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Possible Role of Natural Nephroprotective; *Hemidesmus indicus* in Congestive Heart Failure

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ABSTRACT

Hemidesmus indicus R.Br. is a treasure of the forest and herbal wealth. It is being used as folk medicines and as an ingredient in Ayurveda and Unani preparations against diseases of blood, inflammation, diarrhea, urinary disorders and rheumatism etc. The objective of the present study was to evaluate the role of natural nephroprotectant *Hemidesmus indicus* for the treatment of congestive heart failure. *Hemidesmus indicus* extracts were evaluated by using salt water induced left ventricular hypertrophy in Spargue dawley rat model. The property was evaluated using serum creatinine, urea, SGOT, SGPT and urine albumin, creatinine, Na^+, K^+ and Ca^{++} as biochemical parameters and histopathological changes including myocyte diameter, neutrophil infiltration and cardiac hypertrophy. Results obtained from the present study indicates that aqueous and methanolic extracts have more significant inhibitory effect on salt water feeding induced severity of microalbuminuria, serum urea and creatinine, myocyte diameter and retention of Na^+ and water and increases the serum calcium level. We conclude from the present study that, potent natural nephroprotectant *Hemidesmus indicus* is also a potent cardioprotective.

Keywords: *Hemidesmus indicus,* left ventricular hypertrophy, myocytes, microalbuminuria, Cardioprotective.

INTRODUCTION

Heart failure (HF) is one of the most common causes of death and disability in industrialized nations and is among the syndromes most frequently encountered in clinical practice (1,2). A number of blood tests are routinely performed in the diagnosis of heart failure; blood gas analysis to assess respiratory gas exchange; serum creatinine and urea to assess renal function, serum alanine and aspartate-aminotransferase plus other liver function test (3). The magnitude of albumin excretion is directly corrected with risk for end stage renal disease and the rate for progression to renal failure (4). The greater the magnitude of albuminuria, the faster the decline in renal function. In adults, persistent microalbuminuria suggests not only the existence of renal disease, but also an increase

risk for heart failure; therefore microalbuminuria is a strong and independent predictor of clinical HF (5).

It has been clinically proved that profound reduction of cardiac output and arterial hypotension in severe HF may lead to acute renal failure (6). Early complications of cardiac insufficiency include renal vasoconstriction and the development of sodium and water retention, which are hallmark of the very early stages of congestive heart failure (CHF). The interplay between the direct consequences of renal disease and other risk factors for HF are likely to contribute to this phenomenon (7). Moreover, HF and kidney failure shall be managed by similar means of non-pharmacological and pharmacological therapeutic interventions. There are some natural drugs they have been shown both cardioprotective and nephroprotective action including; *Curcuma longa* (8, 9), *Ginkgo-biloba* (10), *Ocimum* sanctum (11, 12), *Genus Angelica* (13) *and Rheum emodi* (14, 15). With this background the present study has been designed to evaluate the potent and scientifically proven natural nephroprotective drug of Indian system of medicine, *Hemidesmus indicus* R.Br (16) (family: Asclepiadaceae) for its scientific applicability in the treatment of CHF.

MATERIALS AND METHODS

Materials

UV- Spectrophotometer (SHIMADJU-1601), Flame photometer (Elico CL-361), Research centrifuge (REMI-24), Soxhlet extractor, Research microscope (Medzer).

Commercial enzyme kits including Creatinine, Urea, and SGOT & SGPT purchased from Span Diagnostic Ltd. Surat, India. All chemicals used in this study were of AR grade.

Methods

In the present study, the roots of *Hemidesmus indicus* were collected from the forest area of Gandhinagar, Gujarat. The roots were authenticated by Taxonomist available in B.V.V.S. Ayurvedic College, Bagalkot, Karnataka. Soon after authentification, all roots were dried at room temperature, until they were free from the moisture. Finally roots were subjected to get coarse powder and then passed through sieve no.40 to get uniform powder. The sieved powder was stored in airtight, high-density polyethylene containers before extraction.

Plant material

The roots of *Hemidesmus indicus* were collected from the forest area of Junagadh district, authenticated and roots were subjected to get coarse powder and then passed through sieve no: 40 to get uniform powder. The sieved powder was stored in airtight, high density polyethylene container before extraction.

Plant preparation

The powder was subjected to hot continuous extraction (Soxhlet) 12 hours cycle with 90% methanol (50–55°C); finally the drug was macerated with water. After the extraction the solvent was distilled off & excess solvent completely removed by using rotary flash evaporator to get semisolid mass, % yield was calculated and extracts were preserved in refrigerator below 10°C for further study. The suspension of methanol extract was prepared by using 0.5% Tween- 80 in normal saline and solution of aqueous extract was prepared by using normal saline as solvent for the experiment (17).

