Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2161

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **3 (6):** 156-167 (2015)

International Journal of Pure & Applied Bioscience

Research Article

OPEN ACCESS

Absence of adverse toxic effects of Cardoguard, an Ayurvedic anti-hypertensive formulation, in sub-acute toxicity evaluation in rats

P. Yuvaraj, Therasilin Louis and V. Madhavachandran*

Experimental Pharmacology Unit, Nagarjuna Herbal Concentrates Ltd, Alakkode, kalayanthani P.O., Thodupuzha, Idukki, Kerala-685 588, INDIA

*Corresponding Author E-mail: dr.therasilin@gmail.com

ABSTRACT

Cardoguard, an ayurvedic polyherbal medicine, currently used for hypertension, showed efficacy in experiments on rats. Further, recent studies have shed light on its mechanism of action. The formulation containing 6 traditional medicinal plants as ingredients was not subjected to toxicity evaluation under controlled conditions. Therefore, in the present study, a sub-acute toxicity evaluation was carried out. The sub-acute toxicity study (29 days) in rats did not show any toxic symptoms at 25 mg/kg (which is 4 times higher than the extrapolated therapeutic dose) of Cardoguard as judged from serum biochemical parameters, hematological parameters, behaviour of animals, weight as well as histology of liver, kidneys and heart. However, a marginal decrease in body weight of female rats was observed. At very high doses (50 and 100 mg/kg) serum protein (albumin and globulin) decreased. At these doses, total cholesterol and LDL cholesterol were also decreased in male and female rats which are considered as beneficial effects. In male rats alone, at 100 mg/kg group a small statistically significant decrease in serum urea was observed. Thus the formulation appears to be very safe.

Key words: *Cardoguard, Herbal-formulation, Anti-hypertension, Rats. Toxicity*

INTRODUCTION

In India, traditional health care like Ayurveda, Sidha and local health traditions have strong presence since antiquity. The Ayurvedic system of medicine originated in India more than 2000 years ago and is still being practiced. The major limitation in the widespread acceptance of Ayurvedic medication is the lack of scientific validation for safety and efficacy in light of modern science. Detailed scientific studies in light of modern medical science will help to overcome this lacuna and establish international acceptability.

Hypertension affects a considerable percentage of the middle aged population worldwide and is a leading risk factor for cardiovascular diseases, stroke and kidney failure[1-3]. Various drugs and regimens have been formulated for the control of hypertension. In recent years there is a revival of interest in traditional systems of medicine at a global level.

Cite this article: Yuvaraj, P., Louis, T. and Madhavachandran, V., Absence of adverse toxic effects of Cardoguard, an Ayurvedic anti-hypertensive formulation, in sub-acute toxicity evaluation in rats, *Int. J. Pure App. Biosci.* **3(6):** 156-167 (2015). doi: http://dx.doi.org/10.18782/2320-7051.2161

There is growing evidence to show that low levels of several biologically active phytochemicals present in polyherbal phytomedicines can additively or synergistically act at several targets involved in a disease condition and cure the disease with minimal or without side effects compared to high doses of a single compound acting on a specific crucial target. The chances of developing toxic manifestations are more in the latter case.

Cardoguard is an antihypertensive medicine formulated by Nagarjuna Herbal Concentrates Ltd. Kerala, India. It is a polyherbal formulation containing *Raulwolfia serpantina* root, *Terminalia chebula* fruit, *Terminalia bellarica* fruit, *Terminalia arjuna* bark, *Embelica officinalis* fruit and *Boerhavia diffusa* whole plant. The drug is being prescribed by the physicians at Nagarjuna Herbal Concentrates Ltd. and is found to be very effective in the reduction of blood pressure in human subjects.

A prospective clinical study was conducted in 24 hypertensive individuals by the physicians of the Nagarjuna Ayurvedic Group. The mean systolic and diastolic blood pressures before initiation of treatment were 166.92 ± 17.89 mm Hg and 118.83 ± 21.91 mm Hg respectively. The patients were treated with Cardoguard for 15 days. The patients were asymptomatic, and the systolic and diastolic blood pressures dropped to 138.75 ± 15.41 mm Hg and 98.33 ± 14.51 mm Hg respectively (unpublished observations).

Studies have shown that Cardoguard attenuates negative ionotropic response of rat papillary muscle to reactive oxygen species[4]. Further, Cardogurd treatment resulted in the endothelium dependent vaso-relaxation of rat aorta[5]. Recently, the efficacy of Cardoguard in the prevention of cardiac remodeling was evaluated[6]. Cardoguard prevents cardiac remodeling and ameliorates left ventricular cardiac hypertrophy(LVH) in spontaneously hypertensive rats[5]. Left ventricular hypertrophy (LVH) is a modifiable risk factor, and regression of LVH reduces the propensity for adverse cardiovascular events. LVH strongly predicts cardiovascular morbidity and overall mortality in hypertensive patients[7]. Cardiac output increased in response to treatment with Cardoguard. Immunostaining for the phosphorylated components of major signaling pathways associated with hypertrophy shows that prevention of LVH by Cardoguard is possibly mediated through inhibition of extracellular signal-regulated kinases and protein kinase Cε signaling pathways[6].

