International Journal of Cardiology 126 (2008) 123 – 126

International Journal of Cardiology

www.elsevier.com/locate/ijcard

Letter to the Editor

Cardioprotective effect of *Azadirachta indica* A. Juss. on isoprenaline induced myocardial infarction in rats

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Received 23 November 2006; accepted 5 January 2007
Available online 27 April 2007

Abstract

The present study was designed to evaluate the cardioprotective potential of aqueous leaf extract of *Azadirachta indica* A. Juss. (AI) on the basis of haemodynamic, biochemical and histopathological parameters in isoprenaline induced myocardial infarction in rats and to compare with vitamin E, a known cardioprotective antioxidant. A significant ($p<0.01$) decrease in mean arterial blood pressure (MAP), systolic arterial blood pressure (SAP), diastolic arterial blood pressure (DAP) and increase in heart rate (HR) were observed in isoprenaline control group. Isoprenaline showed significant decrease in the level of cardiac marker enzymes [Lactate dehydrogenase (LDH) and Serum Glutamate Oxalotransaminase (SGOT)] in the heart homogenate with a corresponding increase in their level in serum. In vitamin E control group significant ($p<0.05$) increase in LDH in heart homogenate and decrease of SGOT and LDH in serum was observed. In isoprenaline control group, significant ($p<0.01$) increase in total cholesterol and triglycerides levels while decrease in high-density lipoproteins (HDL) was observed. On histopathological examination, myocardial damage in isoprenaline control group further confirmed cardiotoxic effect of isoprenaline. Our data showed that AI (250, 500 and 1000 mg/kg, p.o.) and vitamin E (100 mg/kg, p.o.) significantly restores most of the haemodynamic, biochemical and histopathalogical parameters. Finally we concluded that AI leaf extract exerts equipotent cardioprotective activity in the experimental model of isoprenalin induced myocardial necrosis in rats as compared to vitamin E, a known cardioprotective antioxidant.

Keywords: Isoprenaline; Myocardial infarction; Cardioprotective; *Azadirachta indica* A. Juss

Azadirachta indica A. Juss. (AI) traditionally employed intensively as folklore remedy for a wide spectrum of diseases like cough, nausea, vomiting, fever, jaundice, gonorrhea, intestinal worm infestation and leprosy in indigenous system of medicine [1]. Aqueous extract of leaves possess anti-inflammatory, antibacterial, [2], antioxidant, hepatoprotective [3], gastric ulceration-healing [4], hypoglycemic, negative chronotropic and inotropic effect on heart [5], AI contains alkaloids, tannins, coumarin, proteins, stigmasterol, flavonoids/polyphenols, saponins and sugars [6]. Taking into consideration the reported activities and the various active chemical constituents, in the present study, it is proposed that AI is beneficial to protect myocardial infarction.

The experiment and protocols described in present report were approved by the Institutional Animal Ethics Committee and in accordance with guidance of Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). The study was carried out in male Wistar rat (150–220 g). All animals were housed in-group of 5 and maintained under standardized condition (12/12 light/dark cycle, 24 °C) with free access to pellet food (CHAKKAN Diet, Pranav Agro Pvt. Ltd, India) and water. Isoprenaline (Torrent Research center, India), Vitamin E (Merk Pharma), Urethane (Willson Lab., India) and all experimental biochemical kits were obtained from Nirmal Lab. India. The leaves of the AI were collected from local area of Pimpri, Pune and

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doi:10.1016/j.ijcard.2007.01.108

