Cardioprotective activity of *Bambusa blumeana* Schultes leaf crude extract against isoproterenol-induced myocardial infarction in Sprague-Dawley rats

Sarah Len O. Rosales*, Buena Fe A. Anog[1], Rosmaine M. Blanza[1], Mary Ann D. Cuartero[1], Ma. Chermie Landicho[1], Erma Grace D. Talledo[1], Reby A. Cabanela[2], Oliver Shane R. Dumaoal[2] and Carina R. Magbojos[2]

Medical Laboratory Science Department, College of Allied Medical Professions, Lyceum of the Philippines University, Capitol Site, Batangas City, Philippines

[1] Student Researcher, [2]Faculty Researcher

* Correspondence: *sarahlen_r@yahoo.com*

Abstract: Cardiovascular diseases (CVD) are the major health problems in the Philippines and are now the leading cause of death in the country. Moreover, more than 80% of CVD occurs in underdeveloped and developing countries. Plants have a long history of use all over the world for the treatment of different diseases and complaints. Flavonoids are bioactive compound which are widely distributed in plants. It was reported to have antioxidant activities and had effects of treating cardiovascular diseases and different types of cancer preventive activity. Flavonoids contained in bamboo leaves have been found to have therapeutic effects. The present study was designed to investigate cardioprotective activity of *Bambusa blumeana* Schultes leaf crude extract against isoproterenol-induced myocardial infarction in Sprague-Dawley rats. Rats were pretreated with leaf extract (200 mg/kg, 300 mg/kg and 400 mg/kg, orally) and Tocopherol (60 mg/kg, orally) daily for 21 days. After treatment, isoproterenol (85 mg/kg body weight, s.c.) was injected to rats at 22nd and 23rd day to induce myocardial infarction. Cardioprotection was investigated by measuring the activities of serum aspartate aminotransferase, lactate dehydrogenase and creatine kinase--MB together with histopathologic techniques. The activities of serum marker enzymes were increased significantly (p<0.05) in isoproterenol-induced rats while *B. blumeana* Schultes leaf crude extract (200 mg/kg, 300 mg/kg and 400 mg/kg) treated rats have normal cardiac enzyme levels. This study revealed that at 200 mg/kg body weight of *B. blumeana* leaf crude extract exhibited its cardioprotective effect but with greater effect in reducing the level of cardiac enzymes CK-MB, AST and LDH upon increasing

ISSN: 2094-5906

concentration of 300 mg/kg and 400mg/kg oral extract treatment. The results were confirmed by histopathological evidences and showed decreasing inflammatory condition upon increasing concentrations of extract which revealed severe-to-mild myocardial degeneration and necrosis. This study demonstrated the cardioprotective effect of *B. blumeana* Schultes leaf crude extract against isoproterenol-induced myocardial infarction in Sprague-Dawley rats.

Keywords: *bamboo leaf, flavonoids, cardioprotection, isoproterenol, myocardial infarction*

INTRODUCTION

Cardiovascular diseases (CVD) as it figure prominent problems in public health are the leading causes of death in the Philippines (National Statistical Coordination Board, 2007 as cited by Feranil, Duazo, Kuzawa & Adair, 2011). It is also estimated as the leading cause of disability and death worldwide (Reddy & Yusuf, 1998; WHO, 2003 as cited by Nikolic, Nikić & Petrović, 2008).

Myocardial infarction (MI) is an acute cardiac condition resulted from the imbalance between coronary blood supply and myocardium demand which are caused by necrosis of the myocardium (Patel, Setty & Chakraborty, 2012; Prabhu, Jainu, Sabitha & Devi, 2006). It is part of Acute Coronary Syndromes spectrum of diseases (ACS). This spectrum includes acute myocardial infarction, unstable angina and sudden cardiac death (Petrich, Schanne & Zumino, 1996; Thygesen et al., 2007 as cited by Haleagrahara, Varkkey & Chakaravarthi, 2011). Among different proposed mechanisms, the pathophysiology of acute myocardial infarction has been implicated by accumulation of free radicals (Thygesen et al., 2007 as cited by Haleagrahara, Varkkey & Chakaravarthi, 2011).