Although insights were obtained on the mechanism of action of this ayurvedic type of polyherbal formulation, toxicity, if any, of this medicine was not evaluated. Although the ingredients of cardoguard are well known traditional medicinal plants and one of the ingredients *Phyllanthus embilica* fruit is an ingredient of diet, when a new poly herbal formulation is developed as a therapeutic agent, there is a need to evaluate its toxicity, if any, because in rare cases molecular interactions and formation of new compounds and/or disappearance of anticipated compounds can lead to adverse reactions. Therefore, the present study was undertaken to evaluate sub-acute toxicity, if any, of Cardoguard in rats.

MATERIALS AND METHODS

Cardoguard

Cardoguard is prepared using six medicinal plants given below. The plants were identified, collected, dried, powdered separately, weighed and mixed homogenously with buffer and binding materials, granulated together and made into capsules by Nagarjuna Herbal Concentrates Ltd. Gum acacia was used as the binding material. The capsule has the following composition:

Rauwolfia serpentina root (Sarpagandha/Indian Snakeroot)	(120 mg)
Terminalia arjuna bark (Arjuna/Myrobalan)	(40 mg)
Boerhavia diffusa whole plant (Punarnava/Spreading Hogweed)	(40 mg)
Terminalia chebula fruit (Hareethaki/Chebulic myrobalan)	(40 mg)
Terminalia belerica fruit (Vibheetaki/Beleric myrobalan)	(40 mg)
Emblica officinalis fruit (Amlaki/Indian gooseberry)	(40 mg)

Animals

Male and female Wistar albino rats (140 to 160 g) fed a standard diet and water *ad- libitum* in the animal house facility of our Institute and maintained under standard laboratory conditions were used for

evaluation of toxicity, if any. Institute Animal Ethics Committee (approved by CPCSEA) monitors experiments on animals.

Toxicity evaluation in male and female rats

For evaluation of sub-acute toxicity of the drug, 24 animals were randomized and divided into 4 groups each containing 6 male rats. One group was kept as control and groups 2, 3, 4 received 25, 50 and 100 mg/kg of the drug homogenate respectively. The formulation in 2% gum acacia was administered daily for 29 days (p. o.). Control group received 2% gum acacia in an identical manner. [Human dose: 420 mg/ patient; based on surface area of rat this is equivalent to approximately 6 mg/ kg of adult rat].

Similarly another set of experiment was done using female rats.

The behavior of the animals was observed daily for 1 hr for 29 days. Initial and final body weights, water and food intake, and state of stool were observed. The animals were sacrificed on the 30th day. Blood samples were collected with EDTA and plain tubes, EDTA tubes stored in refrigerator until analysis, plain tubes centrifuged and serum was separated stored in refrigerator until analysis. Liver, heart and kidneys were dissected out, weighed and observed for pathological and morphological changes. These organs were subjected to histo-pathological studies. Thin paraffin sections, processed and stained with hematoxylin and eosin, were observed for histo-pathological changes.

Hematological and serum biochemical parameters were determined. Hemoglobin was measured using haemoglobinometer with comparison standards. Activities of serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were assayed by the method of Reitman and Frankel,[8] and alkaline phosphatase by determining hydrolysed phenol with antipyrine[9]. Urea and cholesterol were determined by conventional methods[10]. Serum lipid parameters, proteins, etc were measured using standard assay kits. Total leukocyte count and differential counts were done as described elsewhere[11].

RESULTS

Feeding for 29 days, daily with the herbal drug (25, 50 or100 mg/kg) did not result in any conspicuous toxic symptoms. The general behavior of the animals was not altered. In the case of male rats, body weights as well as weight of organs were not significantly altered by the drug administration (Table 1).

However, in the case of females, there was a small, but significant reduction in the body weight which was less pronounced in the highest dose treated animals (Table 1).

In females, like body weight decline, organ weights were also decreased. There was a marginal decrease in the weight of heart in 25 or 50 mg/kg treated rats, but there was no significant decrease at 100 mg/kg. The weight of kidneys was also decreased in the treated female rats. The decrease in liver weight was significant at 50 mg/kg only (Table 2). However, when the organ weights were expressed per 100 g body weight, there was no significant change in the weight of organs in the treated groups when compared to that in control group (Table 2).

State of the fecal droppings, and food & water intakes (Table 3) were not altered by the drug treatment in both male and female rats.

The effect of Cardoguard on serum biochemical parameters in male rats is shown in Table 4. Activities of serum GPT, GOT and alkaline phosphatase were not significantly changed by the treatment. Serum levels of urea was decreased in the highest dose (100 mg/kg) treated group. Serum levels of creatinine, glucose, total bilirubin, total lipids, high density lipoprotein (HDL) and triglycerides were not significantly influenced by the drug treatment. Total serum cholesterol, as well as LDL cholesterol, was decreased in 50 and 100 mg/kg drug treated groups. Total protein, albumin and globulin were slightly, but significantly, decreased in the high doses (50 or 100 mg/kg) treated rats. However, the ratio of albumin and globulin was not significantly altered. Thus, the drug did not show any conspicuous toxicity in the serum biochemical parameters. The decrease in cholesterol and urea may be beneficial.