Table 1
Haemodynamic and biochemical parameters in different experimental groups

	Groups /unit	Sham	VitE	AI-250	AI-500	AI-1000	ISP Control	IVitE	IAI-250	IAI-500	IAI-1000
Haemodynamic	SAP (mmHg)	126.80±21.32	103.80±6.05	112.60±5.00	117.60±6.96	119.00±5.91	77.40±2.82$^{\#\#}$	113.00±2.00**	129.20±1.80**	127.80±6.22**	148.80±3.32**
	DAP (mmHg)	100.40±15.06	95.40±3.17	86.20±1.98	90.00±2.55	94.40±7.68	68.40±3.04$^{\#\#}$	87.40±12.92*	107.00±1.81**	100.00±5.01**	109.20±5.36**
	MAP (mmHg)	105.20±15.21	112.80±3.09	120.80±3.82	121.40±5.30	123.80±5.16	82.20±2.13$^{\#\#}$	117.80±4.87**	111.00±4.39**	109.80±6.76**	128.00±4.66**
	Heart rate (bpm)	374.00±12.49	360.00±3.53	358.60±17.45	351.20±8.72	353.40±5.20	500.00±6.93$^{\#\#}$	407.60±6.02**	312.00±7.69**	354.80±6.08**	369.00±9.78**
Biochemical parameters in serum	SGOT (U/l)	91.20±3.30	69.80±3.45 $^{\#}$	85.20±2.41	79.60±1.72	73.80±3.96	201.40±4.00$^{\#\ \#}$	99.60±2.07**	151.20±2.41**	131.80±2.28**	101.80±2.54**
	LDH (U/l)	74.40± 2.50	56.80±2.13$^{\#\ \#}$	71.60±1.36	69.00±1.87	62.80±1.77	190.00±4.23$^{\#\ \#}$	83.40±1.07**	123.60±1.36**	110.00±1.81**	93.80±1.77**
	Triglycerides (Mg/dl)	189.50±2.07	129.33±2.04$^{\#\#}$	183.97±1.34	181.76±1.97	179.31±1.96	321.62±24.90$^{\#\#}$	191.25±2.14**	245.76±3.53**	215.92±2.42**	205.93±1.54**
	Total Cholesterol (Mg/dl)	95.36±2.92	46.29±1.09$^{\#\ \#}$	82.21±1.59	79.56±2.70	69.17±1.64$^{\#}$	142.65±1.9$^{\#\#}$	79.36±1.37**	92.82±1.35**	85.84±1.93**	81.19±1.69**
	HDL(Mg/dl)	76.46±3.06	80.25±4.03	60.56±4.65	66.46±1.95	70.88±5.19	13.40±1.47$^{\#\#}$	73.25±2.93**	51.86±1.56**	56.30±3.99**	65.72±5.27**
Biochemical parameters in heart homogenate	SGOT (U/l)	206.00±1.94	217.00±5.55	189.00±4.90	194.00±3.12	200.20±5.45	39.80±3.12$^{\#\ \#}$	202.52±3.58**	152.80±2.43**	187.40±2.87**	194.80±3.63**
	LDH (U/l)	203.40±3.40	223.40±8.71$^{\#}$	184.60±2.06	189.60±1.43	193.80±4.64	60.20±4.35$^{\#\ \#}$	195.60±2.54**	141.40±2.08**	159.80±1.65**	179.20±1.15**

$n=5$ $^{\#}p<0.05$,$^{\#\ \#}p<0.01$ versus Sham; *$p<0.05$, **$p<0.01$ versus ISP Control VE: Vitamin E 100 mg/kg, p.o., AI-250: *Azadirachta indica* A. Juss. extract 250 mg/kg, p.o., AI-500: *Azadirachta indica* A. Juss. extract 500 mg/kg, p.o., AI-1000: *Azadirachta indica* A. Juss. extract 1000 mg/kg, p.o., ISP: Isoprenaline 25 mg/kg, s.c., IVI-100: Vitamin E 100 mg/kg, p.o.+Isoprenaline 25 mg/kg, s.c., IAI-250: *Azadirachta indica* A. Juss. extract (250 nmg/kg, p.o.)+Isoprenaline 25 mg/kg, s.c., IAI-500: *Azadirachta indica* A. Juss. extract (500 mg/kg, p.o.)+Isoprenaline 25 nmg/kg, s.c., IAI-1000: *Azadirachta indica* A. Juss. extract (1000 mg/kg, p.o.)+Isoprenaline 25 mg/kg, s.c.