Animal selection

Spargue dawley rats of 2 weeks age of either sex were used in this study. The animals were maintained under suitable nutritional and environmental conditions throughout experiment. All the pharmacological experimental protocols were approved by the Institutional animals ethics committee (Reg no: 821/01/a/ CPCSEA, dated 6th August, 2007) H.S.K. College of Pharmacy, Bagalkot. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd)

Acute toxicity study

Healthy Swiss albino mice of either sex weighing 15–20 g, starveted overnight were divided into 3 groups (n=6) and fed with increasing doses (1, 2, and 5 mg/kg) of extract and the toxicity was evaluated as per the guidelines for non-clinical toxicity investigation of Herbal Medicine (Annexure-I) given by ministry of Health and family Welfare Govt. of India (18).

Experimental design

All the animals were randomly divided into the nine groups of six animals each, in which one group was common for both the extract and served as normal rats which received only vehicle (0.5% Tween- 80, 2 ml/kg p.o). From remaining eight groups four groups for each extract in which one group served as control which received salt water and vehicle while remaining three groups received drug extract in a dose of 100, 200 and 400 mg/kg p.o. along with salt water.

Organ to body weight ratio

Body weight of each animal was measured before the salt water and drug treatment were started. Percentage change in body weight was calculated by weighing animal just before scarifying them. After sacrifying each animal, the vital organs; heart, liver, lungs, kidneys were isolated and weighed them in a wet condition to measure organ to body weight ratio (19).

Biochemical parameters

Urine was collected for 24 hrs. using metabolic cages after 8th week of study. Serum was obtained from blood collected by retroorbital method under light ether anesthesia. Urine albumin (20), creatinine (alkaline picrate method), urea (DAM method), SGOT, and SGPT (2, 4-DNPH method) were estimated by using commercial enzyme kits (Span Diagnosis Ltd.). sodium, potassium and calcium ion concentration was estimated by flame photometer.

Histology

Samples of heart tissue were excised and rinsed in 0.9% saline blotted dry of saline and excess blood. They were fixed in 12% formalin for 24 hr. the tissues, after fixation, were washed in water to remove excess fixative. Washed tissues were then dehydrated through a graded series of ethyl alcohol, cleared with xylene and embedded in paraffin wax. Sections were cut at 3μm with microtome blade, and mounted on clean glass slide. The sections were routinely stained with haemotoxyllin and eosin. The stained slides were observed (400X) in research microscope and photographed.

Myocytes diameter

To assess myocardial hypertrophy quantitatively, the short axis diameters of myocytes were measured at the level of the nucleus. In each animal, 100 myocytes were sampled in each slice at papillary muscle level so that the sampling points were evenly distributed from the subendocardium to the subepicardium.

Statistical analysis

Data was expressed as the means ± standard error of means (S.E.M.) and statistical analysis was carried out using student's t-test. P values of less than 0.01 were considered to be statistically significant.

RESULTS

Serum and urine biochemical parameters

Feeding of salt water for six weeks significantly increased urine albumin/creatinine ratio, serum creatinine & urea. Salt water feeding also decreased serum ca^{++} level than compared to normal rats without affecting significantly the serum level of Na^+ and K^+ (Table 1). Increased urine albumin/creatinine ratio by salt water feeding was successfully reduced by 100, 200 and 400 mg/kg aqueous extract. Methanolic extract also significantly increased the urine Na^+ & K^+ level than compare to salt water induced urine electrolyte retention. Serum creatinine and urea was also decreased by the treatment of methanolic extract. Significant increase in level of serum Ca^{++} was also observed with methanolic extract treatment (Table 1).

Organ weight to body weight ratio

In salt water given rats wet weight of heart to body weight ratio was decreased significantly when compared to normal water fed rats. Higher dose of aqueous extract significantly reduced wet weight of heart than compared salt water fed rats. All three doses of aqueous extract significantly reduced wet weight of liver than compare to vehicle treated group. Methanolic extract has no significant effect on heart when compared to salt water fed rats. Regular treatment of animals with aqueous extract also inhibited salt water induced decreased body weight (%) significantly at medium and maximum dose. But, methanolic extract potentiated percent decrease in body weight initiated by salt water administration (Table).