The effect of Cardoguard on serum biochemical parameters in female rats is shown in Table 5. Activities of serum GPT, GOT and alkaline phosphatase were not significantly changed by the treatment. Serum levels of creatinine, urea, glucose, total bilirubin, total lipids, high density lipoprotein (HDL) and

triglycerides were not significantly influenced by the drug treatment. Total cholesterol, as well as LDL cholesterol in the serum, was decreased in 50 and 100 mg/kg drug treated groups. There was no significant decrease at 25 mg/kg drug treated group. Total protein, albumin and globulin were slightly, but significantly, decreased in the highest dose (100 mg/kg) treated rats. However, the ratio of albumin globulin was not significantly altered. Thus, the changes in serum biochemical parameters in female rats were almost like those in male rats. The major difference is the decrease in urea observed in the high dose treated male rats. This decrease was not observed in the females.

There was no change in the hemoglobin levels and WBC counts in the treated groups compared to untreated control group both in males and females (Table 6).

Effect of the drug treatment on differential count is given in Table 7. There was no significant change in the differential count expect a marginal increase in neutrophils and a decrease in lymphocytes at the highest dose (100 mg/kg) studied. The same patenting occurred in female rats also (table not given here).

Table 1. Effect of Cardoguard treatment on body weight of male and female rats

	Weight of male rats (in gram)			Weight of female rats (in gram)		
Groups	Initial	Final	Weight gain	Initial	Final	Weight gain
Control	157.0 ± 7.6	246.3 ± 9.9	89.4± 14.3	142.3 ± 5.2	193.4 ± 8.7	48.3± 8.8
Cardoguard Treated						
25 mg/kg	168.3 ± 17.0	265.9 ± 17.9	87.2 ± 15.5	142.0 ± 8.8	168.2 ± 9.5*	26.2 ± 2.2*
50 mg/kg	142.3 ± 13.6	250.5 ± 30.8	82.3 ± 19.7	142.0 ± 3.2	166.4 ± 10.7*	24.4 ± 10.9*
100 mg/kg	177.5 ± 19.0	272.7 ± 21.1	95.0 ± 7.3	140.8 ± 6.9	168.6 ± 13.7*	31.9 ± 9.0
F value	1.82	1.38	0.51	0.547	5.65	6.82
P value	0.20	0.30	0.69	0.66	0.012	0.006

Values are Mean ± SD, n=6 animals

*, P ≤ 0.05 (compared to control)

Table 2. Effect of Cardoguard treatment on weight of liver heart and kidneys of male and female rats

	Weight of male rat organs (in gram)			Weight of female rat organs (in gram)		
Groups	Liver	Heart	Kidneys	Liver	Heart	Kidneys
Control	8.01 ± 0.86 [3.25]	0.75 ± 0.05 [0.30]	1.56 ± 0.16 [0.63]	6.86 ± 0.25 [3.54]	0.59 ± 0.04 [0.31]	1.28 ± 0.06 [0.66]
Cardoguard Treated						
25 mg/kg	9.06 ± 0.34 [3.41]	0.77 ± 0.03 [0.29]	1.62 ± 0.13 [0.61]	6.07 ± 0.45 [3.61]	0.52 ± 0.02* [0.31]	1.09 ± 0.05* [0.65]
50 mg/kg	8.55 ± 0.71 [3.42]	0.76 ± 0.07 [0.30]	1.58 ± 0.12 [0.63]	5.90 ± 0.39* [3.55]	0.53 ± 0.02* [0.32]	1.07 ± 0.07** [0.65]
100 mg/kg	8.99 ± 0.66 [3.31]	0.76 ± 0.06 [0.28]	1.63 ± 0.10 [0.60]	6.33 ± 0.45 [3.75]	0.56 ± 0.01 [0.33]	1.10 ± 0.07* [0.65]
F value	2.1	0.067	0.271	4.459	4.04	8.51
P value	0.156	0.976	0.845	0.025	0.034	0.003

Values are Mean ± SD, n=6 animals

*, P ≤0. 05; **, P ≤ 0.01 (compared to control)

Figures in parentheses represent the weight of organs per 100 g body weight.

Table 3. Effect of Cardoguard treatment on food and water intake in male and female rats

	M ale		Female	
	Food intake [g/day/rat]	Water intake [ml/day/rat]	Food intake [g/day/rat]	Water intake [ml/day/rat]
Control	16.0	32.3	11.9	17.5
Cardoguard Treated				
25 mg/kg	16.1	37.5	09.6	18.8
50 mg/kg	15.3	37.8	07.9	17.4
100 mg/kg	15.6	32.5	09.5	16.1

Values are mean of 6 animals in each group. Food and water intake for 6 animals was taken together for 24 hrs [on the 28th day of treatment].