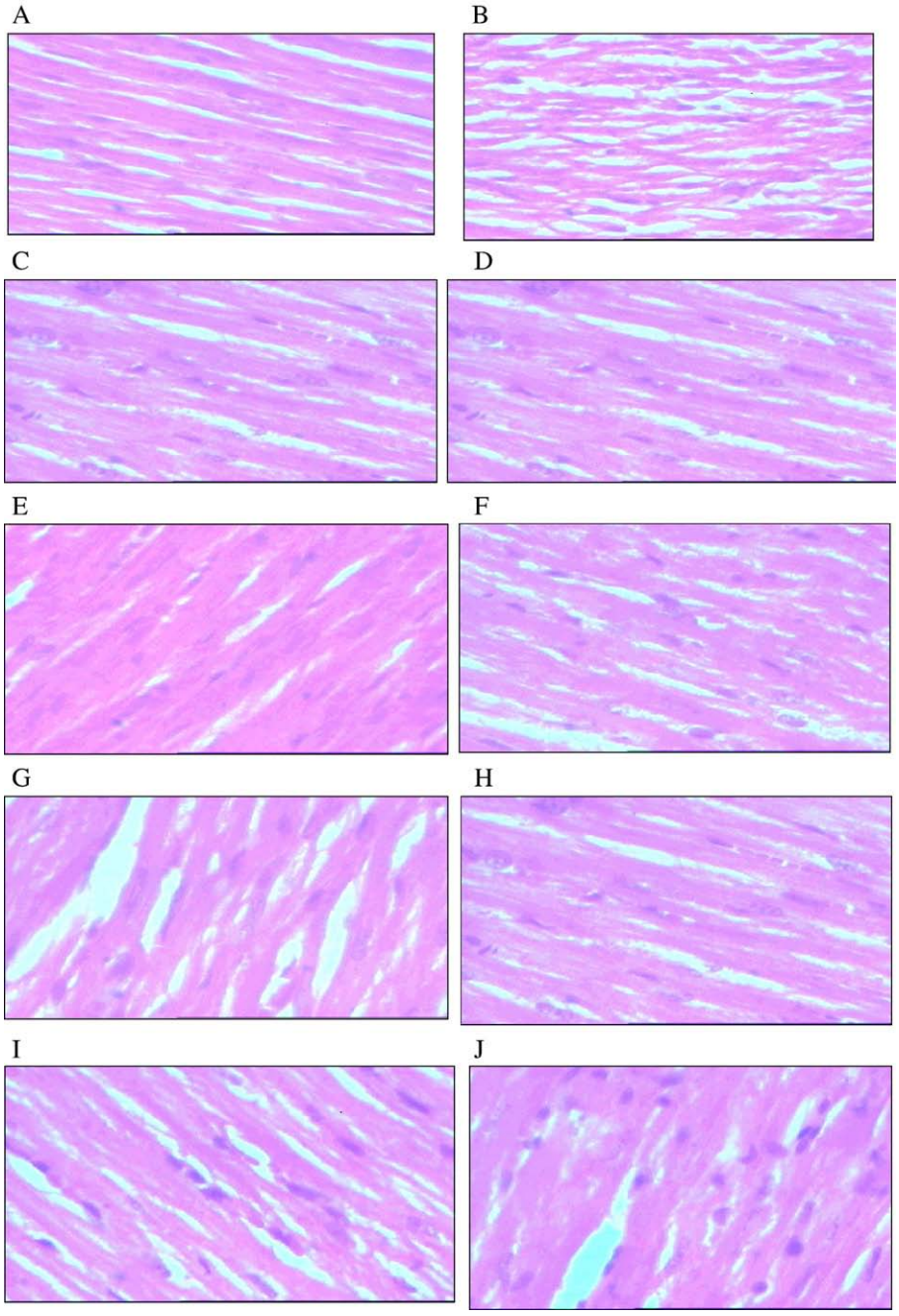

Fig. 1. Histopathology of left ventricle of heart: (A) Sham (distilled water, 1 ml/kg, p.o.), (B) Isoprenaline control (25 mg/kg, s.c.), (C) Vitamin E control histology (VE), (D) *Azadirachta indica* A. Juss. extract (250 mg/kg, p.o.), (E) *Azadirachta indica* A. Juss. extract (500 mg/kg, p.o.), (F) *Azadirachta indica* A. Juss. extract (1000 mg/kg, p.o.), (G) Vitamin E (100 mg/kg, p.o.)+Isoprenaline (25 mg/kg, s.c.), (H) *Azadirachta indica* A. Juss. extract (250 mg/kg, p.o.)+ Isoprenaline (25 mg/kg, s.c.), (I) *Azadirachta indica* A. Juss. extract (500 mg/kg, p.o.)+Isoprenaline (25 mg/kg, s.c.), (J) *Azadirachta indica* A. Juss. extract (500 mg/kg, p.o.)+Isoprenaline (25 mg/kg, s.c.) [Stains: H & E X100].

authenticated by Botanical Survey of India, Pune (voucher specimen number as *A. indica* A. Juss. — 68240.) Leaves were extracted by maceration process. % Yield: 9.2% w/v.

