Tropical Journal of Pharmaceutical Research January 2015; 14 (1): 63-72
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v14i1.10

Original Research Article

Protective Effect of *Bombyx mori* L Cocoon (Abresham) and its Formulations against Isoproterenol-Induced Cardiac Damage

Tarique Mahmood[1]*, Hefazat H. Siddiqui[1], Rakesh Dixit[2], Paramdeep Bagga[1] and Md. Sarfaraj Hussain[1]

[1]Faculty of Pharmacy, Integral University, Dasauli, Kursi Road, Lucknow-226026, [2]Department of Pharmacology, King George Medical University, Lucknow-226018, Uttar Pradesh, India

For correspondence:* ***Email:* *ansaritariq79@yahoo.co.in;* ***Tel:*** *+91-9918681701;* ***Fax:*** *0522-2890809*

Received: 27 March 2014 *Revised accepted: 21 November 2014*

Abstract

Purpose: *To investigate the potential activity of Bombyx mori and its formulations against isoproterenol (ISO) induced cardiotoxicity.*
Methods: *Wistar rats were orally pretreated with the ethanol extract of Bombyx mori cocoons in two doses (250 and 500 mg/kg) for 30 days; rats were similarly pretreated with its polyherbal formulations incorporating Khamira Abresham sada (KAS) and Khamira Abresham Hakim Arshadwala (KAHAW) (800 mg/kg), standard drug metoprolol (10 mg/kg) and normal saline for 30 days. Cardiotoxicity was induced by administration of isoproterenol (ISO, 85 mg/kg, subcutaneous) given twice on days 29 and 30 in all six pre-treated groups (n = 6) except the normal control. Cardiotoxicity was assessed by morphological and biochemical evaluation and further confirmed by histopathological studies.*
Results: *Pretreatment with Bombyx mori (500 mg/kg), KAHAW and KAS significantly decreased ($p < 0.01$) the heart weight:body weight (HW:BW) ratio; significantly decreases the elevated activities of the cardiac marker enzymes, namely, asparate transaminase (AST) ($p < 0.01$), alanine transaminase (ALT) ($p < 0.01$), lactate dehydrogenase (LDH) ($p < 0.01$) ,creatinine kinase (CK-MB) ($p < 0.01$) and thiobarbituric acid reactive substances (TBARS) ($p < 0.01$) similar to the standard drug metoprolol ($p < 0.01$) in ISO-injected rats. Pre-treatment of rats with Bombyx mori (500 mg/kg), KAS, KAHAW and metoprolol challenged with ISO also showed absence of troponin. Pretreatment with B. mori (500 mg/kg), KAHAW and KAS significantly increased the activities of Superoxide dismutase (SOD) ($p < 0.01$), Tissue glutathione (GSH) ($p < 0.01$) and catalase ($p < 0.01$) similar to the standard drug metoprolol ($p < 0.01$).*
Conclusion: *The findings of this study indicate that Bombyx mori as well as its polyherbal formulations exerts potent cardioprotection against isoproterenol-induced cardiotoxicity. This effect is comparable with that of metoprolol.*

Keywords: *Bombyx mori, Myocardial necrosis, Oxidative stress, Cardiotoxicity, Khamira Abresham, Metoprolol, Isoproterenol*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

In modern times, cardiac disease has emerged as the leading cause of death worldwide, particularly in developed countries. The World Health Organization reported that 16.7 million deaths in 2003 (29.2 % of total global deaths) were caused by some form of cardiovascular

disease [1]. Myocardial infarction or acute myocardial infarction, commonly known as heart attack is due to the interruption of blood supply to the heart, causing anoxia and death of heart cells [2]. Isoproterenol (ISO), a synthetic catecholamine and nonselective b-adrenoceptor agonist, at high doses, has been reported to produce weakening of endogenous antioxidant system, contractile dysfunction, cardiotoxicity, and cardiac damage (infarct-like lesions) due to positive chronotropic and inotropic effects [3].

Herbal treatment for heart disease is not a new concept, people have been using herbs for hundreds of years to treat many chronic diseases, including the cardiovascular ones [4]. *The Canon of Medicine (Al-Qanoon fi al tib)* is the most famous single book in the history of medicine written by *Avicenna*. In this book, *Avicenna* mentioned *a tract of cardiac drugs*, which contains 64 drugs of herbal and mineral origin. *Bombyx mori* (Abresham) cocoon is one of the main drugs of this tract [5]. The silkworm, *Bombyx mori* (Lepidoptera: Bombycidae), is one of the most important insects feed on the leaves of the mulberry family Moraceae, genus Morus [6].

The cocoon shell of the silkworm *Bombyx mori* consists of silk fibroin fiber (70 %) surrounded by a sericin layer made up of sericin (25 %) and non-sericin (5 %) components. The non-sericin component consists of carbohydrate, salt, wax, flavanoids and derivatives [7]. Khamira Abresham which is very popular has been used for decades for cardiac problems and is available in two forms, Sada and Khamira Abresham Hakim Arshadwala. Pharmacological work on Khamira Abresham Arshadwala was initiated by *Siddiqui HH 1964* [8] to provide scientific basis for the cardiovascular uses of these drugs in unani clinical practice but in spite of the extensive clinical use of these formulations in unani system of medicine, Abresham (*Bombyx mori* cocoon), the major constituent of Khamira Abresham Hakeem Arshadwala present in all the preparations has not been subjected to detailed investigation for its cardiovascular effects and its role in these preparations. Hence the present work was designed to investigate the cardioprotective potential of *Bombyx mori* (Abresham) and compare it with two of its widely used formulations, KAS and KAHAW.