Table 4. Effect of Cardoguard treatment on serum biochemical parameters in male rats

Biochemical Parameters	Groups				F and P value
	Control	Cardoguard Treated (mg/kg)			
		25	50	100	
Total protein (g/dl)	6.23 ± 0.40	5.83 ± 0.31	5.35 ± 0.39*	5.31 ± 0.40*	F: 5.37, P: .014
Albumin (g/dl)	3.97 ± 0.06	3.89 ± 0.08	3.73 ± 0.14*	3.72 ± 0.13*	F: 5.31 P: .015
Globulin (g/dl)	2.26 ± 0.35	1.93 ± 0.23	1.86 ± 0.21*	1.57 ± 0.14*	F: 4.35 P: .024
Albumin /globulin (ratio)	1.79± 0.25	2.04 ± 0.20	2.12 ± 0.55	2.45 ± 0.50	F. 1.83 P .196
Glucose (mg/dl)	91.48 ± 6.54	94.35 ± 6.88	97.28 ± 4.46	97.90 ± 5.48	F: 1.0 P: .43
SGPT (U/L)	47.65 ± 4.79	45.78 ± 5.22	41.43 ± 1.67	40.23 ± 4.39	F: 2.09 P: .148
SGOT (U/L)	63.28 ± 3.22	66.10 ± 9.02	73.20 ± 6.89	66.83 ± 6.24	F: 1.59 P: .244
AP (KA Units)	9.75 ± 0.42	9.43 ± 0.80	9.75 ± 0.58	9.82 ± 0.75	F: .28 P: .840
Total bilirubin (mg/dl)	1.25 ± 0.06	1.14 ± 0.15	1.04 ± 0.12	1.51 ± 0.25	F: 6.37 P: .008

Urea (mg/dl)	39.16 ± 1.82	38.77 ± 2.03	35.88 ± 2.43	33.75±1.62*	F: 4.15 P: .021
Creatinine (mg/dl)	1.65 ± 0.14	1.57 ± 0.15	1.69 ± 0.15	1.63 ± 0.11	F: .60 P: .630
Total lipids (mg/dl)	392.9 ± 18.0	358.9 ± 24.5	372.4 ± 21.2	357.4 ± 9.6	F: 2.95 P: .075
Total cholesterol (mg/dl)	122.5± 2.87	118.0± 2.97	102.5±3.68*	98.1±3.96*	F: 4.86 P: .019
HDL (mg/dl)	32.57 ± 3.01	32.67 ± 3.15	30.28 ± 3.09	34.29±3.01	F: 1.11 P: 0.38
LDL (mg/dl)	60.88 ± 5.26	57.50 ± 8.28	48.22 ± 5.38*	43.51±5.11*	F: 4.42 P: .026
Triglycerides (mg/dl)	145.4 ± 6.6	134.9 ± 10.8	140.1 ± 3.9	147.8 ± 5.7	F: 2.51 P: .120

Values are Mean ± SD, n=6 animals

*, $P \leq 0.05$ (compared to control)

Table 5. Effect of Cardoguard treatment on serum biochemical parameters in female rats

Biochemical Parameters	Groups				F and P value
	Control	Cardoguard Treated (mg/kg)			
		25	50	100	
Total protein (g/dl)	5.86± 0.30	5.79± 0.18	5.59 ± 0.22	5.27± 0.16*	F: 5.81 P: .011
Albumin (g/dl)	3.68 ± 0.14	3.51 ± 0.08	3.64 ± 0.09	3.48 ± 0.11*	F: 4.04 P: .032
Globulin (g/dl)	2.19 ± 0.39	2.28 ± 0.15	1.92 ± 0.24	1.78 ± 0.06*	F: 3.48 P: .050
Albumin /globulin (ratio)	1.73 ± 0.31	1.54 ± 0.09	1.92 ± 0.31	1.96 ± 0.06	F: 2.84 P: .083
Glucose (mg/dl)	96.9 ± 5.6	103.9± 3.4	103.0 ± 3.3	102.6 ± 2.3	F: 2.60 P: .086
GPT (U/L)	39.9 ± 2.1	39.7 ± 2.2	37.6 ± 2.8	37.8 ± 3.3	F: .840 P: .500
GOT (U/L)	61.8 ± 3.4	64.5 ± 4.3	66.0 ± 5.0	66.8 ± 4.1	F: 2.96 P: .096
AP (KA Units)	10.8± 0.55	9.80 ± 0.61	10.28 ± 0.74	9.85 ± 0.83	F: 1.92

					P: .180
Total bilirubin (mg/dl)	1.4 ± 0.17	1.45 ± 0.27	1.34 ± 0.11	1.23 ± 0.14	F: 1.076
					P: .416
Urea (mg/dl)	34.6 ± 2.0	33.0 ± 0.6	33.7 ± 1.4	33.3 ± 0.8	F: 1.153
					P: .368
Creatinine (mg/dl)	1.59 ± 0.03	1.55 ± 0.19	1.53 ± 0.11	1.61 ± 0.07	F: .348
					P: .791
Total lipids (mg/dl)	395.7±20.9	368.1±22.5	363.4±12.7	364.8±15.1	F: 2.79
					P: .086
Total cholesterol (mg/dl)	118.8±10.9	115.4± 8.4	106.5 ± 4.5	102.9 ± 5.3*	F: 3.766
					P: .041
HDL (mg/dl)	36.5 ± 2.1	35.7 ± 2.7	35.2 ± 2.3	39.0 ± 1.6	F: 2.368
					P: .122
LDL (mg/dl)	53.0 ± 10.0	49.9 ± 10.8	41.8 ± 4.5*	34.5 ± 5.6*	F: 4.14
					P: .031
Triglycerides (mg/dl)	146.6±10.1	149.4± 4.3	147.6± 4.6	147.1 ± 7.1	F: .128
					P: .942