All animals were divided into six main groups: sham, isoprenaline control, AI/vitamin E control and AI/vitamin E treatment groups. AI control and treated group were again subdivided into three subgroups: AI-250, 500 and 1000. AI leaf extract was administered at a dose of 250, 500 and 1000 mg/kg, p.o. and vitamin E at a dose of 100 mg/kg, p.o. for 4 weeks in their respective groups. On day 29 and 30, the rats in the isoprenaline control and AI/vitamin E treated groups were given isoprenaline 25 mg/kg, s.c. at an interval of 24 h. On 31 day, haemodynamic, biochemical and histopathological-changes in heart were recorded.

The results are expressed as Mean ± SEM. The difference between groups was analyzed by One-way analysis of

Variance (ANOVA) followed by followed by Dunnet's *t*-test using INTA software and *p* value. $p<0.05$ has been considered as a statistical significance level.

A significant ($p<0.01$) decrease in mean arterial blood pressure (MAP), systolic arterial blood pressure (SAP), diastolic arterial blood pressure (DAP) and increase in heart rate (HR) were observed in isoprenaline control group. Isoprenaline showed significant decrease in the level of cardiac marker enzymes [Lactate dehydrogenase (LDH) and Serum Glutamate Oxalotransaminase (SGOT)] in the heart homogenate with a corresponding increase in their level in serum. In vitamin E control group significant ($p<0.05$) increase in LDH in heart homogenate and decrease of SGOT and LDH in serum were observed. In isoprenaline control group, significant ($p<0.01$) increase in total cholesterol and triglycerides levels while decrease in high-density lipoproteins (HDL) was observed (Table 1). On histopathological examination, in isoprenaline control group, significant myocardial damage and infiltration of inflammatory cells as compared to sham group was observed. Extensive myonecrosis with fibroblastic proliferation and presence of chronic inflammatory cells was also observed in isoprenaline control group. Our data showed that AI (250, 500 and 1000 mg/kg, p.o.) and vitamin E (100 mg/kg, p.o.) significantly restore most of the haemodynamic, biochemical and histopathalogical parameters.

Isoprenaline, a synthetic catecholamine has toxic effect on the myocardium. Amongst the various mechanisms proposed to explain isoprenaline-induced cardiac damage, generation of highly cytotoxic free radicals through auto-oxidation of catecholamines has been implicated as one of the important causative factor. In the present study, isoprenaline was administered in the dose of 25 mg/kg, s.c. for inducing myocardial infraction [7]. In case of isoprenaline control group, in haemodynamic parameters myocardial dysfunction was clearly evident by a significant fall in MAP, SAP, DAP and significantly increased heart rate (Table 1). It is speculated that deteriorating myocardial contractile status following isoprenaline-induced necrosis might be responsible for the significant fall in MAP. Normally fall in mean arterial pressure increases the heart rate and myocardial contractility due to reflux sympathetic action [8]. In case of Biochemical parameters, isoprenaline was showing significant decrease in the activities of LDH and SGOT (important markers of myocardial infarction) in heart with subsequent increase their activities in serum when compared with Sham. Increase in the activity of these enzymes in serum could be due to the leakage of these enzymes from the heart as a result of isoprenaline-induced necrosis [8]. Administration of isoprenaline (25 mg/kg, s.c.) produced significant increase in the serum total cholesterol and triglyceride levels and decreased the HDL level. *A. indica* A. Juss. leaf extract (250, 500 and 1000 mg/kg, p.o.) and vitamin E (100 mg/kg, p.o.) maintained all haemodynamic, biochemical and histopathological parameters near normal as compared to sham group. In histopathological examination, the presence of focal myonecrosis with myophagocytosis and lymphocytic infiltration (myocarditis) in the subendocardial region was observed in isoprenaline control group. Administration of isoprenaline (25 mg/kg, s.c.) failed to produce significant change in haemodynamic, biochemical and hstopathological parameters in AI (250, 500 and 1000 mg/kg, p.o.) leaf extract and vitamin E pretreated rats, indicating cardioprotective activity of AI leaf extract (Fig. 1).

This study thus demonstrates the cardioprotective effect of *A. indica* A Juss. leaf extract (250, 500 and 1000 mg/kg, p.o.). This extract was found to be most effective in the functional recovery of the heart and restoration of biochemical and histopathological alterations. Further isolation, characterization and purification of the active constituents and further experimentation would be necessary to elucidate the exact mechanism of action of *Azadirachta indica* A Juss.

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