EXPERIMENTAL

Test materials

Fresh *Bombyx mori* cocoons (Abresham) were purchased from the local market of Lucknow in the month of December 2011. The test material was authenticated by Dr. A.K.S Rawat, Pharmacognosy Division, National Botanical Research Institute, Lucknow, India. A voucher specimen no NBRI/CIF/RB-2-167/2011 has been deposited at the herbarium of Faculty of Pharmacy, Integral University, Lucknow, India. Khamira Abresham Arshadwala and Sada (Hamdard Laboratories, New Delhi,India) were purchased from local market.

Extraction of *Bombyx mori* cocoons (Abresham)

The dried material (500 g) was cut into very small pieces. The pieces were packed in muslin cloth and subjected to a Soxhlet extractor for continuous hot extraction with 80 % ethanol for 72 h at 30 °C. Thereafter ethanol extracts of *Bombyx mori* cocoons were filtered through Whattman filter paper no. 42 and the resultant filtrates were concentrated under reduced pressure and finally vacuum dried. The yield of the ethanol extract was 9.2 % w/w.

Drugs and chemicals

Isoproterenol and Metoprolol were purchased from Sigma Chemical Co, St Louis, USA. All other chemicals are of analytical grade, purchased from Merck, SD Fine chemicals, Qualigens and Hi media Pharmaceuticals. The enzymatic kits purchased from Span Diagnostics, Surat and Reckon Diagnostics, Mumbai

Experimental animals

Male Wistar albino rats (150 - 200 g) were obtained from the Central Animal House of CDRI, Lucknow; Animals were maintained in polypropylene cages, each containing a maximum of 4 animals, housed in the departmental animal house under controlled conditions of temperature at 25 ± 2 ºC, relative humidity of 55 ± 10 % and light-dark cycle of 12:12 h. Standard pellet diet and tap water were available *ad libitum*.

All experimental protocols were reviewed and approved by the Institutional Animal Ethical Committee (IAEC). The approval no. is IU/PHARM/PhD/CPCSEA/12/01, Faculty of Pharmacy Integral University, Lucknow, and according to the Guidelines of the National Institutes of Health [9].

Experimental protocol

A total of 54 rats were used and randomly divided into nine groups, each containing six

animals. After one week of acclimatization, the pre treatment started orally with normal saline, test extract, standard drug along with subcutaneous isoproterenol injection for induction of myocardial injury on the scheduled days [10,11].

Group I – Normal Control (Sham): Pretreated with normal saline orally

Group II – ISO Control: Pretreated with normal saline orally and administered isoproterenol 85 mg/kg, subcutaneous, given twice with a 24 h interval on day 29 and 30

Group III – *Bombyx mori*: Pretreated with test extract (250 mg/kg) orally, given daily for 30 days and administered isoproterenol 85 mg/kg, subcutaneously, on days 29 and 30 reference for dose of ISO used.

Group IV - *Bombyx mori*: Pretreated with test extract (500 mg/kg) orally, given daily for 30 days and administered isoproterenol 85 mg/kg, subcutaneously on days 29 and 30

Group V – KAS - Pretreated with KAS (800 mg/kg) orally, given daily for 30 days and administered isoproterenol 85 mg/kg, subcutaneously on days 29 and 30.

Group VI - KAHAW- Pretreated with Khamira Abresham Arshadwala (800 mg/kg/day) orally, given daily for 30 days and administered isoproterenol 85 mg/kg, subcutaneously, on days 29 and 30.

Group VII - Standard drug - Pretreated with metoprolol 10mg/kg reference for dose used orally, given daily for 30 days and administered isoproterenol 85 mg/kg, subcutaneously on days 29 and 30.

Group VIII – *Bombyx mori* (per se effect) - Pretreated with test extract (250 mg/kg) orally, given daily for 30 days

Group IX - *Bombyx mori* (per se effect) - Pretreated with test extract (500 mg/kg) orally, given daily for 30 days.

On day 30, 4 h after administration of ISO, blood samples were collected from the tail vein of rats and presence of troponin was monitored with a strip kit. At the end of the experimental period (day 31) the rats were weighed and then sacrificed under diethyl ether anaesthesia. Blood samples were withdrawn via cardiac puncture and allowed to clot and the serum was separated using a centrifuge at 2500 rpm for 15 min.

Gross examination of hearts

The hearts were excised, washed in an ice cold normal saline solutions, blotted with filter paper and weighed, photograph were taken for gross examination. Grading parameter were Grade 0: No Lesion, Grade 1: Inflammation, redness, capillary dilations, Grade 2: Edema, yellowish ventricle portion, Grade 3: Atrium and ventricle turns yellow, scar formation and Grade 4: Diffuse heart, absolute scar formation, increased necrosis portion [10].