Isoproterenol (ISO) is a beta adrenergic agonist and synthetic catecholamine that produces MI by generating highly cytotoxic free radicals, increased lipid peroxidation, causing cardiac dysfunction and altered activities of cardiac enzymes and antioxidants when administered in large doses (Rathore et al., 1998 as cited by Li et al., 2012). The morphological and pathophysiological changes in the myocardium after administration of ISO is said to be similar to those that occur in human myocardial infarction (Wexler, 1987 as cited by Li et al., 2012). Isoproterenol-induced myocardial injury in the study of beneficial effects of many drugs and cardiac functions is considered a well standardized model (Li et al., 2012).

Creatine kinase-MB (CK-MB), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and alanine aminotransferase

ISSN: 2094-5906

(ALT) are cytosolic enzymes that are said to be sensitive indices used in myocardial infarction to assess its severity. Loss of permeability, functional integrity and cellular damage are indicated by increased level of these enzyme markers in serum (Sabeena et al., 2004 as cited by Li et al., 2012).

Flavonoids are defined as "nature's biological response modifiers" due to strong evidence from their experimental inherent ability to alter the reaction of the body to viruses, allergens, and carcinogens. Their anti-inflammatory, anti-cancer (Cushnie et al., 2005 as cited by Bello, Ndukwe, Audu & Habila, 2011; Stalikas, 2007; Zhang, Wu & Yu, 2010), anti-oxidant activities and their effects on treating cardiovascular diseases have also been reported (Sun, Yue, Tang & Guo, 2010).

The leaves of bamboo are clinically significant in other Asian countries in treating fever and hypertension (Kweon et al., 2001 as cited by Wang, Yue, Tang & Sun, 2012). Some of the functional components of bamboo leaves are flavonoids, lactone and phenolic acid (Singhal, Satya & Sudhakar, 2011; Zhang et al., 2008 as cited by Wang, Yue, Tang & Sun, 2012). Over 1250 bamboo species are found worldwide. Bamboos are tall grasses that belong to the family of Poaceae. This fibrous grass abundantly grows in the tropical and subtropical areas of the world (Liu, Jiang, Ren & Ma, 2011). It is a grass which is beneficial in every industry. They are very distinct in their life form, ecologically important and widely used by humans (Sastry, 2008 as cited by Chongtham, Bisht & Haorongbam, 2011).

Figure 1. *Bambusa blumeana* A. tree B. leaves

Bambusa blumeana Schultes, locally known as kawayan or kawayang tinik, is the most common bamboo specie in the Philippines which grow abundantly on the hillsides and mountainsides (Malab, Batin, Malab, Alipon & Midmore, 2009). The past studies conducted about Philippine bamboos are limited only to its industrial purpose (Alipon, Bausa & Sapin, 2011), plantation and production (Decipulo,

ISSN: 2094-5906

Ockerby, Midmore, 2003; Malab, Batin, Malab, Alipon & Midmore, 2009) but their pharmaceutical potentials have not yet been reported.

The Philippine bamboo species have been studied well for their industrial purposes, plantation and production. However, the pharmaceutical potential, specifically the cardioprotective activity of the plant have not yet been studied. With the view that bamboo leaves contain biologically active components like flavonoids with an anti-oxidant effect, this study assessed the cardioprotective activity of *B. blumeana* Schultes leaf against isoproterenol-induced myocardial infarction in experimental animals. The present study aims to contribute knowledge on effects of bamboo leaf extract on certain cardiac parameters in Sprague-Dawley rats of both sexes with isoproterenol-induced myocardial infarction and may be significant as a potential drug or food component to decrease the incidence of cardiovascular diseases in the Philippines.

MATERIALS AND METHODS

Chemicals

Isoproterenol hydrochloride (ISO) and Tocopherol were purchased from Sigma-Aldrich Co. Singapore. The reagents (Randox, UK) for cardiac enzymes were used for enzyme assay of LDH, AST and CK-MB. Other used chemicals being purchased from local suppliers were ethanol, magnesium ribbon and concentrated hydrochloric acid. All chemicals used were of analytical grade.