Myocyte diameter

Cardiac hypertrophy was evidenced in salt water fed rats with increased diameter of myocytes measured at center of nucleus presence in 100 nos. of myocytes. All three doses of aqueous extract inhibited salt water induced hypertrophy significantly. However, effect was much higher in small dose than compare to moderate and higher doses. Methanolic extract also significantly decreased myocardial cellular diameter than compare to salt water fed group. But, much higher effect was observed with higher dose of methanolic extract. (Table 3)

Histopathology

In salt water fed rat (Figure 1 & Figure 2) significantly increase in diameter of myocytes and also the gap between myocytes, appeared when compared to normal water fed rat (Figure 1). In aqueous treated group significantly decrease in gap between myocardial fibers and appears to be normal with 400 mg/kg dose (Figure 5), but it fails to reduce gap between myocardial fibers at 100 & 200 mg/kg dose (Figure 3 & 4 respectively). In addition there was also arrangement of myocytes nucleus in parallel manner with 400 mg/kg dose. In methanolic extract at 100 mg/kg dose (Figure 7) perivascular neutrophil infiltration

Table 1: Effect of Hemidesmus indicus Aqueous Extract on Serum and Urine Biochemical Parameters

Treatment	Urine albumin (gm %)	Urine Creatinine (gm %)	Albumin/ creatinine	Urine Na^+(mEq/L)	Urine K^+(mEq/L)	Serum creatinine (mg/dl)	Serum Urea (mg/dl)	Serum SGOT (U/ml)	Serum SGPT (U/ml)	SerumNa^+ (mEq/L)	SerumNa^+ (mEq/L)	SerumCa^{++} (mEq/L)
Water (2ml/kg;po)	0.54	0.045	92	99.0	110.9	0.88±0.107	40.30±1.44	50.06±5.69	80.61±7.32	129.06±12.43	139.98±22.25	11.78±1.04
Salt & Water (po)	6.01	0.030	200.33	35.6	60	2.76±0.18***	76.13±9.71*	51.24±2.22	90.36±3.34	135.4±7.44	151.28±19.05	5.30±0.48**
AEHI (100 mg/kg)	3.12	0.072	43.33	869.61	125.9	1.25±0.16**	45.2±2.77*	86.03±5.81**	143.25±6.12***	76.73±7.27**	104.18±13.19	10.46±0.37***
AEHI (200 mg/kg)	29	0.072	40.27	932.59	103.8	0.90±0.098***	45.55±3.16	98.66±6.26***	146.36±7.05***	69.75±11.95*	81.7±7.93*	10.55±0.35***
AEHI (400 mg/kg)	3.27	0.061	53.60	872.77	136.5	0.97±0.07***	44.13±1.47*	85.28±2.61***	125.58±3.19***	86.18±5.25**	134.43±3.93	7.5±3.09*
Salt & Water (po)	6.23	0.019	327.89	27.2	42.5	2.65±0.19***	64.05±5.39*	56.11±3.49	89.76±1.29	144.95±11.51	131.01±14.20	5.73±0.32**
MEHI (100 mg/kg)	2.9	0.058	50	520.21	98.39	1.31±0.13**	45.08±2.62*	57.47±6.43**	81.71±2.20***	140.76±4.51**	133.28±13.91	11.45±0.93***
MEHI (200 mg/kg)	3.01	0.055	60	1220	200.0	1.40±0.09***	43.36±2.58	50±4.11***	80.22±3.41***	136±6.39*	122.51±10.31*	10.8±0.64***
MEHI (400 mg/kg)	3.65	0.056	65.17	2887	353.9	0.99±0.07***	41.21±1.09*	46.11±2.61***	80.63±2.51***	94.43±5.97**	100.91±2.38	11.5±0.53*

Aqueous extract of dose 100, 200 7 400 mg/kg; po was administered daily by oral route for 4 weeks. Urine was collected for 24 hrs. using metabolic cages after 4th week of study. Na^+, k^+, ca^{++} were estimated by flame photometer, albumin was estimated by using Biuret test. Serum was obtained from blood collected by retro orbital method was analyzed by flame photometer to estimate Na^+, k^+, ca^{++} concentration. Creatinine, urea, SGOT and SGPT were estimated by using commercial enzyme kits. Results are expressed as mean ± SEM and analyzed by student't' test. Results of salt water group are compared with normal group and extract treated groups are compared with salt water group.