Heart weight: body weight ratio

Heart weight: body weight ratio was determined as a marker of cardiac hypertrophy, body weight is the weight at the end of the experimental period on 31st day and hearts were excised, washed in an ice cold normal saline solution, blotted with filter paper and weighed [11].

Determination of cardiac marker enzymes

Serum was used for determination of enzyme markers of myocardial damage using commercial kits for ALT, AST, CK-MB, LDH (Span Diagnostics, Surat, India); troponin presence was checked by ready to use kit of Reckon diagnostics after 4 - 5 h of last ISO dose

Evaluation of cardiac antioxidants and lipid peroxidation

Homogenate (10 %) of myocardial tissue was prepared in ice chilled phosphate buffer (50 mmol/L, pH 7.4), and was used to estimate the cardiac antioxidants and lipid per oxidation parameters like Superoxide dismutase (SOD), Catalase, Tissue glutathione (GSH) and thiobarbituric acid reactive substance (TBARS) [12-15].

Histopathological studies

The myocardial tissue was immediately fixed in 10 % buffered neutral formalin solution. After fixation, tissues were embedded in paraffin and serial sections of 5-6 µm were cut and each section was stained with haematoxylin and eosin. The slides were examined under light microscope and photographs were taken [16]. Histopathological studies were done from Capital Diagnostics, Lucknow.

Phytochemical profile: Determination of total protein

Total protein in of *B. mori* cocoon (Abresham) was evaluated by Lowry method using bovine serum albumin (BSA) as standard [17].

Extraction and determination of sericin from B. mori cocoons

Dried *Bombyx mori* silk cocoons were cut into small pieces and then treated with boiling aqueous solution of 0.02 M Na_2CO_3 for 20 min with stirring. The whole mass was washed with distilled water repeatedly to remove the glue-like sericin protein [18,19].

Statistical analysis

Data were expressed as mean ± standard deviation (SD, n = 6). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Turkey's Kramer comparison test with the aid of GraphPad Prism Instat Software (version 5.0, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Assessment and grading of heart

Cardiac damage was assessed by grading of the hearts of the rats in the different groups. The hearts of the control group were healthy and graded 0, while ISO caused severe myocardial damage and ISO control had grade 4 damage, *Bombyx mori* (Abresham) pretreated groups shows slight recovery at low dose (250 mg/kg) but high dose (500 mg/kg) of *Bombyx mori*, KAHAW and KAS pre-treated groups showed marked recovery and scored grade 1 as same as standard drug metoprolol. The results are summarised in Figure 1.

Heart weight: body weight ratio

Heart weight: body weight ratio is an important parameter to evaluate the cardiac hypertrophy. ISO caused increase in HW: BW ratio when compared with control ($p < 0.01$). HW: BW ratio was significantly lowered ($p < 0.01$) in pre-treatment groups of *Bombyx mori* 250 and 500 mg/kg, HW/BW ratio was also significantly decreased ($p < 0.01$) in pre-treatment groups of KAHAW and KAS which was same as the standard drug metoprolol ($p < 0.01$) (Figure 2).

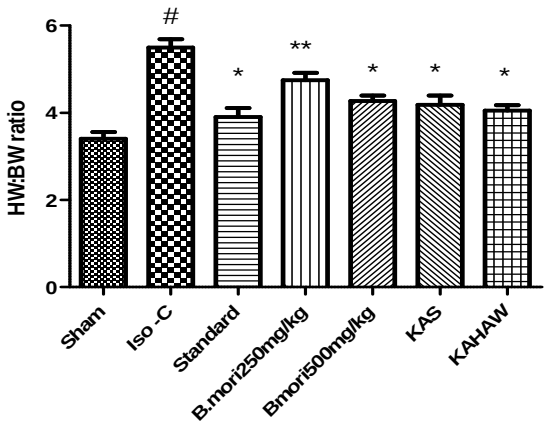

Figure 2: Effect of ethanol extract of *Bombyx mori*, KAS and KAHAWon Heart weight: body weight ratio. [All values are expressed as mean ± SEM for n= 6 animals. $^{\#}p < 0.01$ as compared to sham; $^{*}p < 0.01$ and $^{**}p < 0.05$ as compared to ISO control]