Plant Material

The shady green colored leaves of *B. blumeana* Schultes were obtained around Agoncillo, Batangas. The plant was authenticated by Dr. Wilfredo F. Vendivil of Botany Division of National Museum, Philippines.

Animals

Healthy Sprague-Dawley rats, twelve males and twelve females, (8-10 weeks old; 175-225g) were authenticated at Zoology Division of National Museum, Philippines. The environment was controlled in terms of light (12:12-h light/dark cycle starting at 6:00AM), humidity, and room temperature (20–23°C) (Aubin et al., 2008; Momin, Kalai, Shikalgar & Naikwade, 2011; Zhang et al., 2008). Food and water were given *ad libitum* throughout the experiments (Abdel-Monem et al., 2013; Wong et al., 2011). All experimental protocols were received and approved by the Bureau of Animal Industry.

ISSN: 2094-5906

Plant Extraction

Two kilos of *B. blumeana* Schultes leaves were dried in shade at room temperature. After drying, leaves were pulverized through grinding using blender. A 600 g of dried, pulverized bamboo leaves (de Sousa, Viera, de Pinho, Yamamoto & Alves, 2010) were immersed on 80% ethanol (Peng et al., 2009) for one hour at room temperature (Pineda, 2009 as cited by Perez, Caviteno, Malabanan, Manalo, & Muli, 2011). The ratio of plant material to solvent was 1:15 m/v (Shon et al., 2004 as cited by Goyal, Middha & Sen, 2010). Using a No.1 filter paper (Whatman Inc. Hillsboro, OR, USA), the suspension was filtered twice. The filtrate was incubated at 40°C until the ethanol was completely evaporated (Pineda as cited by Perez, Caviteno, Malabanan, Manalo & Muli, 2011).

Test for Flavonoids

Shinoda test

A 200 mg plant material was extracted with 5 ml ethanol and was filtered; magnesium ribbon plus concentrated hydrochloric acid was added to 1 ml filtrate. A pink or red color production indicated the presence of flavonoids (Evans et al., 1996 as cited by Goyal, Middha & Sen, 2010; Robiel, Merih & Abraha, 2011).

Experimental Protocol

The experimental rats were randomly divided into six groups of four animals each, two males and two females and treated as follows:

Group I - normal control rats with no treatment.

Group II - rats were treated with isoproterenol 85 mg/kg body weight subcutaneously.

Group III - rats were pretreated with *B. blumeana* Schultes leaf extract (200 mg/kg body weight, orally) for 21 days.

Group IV - rats were pretreated with *B. blumeana* Schultes leaf extract (300 mg/kg body weight, orally) for 21 days.

Group V - rats were pretreated with *B. blumeana* Schultes leaf extract (400 mg/kg body weight, orally) for 21 days.

Group VI - rats were pretreated with standard drug, Tocopherol, (60 mg/kg body weight, orally) for 21 days.

On the 22nd and 23rd day, myocardial injury was induced to rats in Group II-VI by injection of isoproterenol (85 mg/kg body weight) subcutaneously at an interval of twenty-four hours for two days

ISSN: 2094-5906

(Kuppusamy et al., 2010; Li et al., 2012; Patel, Setty & Chakraborty, 2012).

Biochemical Studies

Blood samples were collected by retro orbital puncture method (Kuppusamy et al., 2010; Madetoja et al., 2009; Parasuraman, Raveendran, Kesavan, 2010) before and after the experiment proper. Serum was separated by centrifugation and was used for the estimation of marker enzymes, including aspartate aminotransferase (AST), creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH), using a semi-automated machine (Statfax 1604+, Awareness Technology, USA). The activities of CK-MB, LD, and AST in serum were determined. Rats treated with isoproterenol showed increased activities of serum marker enzymes which indicated the onset of myocardial necrosis (Panda & Naik, 2009). The results were expressed in terms of U/L for AST, LDH, and CK-MB. Reference ranges for serum markers of rats ages eight to sixteen weeks old, AST (male: 74-143 U/L; female: 65-203 U/L), CK-MB (male: 162-1184 U/L; female: 163-1085 U/L), LDH (male: 272-1965 U/L; female: 256-1552 U/L) (Giknis & Clifford, 2008).