AEHI: Aqueous extract of Hemidesmus Indus

MEHI: Methanolic extract of Hemidesmus indicus

**P<0.05,*

***P<0.01,*

****P<0.001*

Table 2: Effect of Hemidesmus indicus Aqueous & Methanolic Extract on Organ weight to body weight ratio

Treatment	% Change in body weight	Organ weigh to body weight ratio (mg/gm)			
		Heart/ BW	Liver/BW	Lung/BW	Kidney/BW
Water(2ml/kg;po)	17.35 ± 1.68	4.18±0.25	43.22±2.03	7.18±0.52	4.55±0.32
Salt & Water (po)	-33.29±2.31**	3.82±0.18	48.63±1.77	6.22±0.42	4.24±0.21
AEHI (100mg/kg; po)	-26.06±3.51	3.33±0.16	33.92±1.12***	6.47±0.65	3.72±0.25
AEHI (200 mg/kg; po)	-17.97±3.05*	3.58±0.30	36.71±1.87**	8.12±0.65	4.20±0.24
AEHI (400 mg/kg; po)	-13.67±3.93	3.16±0.06*	33.78±0.58***	8.06±1.13	3.94±0.12
Salt & DMSO 10% (po)	-3.84±2.36	3.24±0.05*	45.05±1.13	6.58±0.54	4.23±0.07
MEHI (100 mg/kg; po)	-31.35±1.65	3.55±0.18	50.23±2.41	5.96±0.30	4.69±0.18
MEHI (200 mg/kg; po)	-29.57±1.85*	3.45±0.28	50.57±2.59	5.87±0.09	4.87±0.33
MEHI (400 mg/kg; po)	-23.32±2.84	3.70±0.37	54.01±3.27*	5.79±0.29	5.41±0.47

Aqueous extract of dose 100, 200 7 400 mg/kg; po and Methanolic extract of dose 100 and 400 mg/kg; po was administered daily by oral route for 4 weeks. Results are expressed as mean ± SEM and analyzed by student't' test. Results of salt water group are compared with normal group and extract treated groups are compared with salt water group. Negative sign indicates decrease in body weight when compared to body wt. of animals prior to studies.

AEHI: Aqueous extract of Hemidesmus Indus

MEHI: Methanolic extract of Hemidesmus Indus

**P<0.05,*

***P<0.01,*

****P<0.001*

Table 3: Effect of Hemidesmus indicus Aqueous & methanolic Extract on Myocyte diameter

Treatment	Diameter (micron)
Water (2ml/kg; po)	21.45±0.59
Salt & Water (po)	32.83±0.68***
AEHI(100mg/kg; po)	24.53±0.60***
AEHI (200 mg/kg; po)	26.60±0.66**
AEHI (400 mg/kg; po)	25.90±0.61***
DMSO 10% (2 ml/kg; po)	21.45±0.59
Salt & DMSO 10% (po)	30.76±0.90***
MEHI (100 mg/kg; po)	27.36±0.50*
MEHI(200 mg/kg; po)	26.89±0.58*
MEHI (400 mg/kg; po)	23.18±0.63***

Aqueous extract of dose 100, 200 7 400 mg/kg; po and Methanolic extract of dose 100,200 and 400 mg/kg; po was administered daily by oral route for 4 weeks. Results are expressed as mean ± SEM and analyzed by student 't' test & results of salt-water group are compared with normal group and extract treated groups are compared with salt water group.

AEHI: Aqueous extract of *Hemidesmus Indicus*

MEHI: Methanolic extract of *Hemidesmus Indicus*

*P<0.05,

**P<0.01,

***P<0.001

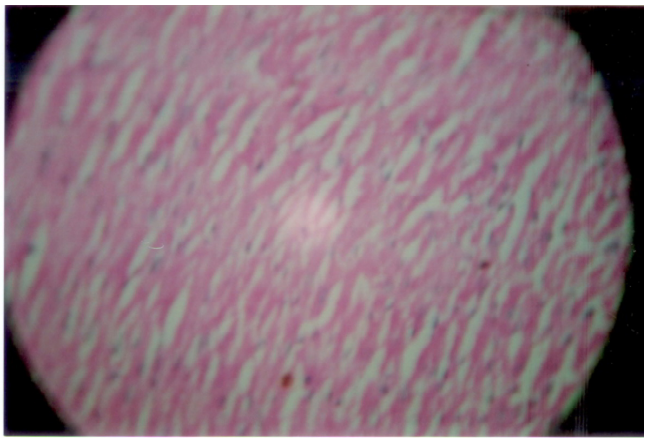

Figure 1: Effect of vehicle (Normal group)

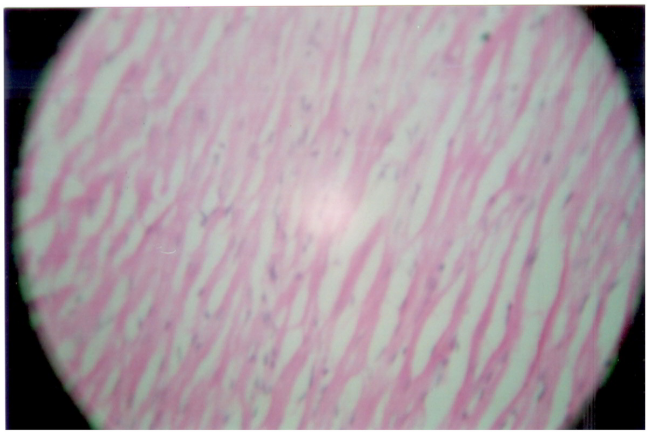

Figure 2: Effect of salt and vehicle (water)