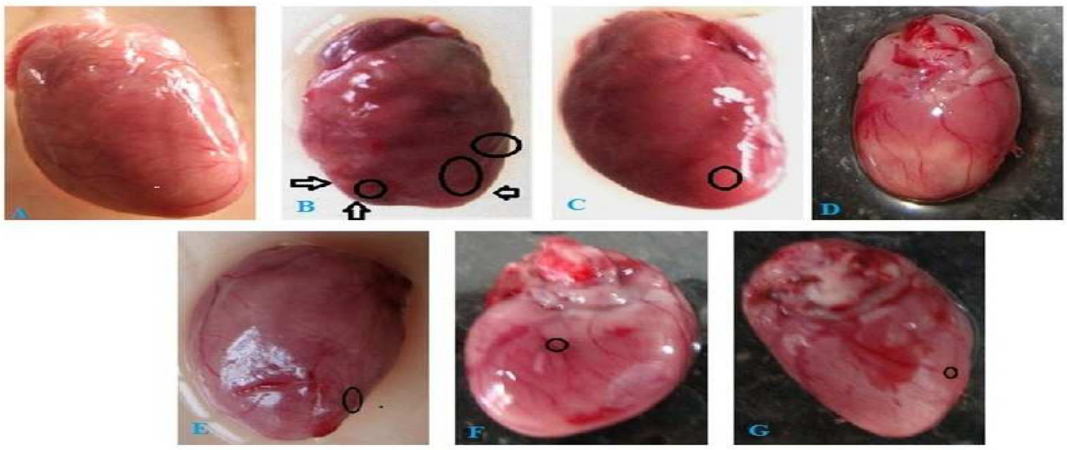

Figure 1: Photographs of assessment and grading of hearts of different experimental group (a) Normal control (b) Isoproterenol (85 mg/kg) treated heart (c) ISO + Metoprolol (10 mg/kg) treated heart; (d) ISO + *B. mori* extract (250 mg/kg) treated heart (e) ISO + *B. mori* (500 mg/kg) treated heart (f) ISO + Khamira sada (800 mg/kg) treated heart (g) ISO + Khamira Abresham arshadwala (800 mg/kg) treated heart

Effect on cardiac marker enzymes

The diagnostic marker enzymes of myocardial damage AST, ALT, LDH and CK-MB are sensitive index to assess the degree of myocardial necrosis. ISO treated rats exhibited marked increase ($p < 0.01$) of these marker enzymes in serum compared to sham control. Pre-treatment with *Bombyx mori* at both doses (250 and 500 mg/kg) to rats challenged with ISO significantly attenuated ($p < 0.05$, $p < 0.01$ respectively) the elevated activities of these marker enzymes AST, ALT, LDH, and CK-MB in serum. KAHAW and KAS had very significant effect ($p < 0.01$) which was same as standard drug, metoprolol ($p < 0.01$, Figure 3).

ISO treated rats exhibited positive test which showed the presence of Troponin in blood. Pre-treatment with *Bombyx mori* at both doses (250 and 500 mg/kg) to rats challenged with ISO showed negative test for Troponin. KAS and KAHAW also exhibited the negative test for troponin as same the standard drug metoprolol. This shows that ISO-treated rats have the myocardial damage but *Bombyx mori*, KAHAW and KAS exhibited protection against ISO induced cardiac damage (Table 1).

Effect on cardiac antioxidants and lipid peroxidation

In the present study there was significant decrease in endogenous antioxidants superoxide dismutase (SOD) and catalase in ISO challenged group when compared to sham control ($p < 0.01$). Pre-treatment with *B. mori* 250 mg/kg increased ($p < 0.05$) while *B. mori* 500 mg/kg, KAHAW and KAS significantly increased ($p < 0.01$) the activities of SOD and catalase level in ISO-injected rats by preventing the depletion of antioxidants which was same as with standard drug metoprolol ($p < 0.01$). In this study there was a significant decrease in glutathione (GSH) level in ISO challenged group compare to sham control ($p < 0.01$).