Histopathological Analysis

At the end of the study, Sprague-Dawley rats underwent cervical decapitation and the hearts were dissected out and submitted to College of Veterinary Medicine, University of the Philippines, Los Baños, Laguna for histopathological analysis to assess the myocardial damage (Kuppusammy et al., 2010).

Statistical Method

Each result was expressed as means ± Standard Error. The grouped data were evaluated statistically using one-way analysis of variance (ANOVA) coupled with Dunnet t-test to correlate the effect of extract on cardiac enzyme markers and Post hoc test (Tukey) to determine the differences on the effect of different concentrations to the cardiac markers where $p<0.05$ was considered significant (Momin, Kalai, Shikalgar & Naikwade, 2011). All computations were done using PASW ver.18

RESULTS AND DISCUSSION

Phytochemical Screening

Figure 2 shows the Shinoda test which was used for the detection of the biochemical compound flavonoid. The extract was treated with concentrated hydrochloric acid and formed an intense red

ISSN: 2094-5906

color after a piece of magnesium ribbon was added. This result indicated the presence of naturally occurring compounds flavonoids in crude leaf extract of *B. blumeana* which has antioxidant activities (Sun, Yue, Tang & Guo, 2010).

Figure 2. Shinoda test

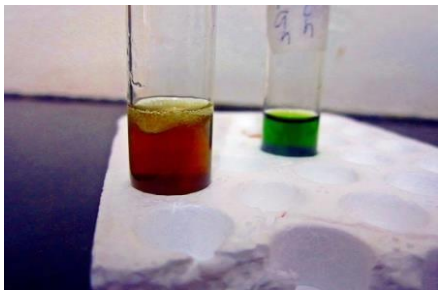

Table 1 shows the effect of *B. blumeana* leaf crude extract and tocopherol on cardiac enzyme markers. The pre test and post test results of cardiac enzyme markers such as CK-MB, LDH and AST were presented. Group I which was used as control group obtained CKMB pre test result of 281.28 IU/L and post test result of 309.59 IU/L, LD pre test of 596.06 IU/L and post test result 581.75 IU/L and with AST pre test result of 96.97 IU/L and post test result of 102.73 IU/L. Group II (isoproterenol) obtained CK-MB pre test and post test result of 307.43 IU/L and 1486.26 IU/L, respectively. Group II also obtained LD pre test result of 832.50 IU/L and post test result of 2098.22 IU/L and AST pre test and post test result of 93.70 IU/L and 289.81 IU/L, respectively. CKMB pre test result for Group III (200 mg/kg leaf extract) was 331.25 IU/L and post test result of 856.63 IU/L. LD pre test and post test result for this group were 743.61 IU/L and 1011.10 IU/L, respectively. On the other hand, Group III obtained AST pre test result of 92.76 IU/L and post test result of 125.45 IU/L. Group IV (300 mg/kg leaf extract) pre test result for CKMB was 347.73 IU/L and post test result of 469.66 IU/L, LD result of 643.33 IU/L and 797.30 IU/L, respectively. Pre test AST result for Group IV was 90.97 IU/L and post test of 106.21 IU/L. CKMB pre test result obtained from Group V (400 mg/kg extract) was 409.64 IU/L and 387.38 IU/L post test result. This group also obtained LDH result of 736.68 IU/L and 798.51 IU/L for pre test and post test, respectively while AST result was 87.21 IU/L for pre test and 94.65 IU/L from post test. Group VI (tocopherol) obtained CK-MB pre test and post test result of 363.00 IU/L, 394.99 IU/L, LD of 684.66 IU/L 783.88 IU/L and AST 86.82 IU/L and 103.77 IU/L, respectively.