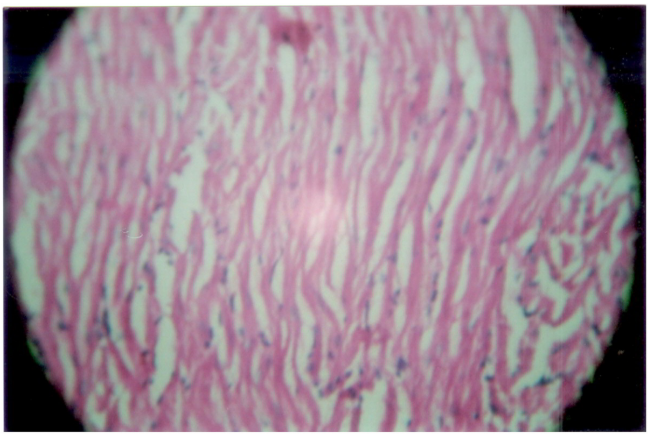

Figure 3: Effect of 100 mg/kg aqueous extract

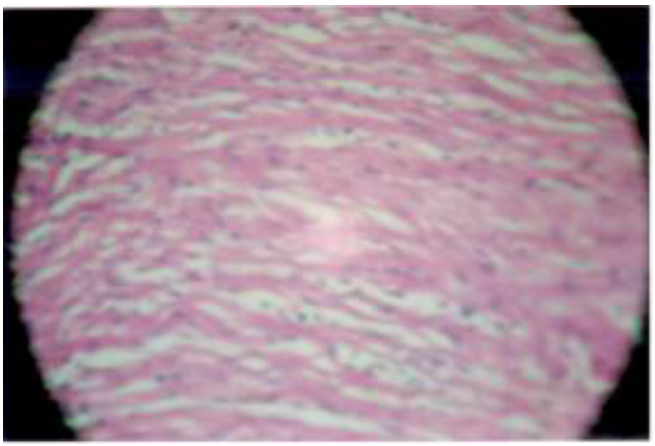

Figure 4: Effect of 200 mg/kg aqueous extract

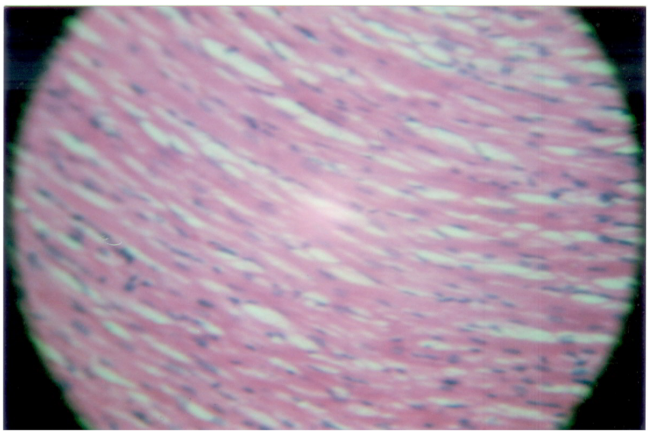

Figure 5: Effect of 400 mg/kg aqueous extract

was evident along with presence of neutrophils in between myocytes. Moderate dose of methanolic extract was associated with myocytes apoptosis/necrosis with the presence of fat deposition, neutrophil infiltration and enlarged diameter of myocytes (Figure 8). 400

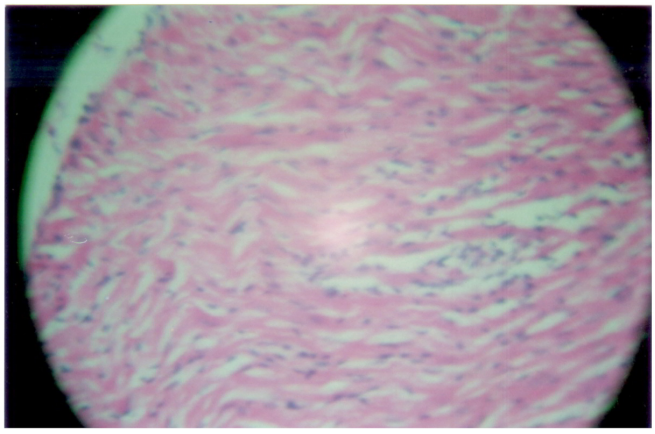

Figure 6: Effect of salt and vehicle (DMSO)