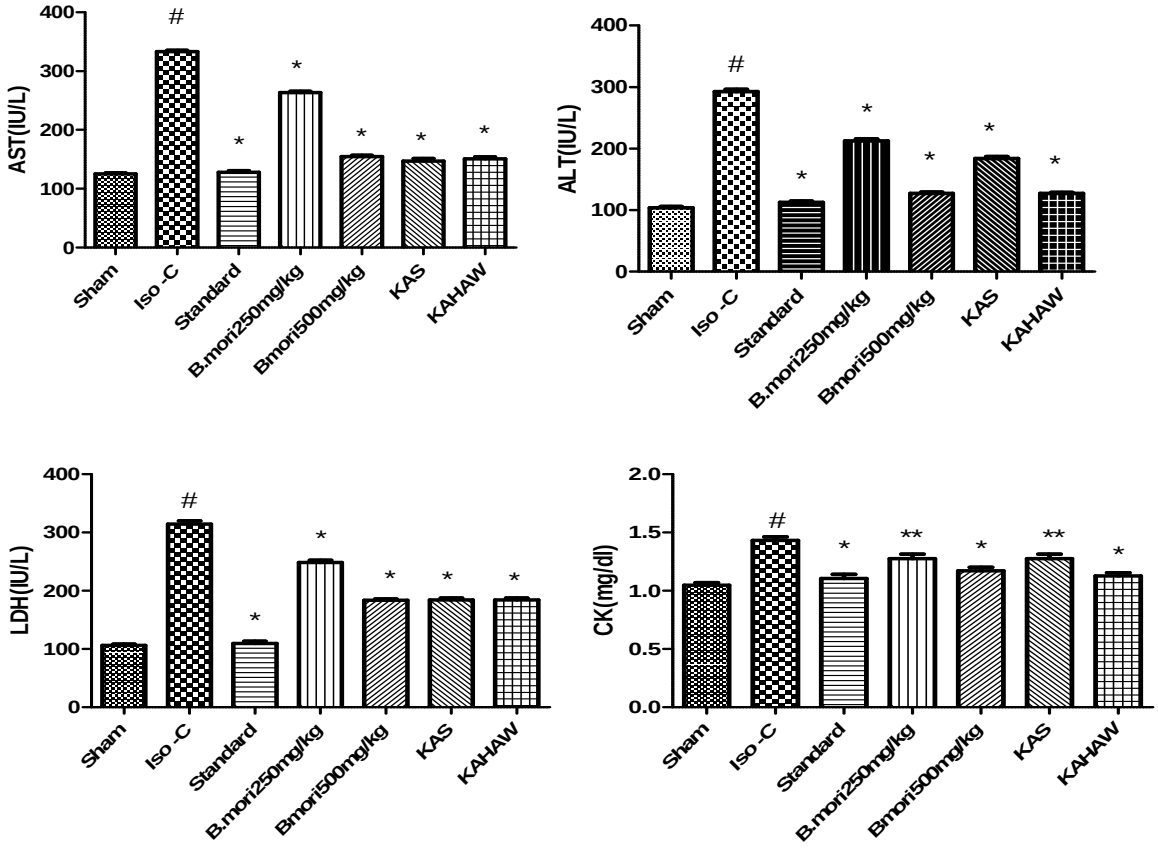

Figure 3: Effect of ethanol extract of *Bombyx mori*, KAS and KAHAW on various cardiac marker enzymes. [All values are expressed as mean ± SEM for n = 6 animals. $^{\#}p < 0.01$ as compared to sham; $^{*}p < 0.01$ and $^{**}p < 0.05$ as compared to ISO control]

Pretreatment with *Bombyx mori* 250 mg/kg increased ($p < 0.05$), *B. mori* 500 mg/kg, KAHAW and KAS very significantly increased ($p < 0.01$) GSH level in ISO-injected rats same as the increase with standard drug metoprolol ($p < 0.01$). ISO treated rats showed significantly elevated levels of thiobarbituric acid reactive substances (TBARS, $p < 0.01$) as a marker of lipid peroxidation in heart when compared with sham. Pretreatment with *B. mori* 250 mg/kg decreased ($p < 0.05$), while *B. mori* 500 mg/kg, KAHAW and KAS significantly decreased ($p < 0.01$) the TBARS level in ISO challenged rats. This was same as the standard drug metoprolol ($p < 0.01$) (Table 2).

Histopathological findings

Photomicrographs of hearts of normal control group rats showed that the endocardium, myocardium, and epicardium as well as papillary muscles and vasculature were all normal and healthy. There was no muscular hypertrophy, myositis, necrosis and round cell infiltrates were visible at 40X and 100X. Photomicrographs of hearts of isoproterenol challenged rats showed focal myonecrosis with myophagocytosis and lymphocytic infiltration. In subendocardium, vacuolar changes and prominent oedema along with chronic inflammatory cells were clearly visible.

Hearts of metoprolol (10 mg/kg) treated rats showed lesser degree of myonecrosis, myophagocytosis, lymphocytic infiltration, oedema and very little infiltration of inflammatory cells. *B. mori* (250 mg/kg) treated rats hearts also showed decreased degree of myonecrosis and lesser infiltration of inflammatory cells but myophagophytosis and subendocardium vacuolar changes were present and visible. *Bombyx mori* (500 mg/kg) treated rats hearts showed little degree of myonecrosis and lesser infiltration of inflammatory cells as well as a decreased myophagophytosis and subendocardium vacuolar changes were present.

Pretreated KAS and Khamira Abresham Arshadwala rats hearts showed far lesser degree of myonecrosis, myophagocytosis, lymphocytic infiltration, oedema and very little infiltration of

Table 1: Effect of ethanol extract of *Bombyx mori,* KAS and KAHAW on cardiac marker enzyme, troponin

No. of animals	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
1	-ve	+ve	-ve	-ve	-ve	-ve	-ve
2	-ve	+ve	-ve	-ve	-ve	-ve	+ve
3	-ve	+ve	-ve	+ve	-ve	-ve	-ve
4	-ve	+ve	+ve	+ve	+ve	-ve	-ve
5	-ve	+ve	-ve	-ve	-ve	+ve	-ve
6	-ve	+ve	-ve	-ve	-ve	-ve	-ve

(+ve = presence of marker enzyme; -ve = absence of marker enzyme); Group I =Normal Control; Group II= Isoproterenol (85mg/kg); Group III= Metoprolol (10mg/kg/day) + ISO; Group IV = B. mori extract (250mg/kg) + ISO; Group V = B. mori extract (500mg/kg) + ISO; Group VI = Khamira Abresham sada (800 mg/kg) +ISO, Group VII = Khamira Abresham arshadwala (800 mg/kg)+ISO)

Table 2: Effect of ethanol extract of *Bombyx mori*, KAS and KAHAW on cardiac antioxidants and lipid peroxidation