ISSN: 2094-5906

Table 1
Effect of *Bambusa blumeana* Leaf Crude Extract and Tocopherol on Cardiac Enzyme Markers

Group no.	Groups	CK-MB (IU/L)		LDH (IU/L)		AST (IU/L)	
		Pre test	Post test	Pre test	Post test	Pre test	Post test
I	Control	281.28	309.59	596.06	581.75	96.97	102.73
II	Isoproterenol	307.43	1486.26	832.50	2098.22	93.70	289.81
III	200 mg/kg Extract +isoproterenol	331.25	856.63	743.61	1011.10	92.76	125.45
IV	300mg/kg Extract + isoproterenol	347.73	469.66	643.33	797.30	90.97	106.21
V	400mg/kg Extract + isoproterenol	409.64	387.38	736.68	798.51	87.21	94.65
VI	Tocopherol + isoproterenol	363.00	394.99	684.66	783.88	86.82	103.77

All pre test result for each group fell within the CK-MB reference range of 162-1184 U/L for male and 163-1085 U/L for female, LD reference range of 272-1965 U/L for male and 256-1552 U/L for female and AST reference range of 74-143 U/L for male and 65-203 U/L for female (Giknis & Clifford, 2008). This demonstrated the normal condition of the rats before the treatment. Based on the post test results there was an increase in serum CK-MB, LDH and AST confirming the acute myocardial infarction in rats. Group II obtained noticeable elevated levels of serum cardiac enzymes where values exceeded the reference ranges. This was due to administration of isoproterenol and lack of treatment. The cells were damaged with increased muscle contractility, which results in increasing the cell membranes permeability allowing cardiac enzymes to leak out into the bloodstream (Haleagrahara, Varkkey & Chakaravarthi, 2011). Pretreatment of *B. blumeana* crude leaf extract (200, 300 and 400 mg/kg) also showed an increase cardiac enzyme markers but fell within reference ranges which were considered to have normal levels of cardiac enzymes.

The results indicate that *B. blumeana* has the tendency to reduce the elevated cardiac marker enzymes proving its cardioprotective effect which was probably due to antioxidant activity of the phytochemical compounds contain in bamboo leaves. The antioxidant activity is mainly due to redox properties, which can play an important role in absorbing and neutralizing free radicals (Goyal et al., 2010). Tocopherol group also obtained increased levels of cardiac enzymes but also fell within reference ranges which also produced

ISSN: 2094-5906

normal levels of cardiac enzymes and when compared with isoproterenol group showed significant ($p<0.05$) difference. Pretreatment of tocopherol, which was used as standard drug, prevent the increase of cardiac enzyme level as it protects the myocardial membrane by nature of its antioxidant ability (Kuppusammy et al., 2010). Values of all groups were increased but with decreasing level upon increasing concentrations and when compared to the value of Group II which did not receive pretreatment of bamboo leaf extract. This may be probably due to the suspected capability of bamboo leaf in protecting the heart against infarction.

Table 2
Comparison of the Effect of *Bambusa blumeana* leaf extract on cardiac enzymes

	p-value	Interpretation
CK-MB	0.002	Significant
LDH	0.011	Significant
AST	0.004	Significant

p-value is significant at <0.05

Table 2 shows the comparison of the effect of *B. blumeana* leaf extract on cardiac enzyme markers from the pre test and post test results. CKMB got the p-value of 0.002 while LDH obtained 0.011 and AST with 0.004. Statistically, it was revealed that there was a significant difference (p-value <0.05) between the groups regarding on the cardiac enzyme markers (CKMB, LDH and AST). Significant difference was due to elevation of cardiac enzymes. On the other hand, values of cardiac enzymes increases but post test results of groups with bamboo leaf extract were normal since it fell within the reference ranges.

When the pre test results and post test results of CKMB, LDH and AST were compared, it reveals significant difference (p-value <0.05) on the levels of those cardiac enzyme markers before and after the treatment which proves the effect of *B. blumeana* Schultes leaf extract at increasing concentrations. Moreover, Table 2 shows that the bamboo leaf extract had the ability to protect the heart from infarction since there was a significant difference on cardiac enzyme markers. Values obtained from pre test and post test results were compared and p-value level of <0.05 is considered significant. This was supported by previous studies that the main phytochemical component of bamboo leaf, flavonoids had antioxidant activity which counteracts the mechanism of myocardial infarction (Bello, Ndukwe, Audu & Habila,

ISSN: 2094-5906

2011; Stalikas, 2007; Sun, Yue, Tang & Guo, 2010; Zhang, Wu & Yu, 2010).

