for serum biochemistry.

Blood Collection

The animals were mildly anesthetized using anesthetic ether before blood collection. Blood was collected from the retro-orbital sinus using heparinised capillaries (Remi, Mumbai). A portion of blood was collected in heparinsed vials for biochemical analysis of glucose (GLU), blood urea nitrogen (BUN), creatinine (CRE), protein (PRO), using kits of Span Dignostics (Mumbai, Maharashatra, India)/Nirmal Laboratories (Chopda, Maharashatra, India) on a Chariot Prince Biochemistry Analyser

Body weight and feed consumption

Body weight and feed consumption of animals were recorded daily throughout the experimental period. The animals were also observed for clinical signs daily throughout the experimental period.

Results and Discussion

Clinical signs: No clinical signs of toxicity and mortality were observed in animals belonging to any group.

Table 2: Changes in Biochemical values in the serum after 8th day of drug treatment

Group	$BUN_{(mg/dl)}$	$CRE_{(mg/dl)}$
Control	18.95±0.62	0.72±0.009
CMC	15.13±1.32	0.72±0.003
GAG	76.13±3.86	1.56±0.08
GM+ Hm I	70.58±4.93	1.18±0.17*
GM+ Hm II	49.15±9.54*	1.11±0.10**
GM+Hm III	22.88±2.95**	0.94±0.01***

GM + Hm – I = 250 mg/kg, GM + Hm – II = 500 mg/kg**,** GM + Hm – III = 1000 mg/kg,Values are expressed as Mean ± S.E Level of significance was evaluated by comparing with GAG*p<0.05, **p<0.01, ***P<0.001

Table 3 Urine analysis after the 8th day of treatment of drug

Group	Urea (mg/dl)	Creatinine (mg/dl)
Control	22.75±0.67	9.28±0.017
CMC	23.20±0.75	9.24±0.008
GAG	38.10±1.72	17.67±0.32
GM+ Hm I	38.98±1.29	17.30±0.15
GM+ Hm II	31.55±1.78*	16.46±0.32*
GM+Hm III	29.80±1.86**	10.51±0.49***

GM + Hm – I = 250 mg/kg, GM+ Hm – II = 500 mg/kg**,** GM + Hm – III = 1000 mg/kg, Values are expressed as Mean ± S.E, (n=6). Level of significance was evaluated by comparing with GAG*p<0.05, **p<0.01, ***P<0.001

Table: 4 Antioxidant activities in the kidney of rats after 8th day of drug treatment.

Groups	Superoxide dismutase (U/mg protein)	Catalase (U/mg protein)
Control	67.98± 4.04	49.22±4.16
CMC	62.67± 4.57	42.03±5.02
GAG	53.75±3.02	30.43±2.90
GM+ Hm I	66.71±3.46	38.96±3.87
GM+ Hm II	67.99±2.26*	46.43±3.46*
GM+Hm III	71.63±3.94**	50.74±0.76***

GM + Hm – I = 250 mg/kg, GM + Hm – II = 500 mg/kg**,** GM + Hm – III = 1000 mg/kg, Values are expressed as Mean ± .S.E (n=6), *P<0.05, **P<0.01, ***P<0.001

Changes in Biochemical values in the serum after 8th day of drug tretment

High level of BUN and creatinine indicate nephrotoxicity. Gentamicin when administered induced a marked renal failure characterized by a significant increase in BUN 76.13±3.86 mg/dl and serum creatinine 1.56±0.08 mg/dl as compared to control group (18.95±0.62 and 0.72±0.009 mg/dl respectively). On treating with different doses of the herbal mixture, the levels of BUN and creatinine were similar to that of respective control at the dose of 1000 mg/kg b.w., (22.88± 2.95 mg/dl 0.94±0.01mg/dl and respectively) whereas, at the other two dose levels these two parameters were higher than that, of the respective control.

Urine analysis after 8th day of treatment of drug.

The Urea and Creatinine were significantly increased in gentamicin alone treated group of animals (38.10 ± 1.72, 5.83 ± 0.18, 7.51 ± 0.14, 5.72 ± 0.05, 17.67 ± 0.32 respectively) when compared to control group of animals 22.75±0.67, 4.11±0.04, 5.12±0.34, 2.88±0.15, and 9.28±0.017 respectively. The changes in Urea (29.80±1.86 ***P*<0.01) and creatinine (10.51 ± 0.49 ****P*<0.001) were revert significantly to normal level on co-administration with the herbal mixture at the dose 1000 mg/kg b.w. as compared with control group of animals (22.75 ± 0.67; 4.11 ± 0.04; 5.12 ± 0.34; 2.88 ± 0.15; 9.28 ± 0.017 respectively).

Antioxidant Activity

Superoxide dismutase (SOD)

The level of SOD is decreesed significantly when administration of GM at a dose 80mg /kg b.w. as compared with control group that is 67.98±4.0 and 53.75±3.0 U/mg protein in GAG group of animals. Level of SOD increased significantly ***P*<0.01

Catalase

Catalase activity was decreased in GAG group of animals (30.43±2.90).Which was recoverd when it was treated with herbal mixture at a dose 1000 mg/kg b.w. in 50.74 ±0.76 U/mg protein [2].