Values are Mean ± SD, n=6 animals

*, $P \leq 0.05$ (compared to control)

Table 6. Effect of Cardoguard treatment on hemoglobulin and total leukocyte count in male and female rats

	Males		Females	
	Haemoglobin (mg/dl)	WBC (mm^3 $x10^{-3}$)	Haemoglobin (mg/dl)	WBC (mm^3x10^{-3})
Control	14.00 ± 0.34	12.73 ± 0.36	14.20 ± 0.12	12.63 ± 0.39
Cardoguard Treated				
25 mg/kg	14.23 ± 0.28	13.25 ± 0.52	14.15 ± 0.13	12.98 ± 0.26
50 mg/kg	14.10 ± 0.12	12.98 ± 0.17	14.16 ± 0.13	12.80 ± 0.23
100 mg/kg	14.33 ± 0.05	13.30 ± 0.66	14.21 ± 0.08	13.13 ± 0.22
F value	1.57	1.31	0.25	2.34
P value	0.25	0.32	0.86	0.13

Values are Mean ± SD, n=6 animals

Table 7. Effect of Cardoguard treatment on differential count in male rats % of total leucocytes

	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
Control	64.00 ± 1. 83	3.4 0 ± 0. 41	> 1	31.25 ± 1. 50	4.78± 0. 96
Cardoguard Treated					
25 mg/kg	66.50 ± 1. 91	3.0 5 ± 0. 53	>1	30.50 ± 1. 00	3.00 ± 1. 15
50 mg/kg	67.25 ± 2. 06	3.2 2 ± 0. 91	>1	29.25 ± 2. 98	3.50 ± 1. 00
100 mg/kg	69.50 ± 1. 92*	3.11 ± 0. 38	> 1	26.25 ± 1. 50*	4.25 ± 0. 92
F	5.51	1.1		5.38	2.32
P	.013	NS	NS	.014	NS

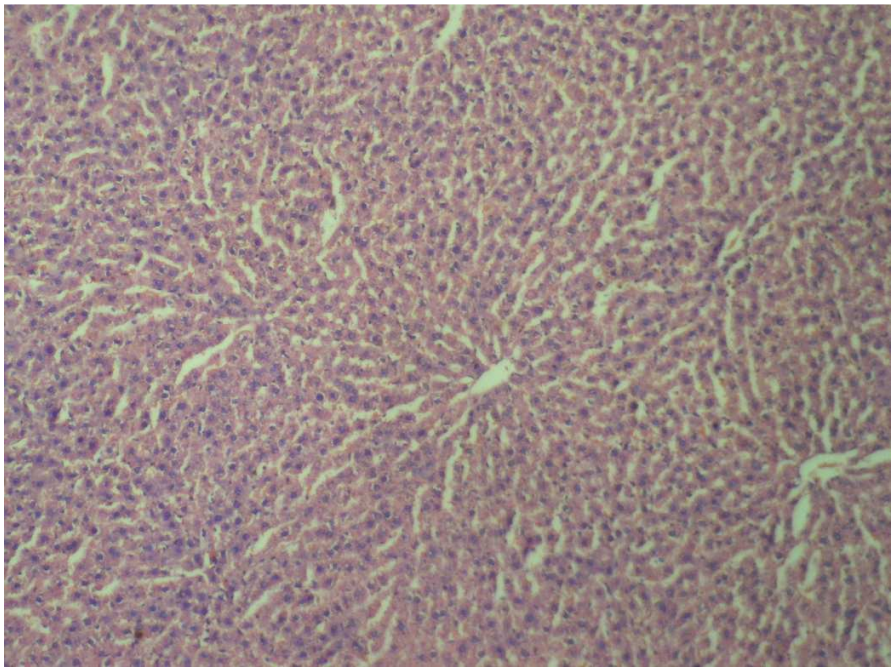
Values are Mean ± SD, n=6 animals

*, $P \leq 0.05$ (compared to control)

Histo-pathological studies:

Histo-pathological studies of liver, kidney and heart at the light microscopical level, did not revel any significant alterations [Fig. 1-3].