Group	TBARS (nmol/g tissue protein)	GSH (µg/g tissue weight)	SOD (unit/mg protein)	Catalase (unit/mg of protein)
Normal control	56.98±0.78	2.93±0.04	7.91±0.22	21.40±0.44
ISO control	172.91±0.64$^{\#}$	1.58±0.12$^{\#}$	3.13±0.29$^{\#}$	11.83±0.35$^{\#}$
Reference (metoprolol)	67.15±0.58*	2.85±0.11*	7.74±0.25*	19.70±0.51*
B. mori (250mg/kg)	111.7±0.47*	1.65±0.15**	4.86±0.27*	16.91±0.56*
B. mori (500mg/kg)	98.56±0.40*	2.71±0.11*	7.46±0.24*	19.37±0.58*
Khamira sada (800 mg/kg)	102.90±0.39*	2.44±0.14*	7.72±0.29*	19.20±0.52*
Khamira arshadwala (800 mg/kg)	96.67±0.46*	2.63±0.11*	7.55±0.20*	19.68±0.49*
B. mori (250mg/kg) *per se*	57.01±0.53	2.93±0.04	7.67±0.20	21.41±0.46
B. mori (500mg/kg) *per se*	54.68±0.53	2.96±0.05	8.05±0.15	23.58±0.44

*All values are expressed as mean ± SEM (n = 6); $^{\#}p < 0.01$, compared to normal control; *$p < 0.01$ and ** $p < 0.05$, compared to ISO control]*

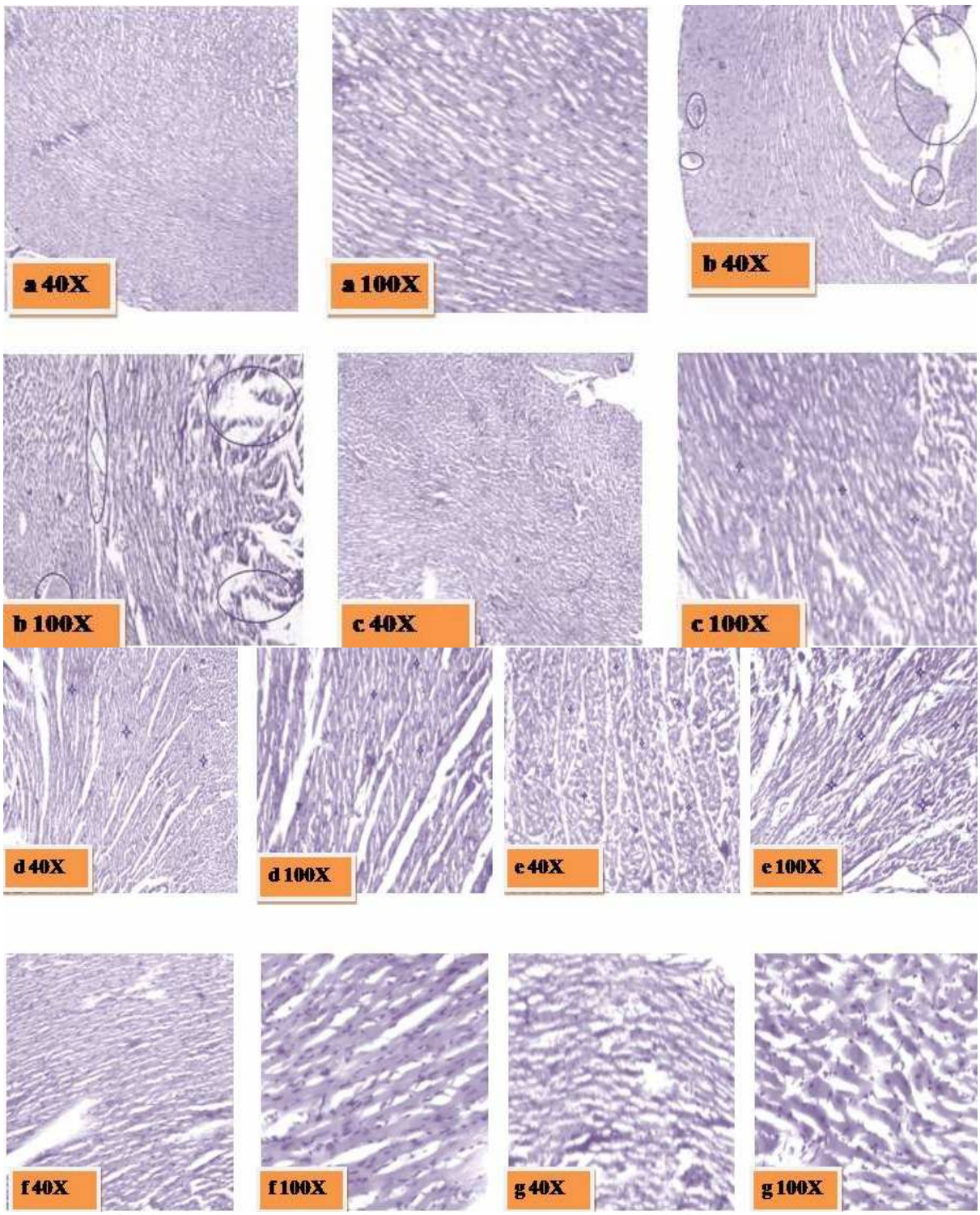

Figure 4: Photomicrographs of histopathological studies at 40X and 100X (a) Normal control, (b) Isoproterenol (85 mg/kg. b. wt), (c) ISO + Metoprolol (10 mg/kg. b. wt), (d) ISO + *B.mori* (250 mg/kg b. wt), (e) ISO + *B.mori* (500 mg/kg b. wt), (f) ISO + Khamira sada (800 mg/kg b. wt), (g) ISO + Khamira Abresham arshadwala (800 mg/kg b. wt)

inflammatory cells and subendocardium vacuolar changes (Figure 4).