Table 3
Multiple Comparisons of The Difference in the Cardioprotective Effect Between Different Concentrations

Concentrations		p-value	Interpretation
tocopherol	Isoproterenol	0.000	Highly significant
	200 mg/kg	0.072	Not significant
	300 mg/kg	0.999	Not significant
	400 mg/kg	1.000	Not significant
200 mg/kg	Isoproterenol	0.000	Highly significant
	300 mg/kg	0.021	Significant
	400 mg/kg	0.010	Significant
	Tocopherol	0.072	Not significant
300 mg/kg	Isoproterenol	0.000	Highly significant
	200 mg/kg	0.021	Significant
	400 mg/kg	0.873	Not significant
	Tocopherol	0.999	Not significant
400 mg/kg	Isoproterenol	0.000	Highly significant
	200 mg/kg	0.010	Significant
	300 mg/kg	0.873	Not significant
	Tocopherol	1.000	Not significant
isoproterenol	200 mg/kg	0.000	Highly significant
	300 mg/kg	0.000	Highly significant
	400 mg/kg	0.000	Highly significant
	Tocopherol	0.000	Highly significant

p-value is significant at <0.05

Table 3 shows multiple comparisons of difference in the cardioprotective effect between different concentrations. Isoproterenol when compared in different concentrations of *B. blumeana* leaf extract obtained the p-value of 0.000 which was considered highly significant (p-value <0.05).

Also showed at Table 3 are the comparisons of the increasing concentrations of *B. blumeana* leaf extract (200, 300 and 400 mg/kg). Extract at 200 mg/kg when compared to 300 mg/kg and 400 mg/kg obtained p-values of 0.021 and 0.010, respectively which reveals that there were significant difference between the increasing concentrations of bamboo leaf extract (p-value <0.05). It was demonstrated that there was no significant difference between 300 mg/kg and 400 mg/kg extract of bamboo leaf with the p-value of 0.873 (p-value >0.05). Therefore, 300 mg/kg and 400 mg/kg extract of *B. blumeana* have actually produced the same effect on the cardiac enzyme level. When 200 mg/kg, 300 mg/kg and 400 mg/kg extract of bamboo leaves were

ISSN: 2094-5906

compared with the standard drug, tocopherol obtained p-values of 0.072, 0.999 and 1.000, respectively.

Statistically, it was revealed that there were no significant difference (p-value >0.05) between the standard drug tocopherol and the increasing concentrations of *B. blumeana* leaf extract (200 mg/kg, 300 mg/kg and 400 mg/kg). In the present study, tocopherol was used as standard as it protects the myocardial membrane against the free radical damage by nature of its antioxidant ability (Kuppusammy et al., 2010). It was noted that *B. blumeana* leaf crude extract exhibited its cardioprotective effect at dose of 200 mg/kg but produced greater effect upon increasing concentrations. When the effects of three different doses of *B. blumeana* leaf crude extract were compared, it was observed that the higher the dose of bamboo leaf extract (300 mg/kg and 400 mg/kg) had greater effect than the lower dose (200 mg/kg).

In summary, *B. blumeana* leaf crude extract (200 mg/kg, 300 mg/kg and 400 mg/kg) when compared with the standard drug tocopherol showed no significant difference. This actually means that three different doses of *B. blumeana* leaf extract can be comparable to the standard drug tocopherol. According to the obtained statistical data, *B. blumeana* leaf crude extract have the significant effect on cardioprotection at 200 mg/kg but with greater effect when increased the concentration at 300 mg/kg and 400 mg/kg. Previous study conducted about cardiovascular disease also used increasing concentrations of bamboo leaves to lower blood triglyceride and cholesterol (Singhal, Satya & Sudhakar, 2011). Increasing concentrations of orientin from bamboo leaves *(Phyllostachys nigra)* showed cardioprotective effect on ischemia/reperfusion (I/R) and inhibits apoptosis by preventing activation of the mitochondrial apoptotic pathway (Fu et al., 2006 as cited by Singhal, Satya & Sudhakar, 2011).