Fig.1: 1.A. Control male rat liver sections showing normal histo-architecture (stained with H&E)



1B. High dose (100 mg/kg) Cardoguard treated male rat liver showing normal histo-architecture (stained with H&E)

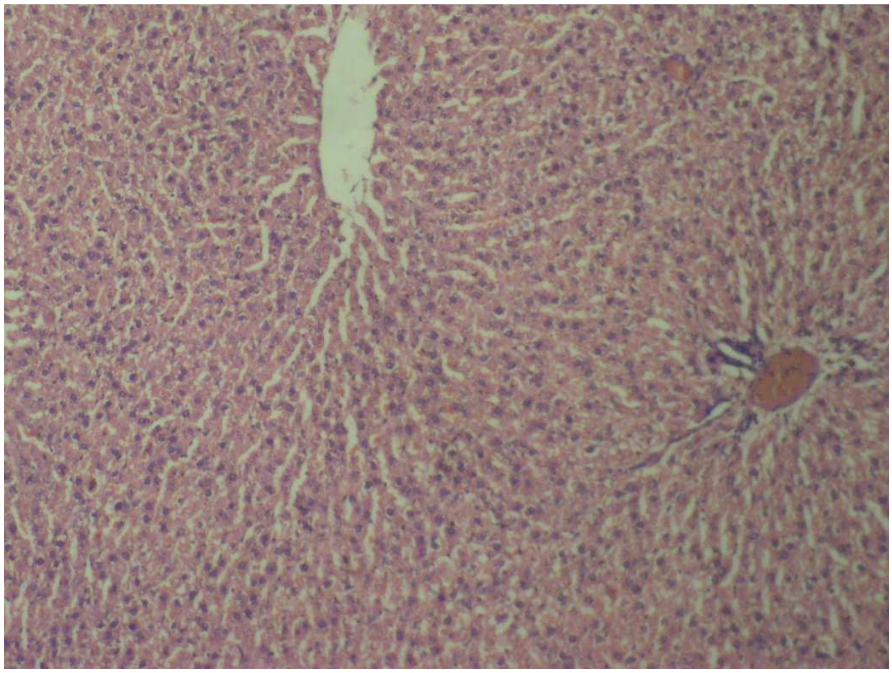
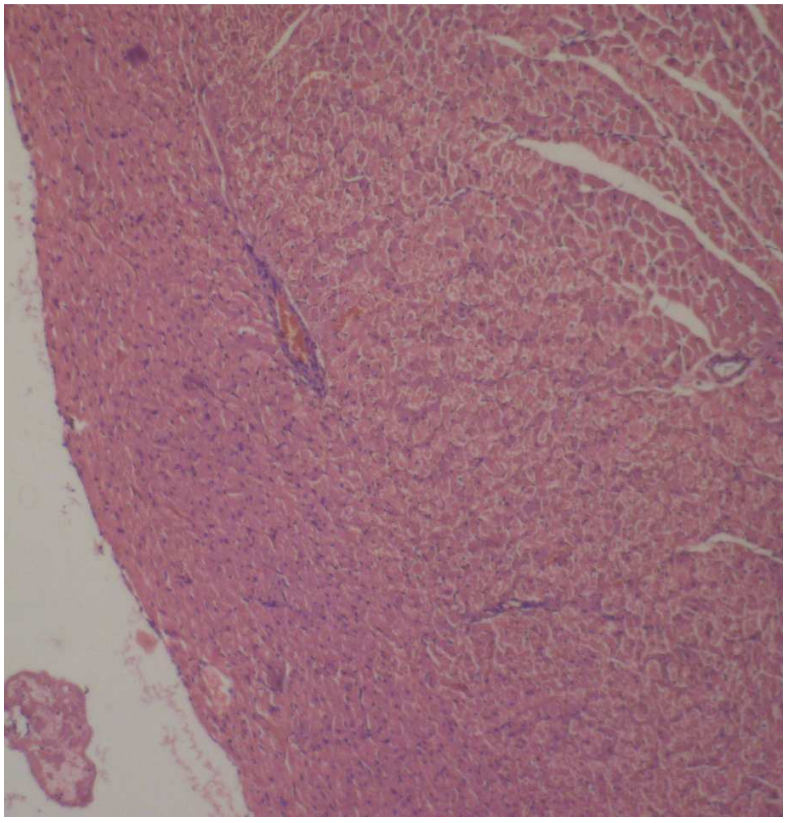


Fig 2: 2 A. Control female rat heart showing normal histo-architecture (stained with H&E)