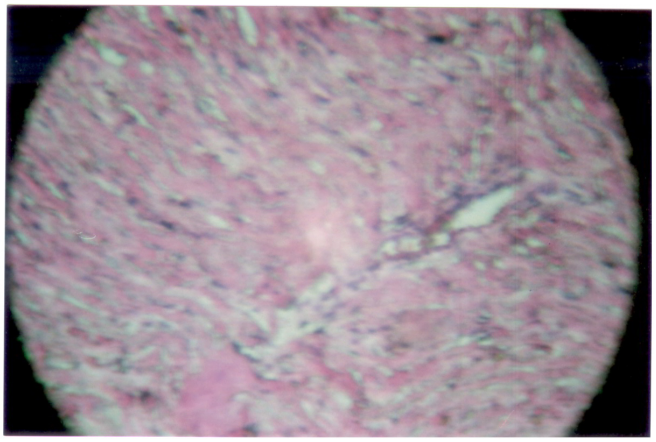

Figure 8: Effect of 200 mg/kg methanolic extract

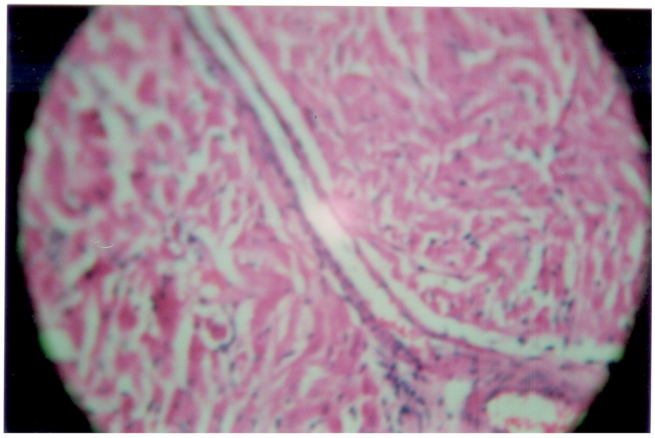

Figure 7: Effect of 100 mg/kg methanolic extract

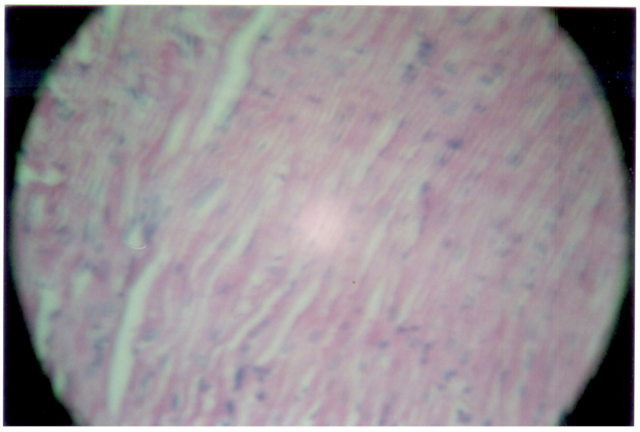

Figure 9: Effect of 400 mg/kg methanolic extract

mg/kg dose of the extract reduce gap in myocyte with potentially infiltration of neutrophils and apoptosis/ necrosis of myocytes.

DISCUSSION

Drugs acting on kidney and its performance have very significant role in control of cardiovascular complications and failure. This is the reason in extensive use of diuretics and ACE inhibitors for HF conditions, where they are able to control symptoms, exercise tolerance, survival in patients without improving functional capacity. With this background present study was carried out to know whether natural nephroprotective can be used in CHF induced by salt water feeding and evaluated its effect by considering organ/Body weight ratio, serum and urine biochemical parameters, myocytes diameter and histopathological conditions.

The Dahl-salt sensitive rats model has some advantages when compared to available models in consistency in the transition from LV hypertrophy to CHF, and compensated circulatory state to decompensated state can be achieved without and invasive and/or specialized surgical procedure therefore, this has been considered in our present study with little modification (20).

Supplementation of high salt diet to immature rats leads to development of systemic hypertension, renin angiotensin system activation and vascular sensitization. These pathologic changes leading to development of LV hypertrophy, decrease contractility of LV myocardium and pulmonary congestion causing shorter life expectancy and death. At the age of 10 weeks animals were started dying just after the salt water feeding for 8 weeks which confirms the earlier model. The postmortem pathological examination confirms these changes and demonstrated pathophysiology of HF also indicating the usefulness

of experimental model for study the mechanism and treatment of HF.