Histopathological Results

Figure 3 shows the histopathological results which were used to assess the myocardial damage after the treatment. Histopathological examination of the myocardium of normal rats (A) showed no inflammation or cell infiltration. It was also observed that there was no degeneration and significant lesion in the myocardium indicating that it is in normal condition. Isoproterenol group (B) showed severe myocardial degeneration and necrosis with minute hemorrhagic foci, many myocytes showed granularity and in some vacuolations of cytoplasm; others showed homogenous dark red cytoplasm with dark shrunken nucleus. In the isoproterenol group there were morphological changes that were strongly suggestive of isoproterenol-induced

ISSN: 2094-5906

myocardial injury (Haleagrahara, Varkkey & Chakaravarthi, 2011). Shrunken myocytes showed infiltration by mononuclear cells and fibrous connective tissue. *B. blumeana* Schultes extract at dosage 200 mg/kg (C) showed moderate myocardial degeneration and necrosis with areas of mononuclear infiltration of myocytes showing homogenous red cytoplasm and dark shrunken nuclei. *B. blumeana* Schultes extract at dosage 300 mg/kg (D) showed moderate myocardial degeneration and necrosis with areas of mononuclear infiltration of myocytes but less extensive than group pretreated with 200 mg/kg (D). The characteristic of group treated with *B. blumeana* Schultes extract of 400 mg/kg (E) was mild myocardial degeneration and necrosis. It was also noted that tocopherol treated group (F) showed moderate myocardial degeneration and necrosis. After administration of isoproterenol, the heart had increased oxygen demand with increase ionotropic effect of the heart, resulting in glucose deprivation and prolonged ischemia. The cells were damaged, increasing the cell permeability and muscle contractility allowing the enzymes to leak out into the bloodstream.

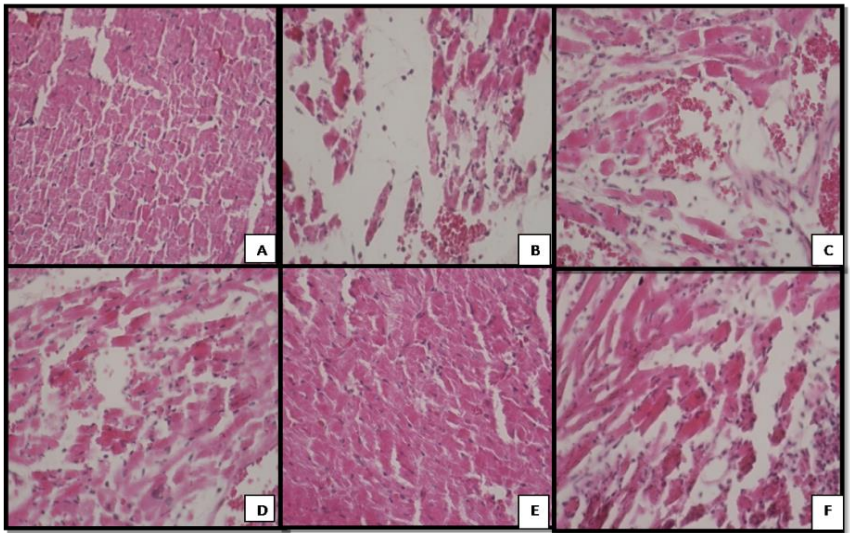

Figure 3. Histopathological Analysis: **A**. Normal rats with no treatment; **B**. Isoproterenol treated rats; **C**. *B. blumeana* extract 200 mg/kg pretreated rats; **D**. *B. blumeana* extract 300 mg/kg pretreated rats; **E**. *B. blumeana* extract 400 mg/kg pretreated rats; **F**. Tocopherol pretreated rats

ISSN: 2094-5906

The pathophysiology of acute myocardial infarction has been implicated by accumulation of free radicals (Haleagrahara, Varkkey & Chakaravarthi, 2011). *B. blumeana* Schultes treatment to isoproterenol groups had shown protection against myocardial injury with lesser damage compared to isoproterenol alone group.

Decreasing inflammatory condition was examined in increasing concentrations of extract which revealed severe-to-mild myocardial degeneration and necrosis. Histopathological results confirmed the cardioprotective activity of *B. blumeana* Schultes leaf crude extract against isoproterenol-induced myocardial infarction in Sprague-Dawley rats.