2 B. High dose(100 mg/kg) Cardogurd treated female rat heart showing normal histoarchitecture (stained with H&E)

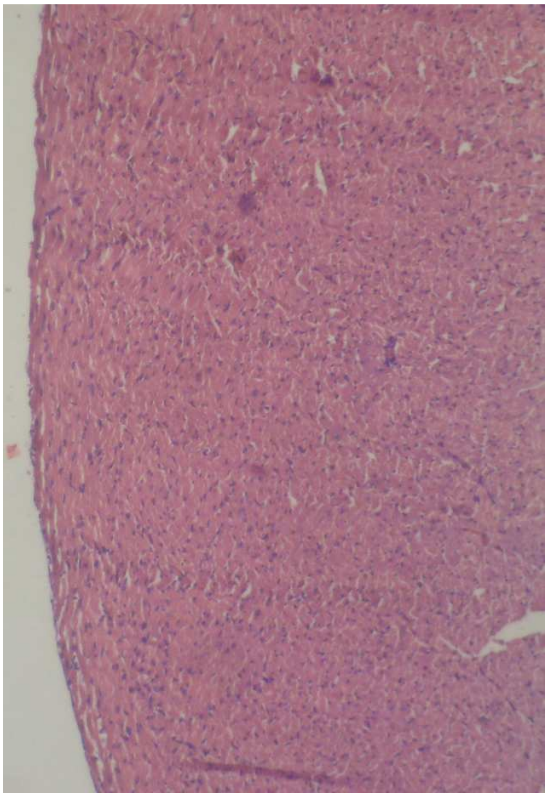
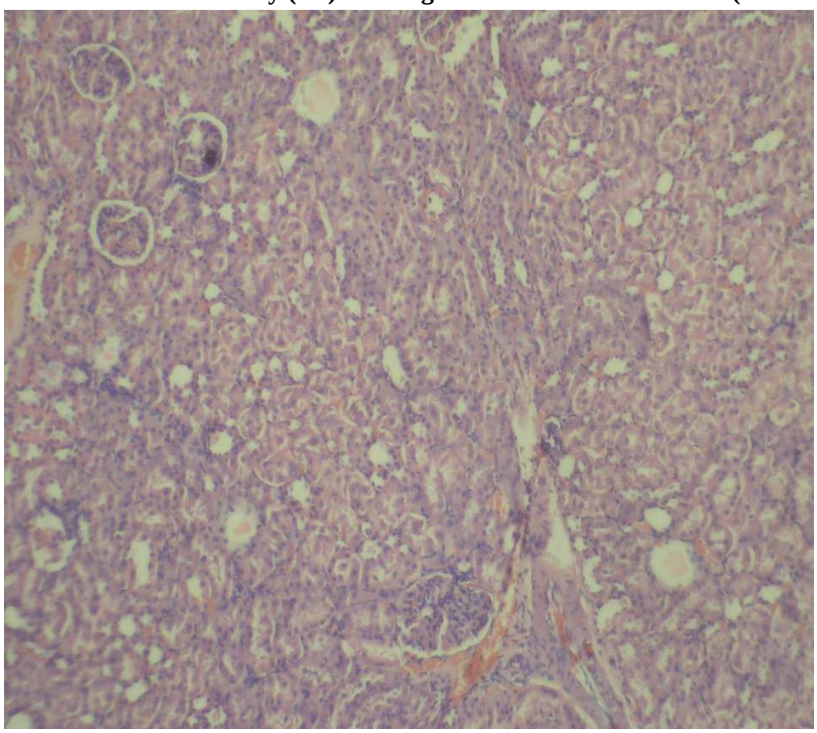
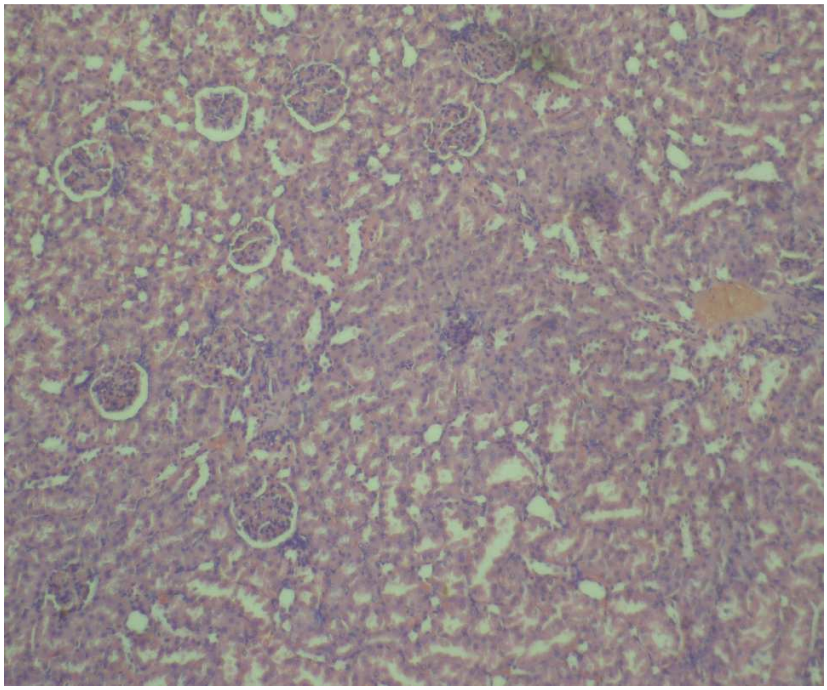


Fig. 3: 3 A. Control female rat kidney (LS) showing normal histo-architecture (stained with H&E)



3 B. High dose (100 mg/kg) Cardoguard treated female rat kidney (LS) showing normal histoarchitecture (stained with H&E)



DISCUSSION

The sub-acute toxicity study (29 days) in rats did not show any toxic symptoms at 25 mg/kg (which is 4 times higher than the extrapolated therapeutic dose) of Cardguard. Thus, this study strengthens the fact that the polyherbal formulation prepared from 6 traditional medicinal plants is very safe.

A marginal decrease in body weight of females, not males, was observed. This could be due to a marginal increase in the metabolic rate; this remains to be confirmed. At high doses (50 and 100 mg/kg) total cholesterol and LDL cholesterol were also decreased in males and females, which are considered as beneficial effects. At these doses, serum protein (albumin and globulin) was slightly decreased in both males and females.