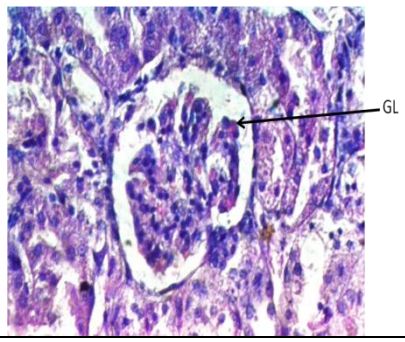

Fig 1: T.S. of kidney of Control group (9th Day).
GL = Glomerulas (100X; H-E Stain)

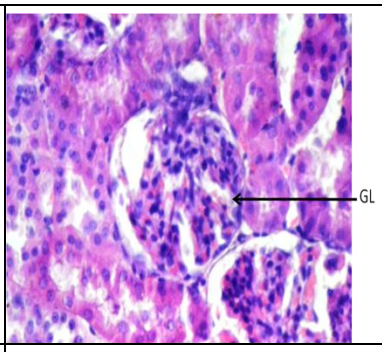

Fig 2: T.S. of kidney of CMC group (9th Day).
Normal Glomerulus (GL) (100X; H-E Stain)

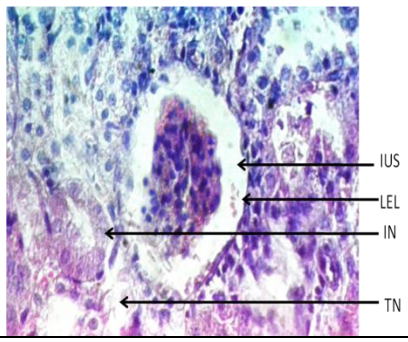

Fig 3: T.S. of kidney of gentamicin 80 mg/kg b.w. group (9th day).
Tubular Necrosis (TN), Increased Urinary Space (IUS), Loss of Epitheilal Lining (LEL) and Inconspicuous Nucleoli (IN).
(100X;H-EStain)

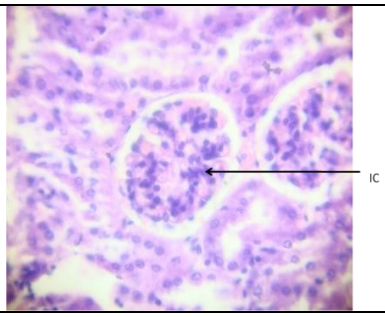

Fig 4: T.S. of kidney of Hm 250 mg/kg b.w. group (9th Day).
Inflammatory cell (IC), (100X;H-EStain)

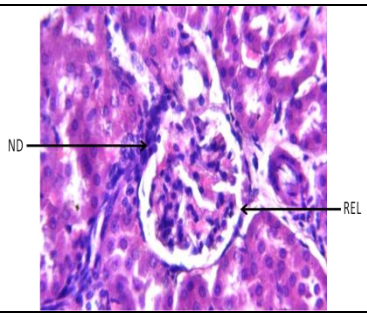

Fig 5: T.S. of kidney of Hm 500 mg/kg b.w. group (9th Day).
Restored Epitheilal Lining (REL) and Nuclear Debris (ND).
(100X;H-E Stain)

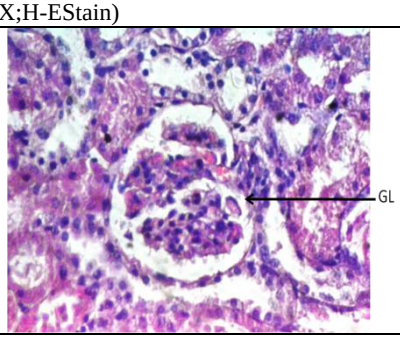

Fig. 6: T.S. of kidney of Hm 1000 mg/kg b.w. group (9th Day).
Normal Glomerulus (GL) (100XH-E Stain)

It is well established that GM induced histomorphologal changes in kidney of rat. After 8 days of treatment histopathological examination of kidney section of rat revealed normal architecture in control group of rats Fig.1 Whereas, severe and generalized tubular changes such as Tubular Necrosis, Increased urinary space, Loss of epithelial lining, and Inconspicuous nucleoli seen in gentamicin treated rats. Fig.2 [3] while, Co-administration of herbal mixture at a dose 250mg /kg shows mild tubular necrosis and nuclear debris Fig.3.Wheras,Hm dose increased at 500 mg /kg in male restored epithelial lining and nuclear debris.Whereas, the Hm at a dose of 1000mg/kg showing normal architecture of glomerulus of kidney seen in rats.Fig.4 and 5 [4].

Conclusion

The present study showed that the polyherbal prepration has antioxidant activity similar to the standard compound. Also it shows good nephroprotective activity. Further investigation of individual compounds and characterization of bioactive compounds responsible for the observed significant efficacy is needed.

References

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