A dynamic reduction of venous capacity results in enhanced venous return, thereby increasing left ventricular end diastolic volume and pressure (21) leads to increased length of left ventricular muscle fibers and myocytes diameter (22), this compensatory mechanism will be lead to HF. At the cellular level changes associated with the transition to contractile dysfunction include deregulation of Ca^{++} homeostasis (23), myocytes enter a maladaptive proliferative phase in which fetal isoforms are expressed evidenced with increased myocytes diameter (24). Physiologically the retention of Na^+, K^+ and water are common to seen excessive in serum level while vasoconstriction is a pathologic phenomenon (25). The profound reduction of cardiac output and arterial hypotension in severe HF may lead to renal failure there conditions will be associated with rise in the blood content of urea, creatinine and phosphate. Microalbuminuria is recognized as an early sign of kidney disease as well as an independent risk factor for CV diseases. The magnitude of microalbuminuria is directly correlated with rate of progression in renal failure, hypertension and CV risk factors leading to HF.

Salt water feeding group shown an increase in serum Na^+ and K^+ level similar to earlier study may be leading to hypertension by increased norepinephrine (NE) vascular sensitivity[25]. Serum Ca^{++} homeostasis was also decreased in this group substantiating the earlier study on change in Ca^{++} homeostasis in HF condition.

Salt water fed rats also shown significant increase in serum creatinine and urea level signaling the kidney failure. Excess loss of albumin through urine was also observed in this group indicating renal damage. Microalbuminuria (the ratio between albumin and creatinine in urine), which is prominent in renal failure condition; this was also supported by their ratio as there is a significant increase in microalbuminuria in this group when compared to control. The enlargement of myocytes was also observed in this group as a compensatory process in HF condition.

The nephroprotective property of *Hemidesmus indicus* was evidenced with aqueous and methanolic extract preparation. Aqueous extract significantly inhibited the effect of salt water feeding modulated biochemical factors and reduced serum urea/creatinine level and also increased the level of urine creatinine. This extract also maintains homeostasis in serum Ca^{++} level (altered by salt water) may increase its cellular availability leading to increased cardiac output proposed in earlier studies. All three doses of Aqueous extract significantly ($P< 0.001$) decreased salt water induced change in liver/body weight ratio, simultaneously increased the level of SGPT and SGOT. Cardiac remodeling property of *Hemidesmus indicus* was also evidenced by its significant reduction in myocytes diameter in salt water induced hypertrophy. Significant inhibitory action of aqueous extract on salt water induced increased serum Na^+ may desensitize the NE action on vascular system and this factor may prevent the disease progression.

This methanolic extract increase urine Na^+ and K^+ level and salt water induced serum Na^+ and K^+ significantly showing its diuretic property which was supported by earlier studies, also inhibited salt water decreased serum Ca^{++} level and brought it to normal; but this extract decreased the body weight of animals higher than that of salt water treated groups showing its inability in improving the health status. More ever all animals in the dose of 200 mg/kg methanolic extract were died in 10^{th} week of study; histopathological examination indicates myocytes apoptosis and necrosis. Compared to methanolic extract aqueous extract of *Hemidesmus indicus* have shown promising effects for the treatment of CHF.

CONCLUSION

From the results obtained in our laboratory we propose the cardioprotective activity aqueous extract of *Hemidesmus indicus* possibly mediated through its diuretic, inhibition of pulmonary congestion, ability in remodeling of cardiac hypertrophy, reduced vascular sensitivity to NE, improved renal performance. Improvement in cardiac performance may also be related to increase in serum calcium level leading to increase in uptake of Ca^{++} by myocytes thereby increase the cardiac output. We conclude from this study that the plant *Hemidesmus indicus* known as a natural nephroprotective could be useful for CHF condition because of its multidimensory, molecular, cellular and pharmacological activities produced by its active constituents.

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REFERENCES

1. Davis R.C., Hobbs F.D.R., Lip G.Y.H. ABC of heart failure, History and Epidemiology. *BMJ*. **320**: 39–420 (2000).
2. Kasper D.L., Braunwald E., Fauci A.S., Hauser S.L., Longo DL, Jameson JL. *Harrison's principles of internal Medicine*, Vol-**I**, (McGraw-Hill: New York, 2005) 1301–1302.