Phytochemical profile

Estimation of total protein and sericin

Total protein was estimated by above mentioned procedure and was found to be 1.01 mg /ml. The sericin layer of protein was extracted and estimated as above mentioned procedure and was found to be 0.28 mg/ml.

DISCUSSION

The present study shows the cardio protective potential of *Bombyx mori* against isoproterenol induced necrosis, oxidative stress and cardiotoxicity. Cardiac damage was assessed by grading of hearts of rats of different groups. Hearts of control group were healthy, while ISO caused severe myocardial damage, *Bombyx mori* pre-treated groups shows slight recovery at low dose but in the higher dose (500 mg/kg) combination with KAHAW and KAS groups, the recovery was high which was similar to the effect of the standard drug, metoprolol (10 mg/kg) group.

The diagnostic marker enzymes of myocardial damage, asparate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) and creatinine kinase (CK-MB) serves as a sensitive index to assess the degree of myocardial necrosis [20]. ISO treated rats exhibited marked increase of these marker enzymes in serum compared to sham. Pretreatment with *Bombyx mori* (500 mg/kg), KAHAW, KAS and metoprolol to rats challenged with ISO significantly attenuated the elevated activities of these cardiac marker enzymes. Apart from these traditional cardiac marker enzymes, one enzyme which is specific for myocardial damage is troponin T & I. Presence of troponin in blood/serum is usually considered a gold marker for cardiac damage. Troponin appears in blood after 4 - 5 h of MI and disappears after 8 - 10 h. ISO-treated rats exhibited positive test result showing the presence of troponin I. Pre-treatment of *B. mori* cocoon (Abresham) at doses 250 and 500 mg/kg to rats challenged with ISO showed negative test result, indicating the absence of troponin. KAS and KAHAW also exhibited negative test result for troponin I, similar to the reference drug, metoprolol. It shows that ISO induced myocardial damage which was protected by *B. mori* cocoon (Abresham), KAS, KAHAW and metoprolol.

Heart weight: body weight ratio (HW: BW) is another important parameter to evaluate the cardiac hypertrophy. ISO causes significant increase in HW: BW ratio as compared to control ($p < 0.01$).HW: BW ratio significantly decreased in pre-treatment groups, $p < 0.01$ in *B. mori* (Abresham) 250 mg/kg and $p < 0.01$ in *B. mori* (Abresham) 500 mg/kg, KAS & KAHAW pre-treated groups.

Isoprenaline is known to induce oxidative stress by generating free radical moieties via its quinine metabolites which react with oxygen to produce superoxide anions and other reactive oxygen species in rat myocardium. Free radical scavenging antioxidants such as SOD, Catalase, and GSH are the first line of cellular defence against oxidative injury. The role of ISO has been well documented in the reduction of myocardial SOD and catalase activities and GSH content, decreased SOD activity in isoprenaline control animals may be due to excessive formation of superoxide anions or the decreased removal of superoxide anions, which can be harmful to the myocardium.

In present study there was a significant decrease in these endogenous antioxidants SOD and catalase in ISO challenged group compared to sham control ($p < 0.01$). Pretreatment with *B. mori* (Abresham) 250 mg/kg increased ($p < 0.01$) and *B. mori* (Abresham) 500 mg/kg, KAS and KAHAW very significantly increased ($p < 0.01$) the activities of SOD and catalase levels in ISO-injected rats by preventing the depletion of antioxidants which is similar to the increase with metoprolol ($p < 0.01$). Glutathione antioxidant system plays a fundamental role in cellular defence against reactive free radicals and other oxidant species. It protects the myocardial cellular membrane against oxidative damage by regulating the redox status of proteins in the cell-surface membrane. In present study there is significant decrease in GSH level in ISO challenged group compared to sham control ($p < 0.01$). Pretreatment with *B. mori* (Abresham) 250 mg/kg increased ($p < 0.01$) and *B. mori* (Abresham) 500 mg/kg, KAS and KAHAW significantly increased ($p < 0.01$) GSH level in ISO-injected rats and preventing the depletion of antioxidant which is as same as the increase with metoprolol ($p < 0.01$). Glutathione depletion further increases the susceptibility of myocardial membrane to reactive oxygen metabolites and lipoperoxidative necrotic damage.

Lipid peroxidation is an important pathogenic event in myocardial necrosis and the

accumulation of lipid hydroperoxides reflects cardiac damage. The increased lipid peroxides in isoprenaline-induced myocardial necrosis might be due to free radical mediated membrane damage. Increased levels of lipid peroxidation products injured blood vessels, causing increased adherence and aggregation of platelets to the injured sites. ISO treated rats showed significantly elevated levels of TBARS ($p < 0.01$) as marker of lipid peroxidation in heart compared to normal control. Pretreatment with *B. mori* cocoon (Abresham) 250 mg/kg increased ($p < 0.05$) and *B. mori* cocoon (Abresham) 500 mg/kg, KAS and KAHAW significantly increased ($p < 0.01$) TBARS level in ISO-injected rats which is similar to the increase produced by metoprolol ($p < 0.01$).