In male rats alone, at 100 mg/kg group a small statistically significant decrease in serum urea was observed. This marginal decrease may not have any adverse effect in the physiology. This may indicate an improvement in urea filtration by kidney. In this connection it should be noted that the individual plants which form ingredients of Cardoguard are known to have several beneficial pharmacological properties.

Boerhavia diffussa is reported to have hepatoprotective action against radiation, anti-oxidant property and diuretic action[12,13]. *Terminalia arjuna* and *Terminalia chebula* are known to posses cardio-protective and anti-oxidant properties[14-20]. *Terminalia arjuna* is reported to have hypocholesterolemic activity also[20]. The poly phenols from *Embelica officinalis* are known to have powerful antioxidant activity[21-24.] *Rauwolfia serpentina* root is reported to cause generalized vasodilation, with a lowering of blood pressure, depressant action on the cerebral centres etc[25]. The toxicity study also suggests that in addition to its anti-hypertensive properly, the polyherbal phytomedicine may have other health promoting properties. Human clinical trials are warranted to establish these health benefits. When taken all together, the drug appears to be very safe and further studies including chemical standardization and fixing expiry date may lead to international acceptability to this valuable herbal medicine.

REFERENCES

1. Brilla, C.G., Janicki, J.S., Weber, K.T., *Circ Res.*, **69:** 107-15 (1991).
2. Haider, A.W., Larson, M.G., Benjamin, E.J., Levy, D,. *J Am Coll Cardiol.*, **32:** 1454-9(1998).

3. Hunter, J.J., Chien, K.R,. *N Engl J Med,*. **341:** 1276-83, (1999).
4. Sankar, V., Nair, R.R., Fernandez, A.C., Kumar, C.S.K., Madhavachandran, V. *Biomedicine,*. **28:** 12-15, (2008).
5. Sankar, V., Nair, R.R., Fernandez, A.C., Kumar, C.S.K., Madhavachandran, V. *Biomedicine,*. **30:** 18-24, (2010).
6. Sankar, V., Nair, R.R., Harikrishnan, V.S., Fernandez, A.C., Kumar, C.S.K., Madhavachandran, V. *Can J physiol Pharmacol*; **90:** 627-35, (2012).
7. Bauml, M.A., Underwood, D.A. *Cleve Clin J Med,*; **77:** 381-7, (2010).
8. Reitman, S., Frankel, S. *Am J Clin Pathol,*. **28:** 56-63 (1957).
9. Kind, P.R.N., King, E.J. *J Clin Pathol*, **70:** 322-30 (1954).
10. Jam, N.C. Schalm's Veterinary Haematology, 4th ed. Lea and Fabiger, Philadelphia, 1986: 43-8.
11. Subramoniam, A., Rajasekharan, S., Latha, P.G., Evans, D.A., Pushpangadan, P. *Fitoterapia*, **57:** 140-4 (1996).
12. Manu, K.A., Leyon, P.V., Kuttan, G. *Integr Cancer Ther*. **6(4):** 381-8 (2007).
13. Satheesh, M.A., Pari, L. *Indian J Exp Biol*. **42(10):** 989-92 (2004).
14. Karthikeyan, K., Bai, B.R., Gauthaman, K., Sathish, K.S., Devaraj, S.N. *Life Sci*. **73(21):** 2727-39 (2003).
15. Sun, F.Y., Chen, X.P., Wang, J.H., Qin, H.L., Yang, S.R., Du, G.H. *Am J Chin Med*. **36(1):** 197-207 (2008).
16. Suchalatha, S., Shyamala Devi, C.S. *Indian J Exp Biol*, **42:** 174-8 (2004).
17. Reddy, V.R.C., Kumari, S.V.R., Reddy, B.M., Azeem, M.A., Prabhakar, M.C., Appa Rao, A.V.N. *Fitoterapia*, **61:** 517-25 (1990).
18. Lee, H.S., Jung, S.H., Yun, B.S., Lee, K.W. *Arch Toxicol*, **81:** 211-8 (2006).
19. Bharani, A., Ganguly, A., Bhargava, K.D. *Int J Cardiol*, **49:** 191-9 (1995).
20. Gupta, R., Singhal, S., Goyle, A., Sharma, V.N. *J Assoc Physicians India*, **49:** 231-5 (2001).
21. Pozharitskaya, O.N., Ivanova, S.A., Shikov, A.N., Makarov, V.G. *J Sep Sci*, **30:** 1250-4 (2007).
22. Anilakumar, K.R., Nagaraj, N.S., Santhanam, K. *Indian J Exp Biol*, **45:** 450-4 (2007).
23. Bhattacharya, S.K., Bhattacharya, A., Sairam, K., Ghosal, S. *Phytomedicine*, **9:** 171-4 (2002).
24. Yokozawa, T., Kim, H.Y., Kim, H.J., Tanaka, T., Sugino, H., Okubo, T., Chu, D.C., Juneja, L.R. *J Agric Food Chem*, **19(55):** 7744-5 (2007).
25. Fahim, M., Khan, M.S., Hameed, H.A. *Indian J Physiol Pharmacol*, **39:** 101-5 (1995).