3. Walker. R., Edwards C., *Clinical pharmacy and Therapeutics*, (Edinburgh: Churchill Livingstone 2003).
4. Ljungman S., Wikstrand J., Hartford M., Berglund G. Urinary albumin excretion-a predictor risk of cardiovascular disease. *Am. J. Hypertens*. **9** (8): 770–778 (1996).
5. Borch J.K., Feldt R.B., Strandgacard S., Schroll M., Jensen J.S. Urinary albumin excretion. An independent predictor of ischemic heart disease. *Arterioscler Thromb. Vascu. Biol*. **19** (8): 1992–1997 (1999).
6. Hall W.D. Abnormalities of kidney function as a cause and a consequence of cardiovascular disease. *Am. J. Med. Sci*. **317**(3): 176–182 (1999).
7. Brenner R.M., Wrone E.M. The epidemic of cardiovascular disease in end-stage renal disease. *Curr. Opin. Nephrol. Hypertens.*, **8**(3): 365–369(1999).
8. Mohanty I., Arya D.S., Gupta S. Effect of curcuma longa and ocimum sanctum on myocardial apoptosis in experimentally induced myocardial ischemic-reperfusion injury. *BMC complem. and Alter. Med*. **6**(3): 243–248 (2006).
9. Ali B.H., Al-Wabel N., Mahmoud O., Mousa H.M., Hashad M. Curcumin has a palliative action on gentamicin-induced nephrotoxicity in rats. *Fundamen. and Clin. Pharmacol.*, **19**(4): 473 (2005).
10. Liebgott T., Miollan M., Berchadsky Y., Drieu K., Culcasi M., Pietri S. Complementary cardioprotective effects of flavonoid metabolites and terpenoid constituents of Ginkgo biloba extract during ischemia and reperfusion. *Bas. Res. Cardiol*; **95**(5): 368–77 (2000).
11. Sharma M., Kishore K., Gupta S.K., Joshi S., Arya D.S. Cardioprotective potential of Ocimum Sanctum in isoproterenol induced myocardial infarction in rats. *Mol. Cell Biochem.***225**(1): 75–83 (2001).
12. Prakash P., Gupta N. Therapeutic uses of Ocimum sanctum Linn (Tulsi) with a note on eugenol and its Pharmacological actions: a short review. *Ind. J. Physio. Pharmacol*. **49**(2): 125–31(2005).
13. Sarkar S.D., Nahar L. Natural medicine: the Genus Angelica. *Cur. medi. Chem*. **11**(11): 1479–1500 (2004).
14. Alam M.M., Javed K., Jafri M.A. Effect of Rheum emodi (Revand Hindi) on renal functions in rats. *J. Ethnopharmacol*. **96**(1–2): 121–5 (2005).
15. Du Y., Ko K.M. Effects of pharmacological preconditioning by emodin/oleanolic acid treatment and/or ischemic preconditioning on mitochondrial antioxidant components as well as the susceptibility to ischemia- reperfusion injury in rat hearts. *Mol. Cell. Biochem*. **12**(7): (2006).
16. Kotnis M.S., Patel P., Menon S.N., Sane R.T. Renoprotective effect of herbal drug used in gentamicin induced renal toxicity. *Nephrology*. **9**: 142–152 (2004).
17. Kokate CK, In: practical pharmacognosy, 3rd ed: New Delhi: Vallabh Prakashan: 107 (1994).
18. *Guidelines for Toxicity Investigation of Herbal Medicine (Annexure-1)* Department of Ayurveda, Yoga, Naturopathy, Unani, Siddha and Homeopathy, Ministry, Government of India 2007. http://indianmedicine.nic.in
19. Inoko M., Kiharay Y., Morii I., Fujiwara H., Dasayana S. Transition from compensatory hypertrophy to dilated failing heart ventricles in Dahi-salt sensitive rats. *Am. J. Physiolol*. **267**(36): H2471–H2482 (1994).
20. Gownelock AH. Varley's Practical Clinical Biochemistry.6th ed. New Delhi: CBS Publishers & Distributors. (1988).
21. Bruntony LL, Lazo JS, Parker KL. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York: Mc Graw- Hill (2006).
22. Pasumarthi K.B.S., Field L.J. Cardiomyocyte cell cycle regulation. *Circ. Res*, **90**: 1044 (2002).
23. Tasic N., Nesovic M., Djuric D., Kanjuh. Changes in calcium levels in blood and urine during various regimens of table salt intake in patients with essential arterial hypertension. *Srp. Arch. Celok. Lek*. **130**(1–2): 7–12 (2002).
24. Coluci W.S., Wright R.F., Braunwald E. New positive inotropic agents in the treatment of congestive heart failure. *N .Engl .J. Med*. **314**: 290–299 (1986).
25. Weber K.T. Aldosterone in congestive heart failure. *N. Engl. J .Med*. **345**: 1689–1697 (2001).