Cardioprotection of *B. mori* cocoon (Abresham), KAS & KAHAW was also confirmed by histopathological studies. Isoprenaline induced rat heart showed the extensive myofibrillar degeneration, severe myocardial necrosis, oedema and separation of myofibrils compare to sham group which showed architecture of myocardium. Pretreatment with *B. mori* cocoon (Abresham) 250 mg/kg shows some degree of control on this cardiac damage and *B. mori* cocoon (Abresham) 500 mg/kg, KAS and KAHAW showed highly significant protection showing very lesser degree of necrosis, edema and myofibrillar degeneration. The reference drug, metoprolol, showed normal myofibrils, in continuity with adjacent myofibrils.

CONCLUSION

Bombyx mori (Abresham) cocoon has potent cardioprotective effect, so also is its formulations, Khamira Abresham sada and Khamira Abresham Arshadwala. The cardioprotective effect of *Bombyx mori* (Abresham) is possibly due to its high protein content and very small quantities of non-sericin components of silk cocoons, especially the flavonoids, which have potential free radical scavenging and antioxidant activities.

ACKNOWLEDGEMENT

The authors thank Professor SK Maulik, Head, Department of Pharmacology, AIIMS, New Delhi, for his valuable guidance, Dr Sajid Khan, Department of Biotechnology, Integral University for his support and Professor SW Akhtar, Vice Chancellor, Integral University, Lucknow for providing the required facilities for this work.

REFERENCES

1. *Thippeswamy S, Thakker SP, Tubachi S, Kalyani GA, Netra MK, Kuma B. In vitro Anthelmintic Activity of ethanolic and aqueous fruit Extract of Terminalia belerica. J. Pharm. Res. 2010; 3(5): 77-82.*
2. *Reznik, AG. Morphology of acute myocardial infarction at pre necrotic stage. Kardiologiia 2010 50(1): 4–8.*
3. *Rona G, David, Khan S, Clifford I, Chappel CI.Study on the healing of cardiac necrosis in the rat. American J. Pathol 1961; 39(4): 473-489.*
4. *Mahmood ZA, Mohammad S, Mahmood SBZ, Karim MA. Herbal treatment for cardiovascular disease the evidence based therapy. Pak. J. Pharm. Sci. 2001; 23(1): 119-124.*
5. *Siddiqui HH, Aziz MA. A note on Avicenna's tract on cardiac drugs. Planta Medica 1963; 4: 437.*
6. *Ali MM, Sarasa B, Arumugam A. Effect of crude extract of Bombyx mori cocoons in hyperlipidemia & atherosclerosis. J Ayuveda Integr Med. 2011; 2(2): 72-78.*
7. *Wang H, Wang Y, Zhou L, Lin Z, Zhang Y. Isolation and bioactivities of a non-sericin component from cocoon shell silk sericin of the silkworm Bombyx mori. Food Funct. 2012; 3(2): 150-158.*
8. *Siddiqui HH. Effect of Khamira Abresham Arshadwala on serum cholesterol levels in rabbits. Planta Med 1964; 12(4): 443-445.*
9. *Guide for the Care and Use of Laboratory Animals. 8th edition, 2011 National Academic Press, Washington DC. 20001*
10. *Suchalatha S, Shyamala CS. Effect of Arogh - A Polyherbal formulation on the marker enzymes in isoproterenol induced myocardial injury. Indian J. Clinical Biochem. 2004; 19(2): 184-189.*
11. *Rona G, Chappel CI, Balazs T, Gaudry R. An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. AMA Arch Pathol 1959; 67: 443-455.*
12. *Khatun S, Chatterjee NC, Cakilcioglu U. Antioxidant activity of the medicinal plant Coleus forskohlii Briq. Afr. J. Biotechnol 2011; 10(13): 2530-2535.*
13. *Shokrzadeh M, Ebadi AG, Mirshafiee SS, Choudhary MI. Effect of the aqueous green leaf extract of green tea (Camellia sinensis) on glucose level of rat. Pak J Biol. Sci 2006; 9(14): 2708-2711.*
14. *Shanmukha I, Siddiq A, Kamlesh G, Khan M, Ramachandra S. Antioxidant and nephroprotective activity of Spathodea campanulata bark against gentamicin induced nephrotoxicity. Pharmacol Online 2010; 1: 666-675.*
15. *Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358.*
16. *Vandana SP, Suresh RN. Evaluation of cardioprotective activity of Gingko biloba & Ocimum sanctum in rodents. Altern Med Rev. 2009; 14(1): 161-171.*

17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-275.
18. Shah MK, Kumar A, Pramanik K. The extraction of fibroin protein from Bombyx mori silk cocoon: Optimization of process parameters. Int J Bioinformatics Res. 2010; 2(2): 33-41.
19. Takasu Y, Yamada H, Tsubouchi K. Extraction and chromatographic analysis of cocoons of silkworm Bombyx mori. J. Insect Biotech. Sericol 2002; 71: 151-156.
20. Rosalki SB, Roberts R, Katus HA, Giannitsis E, Ladenson, JH. Cardiac Biomarkers for detection of myocardial infarction: Perspectives from past to present. Clin. Chem. 2004; 50: 2205-2213.