The result revealed beneficial effect of *B. blumeana* Schultes in isoproterenol-induced myocardial infarction in rats. When results were based on histopathological analysis, tocopherol group shows moderate myocardial degeneration and necrosis in histopathological examination which has the same diagnosis with the groups treated with *B. blumeana* Schultes extract (200 mg/kg and 300mg/kg). The results indicated that *Bambusa blumeana* Schultes leaf extract has the tendency to reduce elevated cardiac marker enzymes proving its cardioprotective effect.

CONCLUSION

This study revealed that at 200 mg/kg of *B. blumeana* leaf crude extract exhibited its cardioprotective effect but with greater effect in reducing the level of cardiac enzymes CK-MB, AST and LDH upon increasing concentration of 300 mg/kg and 400mg/kg oral extract treatment. Histopathological findings of the present study confirmed and indicated that *B. blumeana* leaf has cardioprotective effect against isoproterenol-induced myocardial infarction in rats. Decreasing inflammatory condition was examined in increasing concentrations of extract which revealed severe-to-mild myocardial degeneration and necrosis. The results of the present study revealed that *B. blumeana* Schultes leaves can be used as an easily accessible source of natural antioxidants against myocardial infarction.

RECOMMENDATIONS

The phytoconstituents responsible for the cardioprotective activity as well as its mechanism are not much clear. Therefore, further research and studies focusing on the isolation, characterization and purification of the biochemical compounds responsible for the cardioprotection must be carried out to support the rationality of cardioprotective activity of *B. blumeana* Schultes leaves.

ISSN: 2094-5906

ACKNOWLEDGMENT

The authors express their sincere gratitude to Prof. Reby A. Cabanela and Prof. Oliver Shane R. Dumaoal for their help and support in the researchers' endeavours in accomplishing the works. The researchers would also like to give their wholehearted thanks to Dr. Lorelie A. Villarba, Dr. John Rey R. Sungahed and Ms. Annalie A. Pateña for their support and guidance in the fulfilment of this study.

REFERENCES

Abdel-Monem N.M, Abdel-Azeem, A.M, El-Ashry, E-S, Ghareeb, D.A, Nabil-Adam A. (2013). Pretreatment hepatoprotective effect of the marine fungus derive from sponge on hepatic toxicity induced by heavy metals in rats. Article ID 510879, 1-15.

Alipon, M.A., Bausa, Edna B., and Sapin G.N. (2011). Development of floor tiles from Philippine bamboos. *Philippine Journal of Science.*140(1)

Aubin, M.C., Lajoie, C., Clement, R., Gosselyn, H., Calderone, A., and Perrault, L. (2008). Female rats fed A high-fat diet were associated with vascular dysfunction and cardiac fibrosis in the absence of overt obesity and hyperlipidemia: therapeutic potential of resveratrol. *The American Society for Pharmacology and Experimental Therapeutics*, 325(3), 1-8.

Bae, S., & Zhang, S. (2005). Gender differences in cardioprotection against ischemia/reperfusion injury in adult hearts: focus on Akt and protein kinase C signaling. *The Journal of Pharmacology and Experimental Therapeutics*, 315(3), 1125-1135. doi:10.1124/jpet.105.090803

Bello, I., Ndukwe, G., Audu, O., and Habila, J. (2011). A bioactive flavonoid from Pavettacrassipes K. Schum. *Organic and Medicinal Chemistry Letters*, 1(14).1-5.

Chongtam, N., Bisht, M.S., and Haorongbam, S. (2010). Nutritional properties of bamboo shoots: potential and prospects for utilization as health foods. *Comprehensive Reviews in Food Science and Food Safety*, 10, 153-169.

de Sousa, O., Viera, G., de Pinho, J., Yamamoto C., and Alves, M.(2010). Antinociceptive and anti-inflammatory activities of the ethanol extract of *Annonamuricata* L. leaves in animal models. *International Journal of Molecular Sciences*, 11, 2067-2078. doi:10.3390/ijms11052067

Decipulo, M., Ockerby, S., Midmore, D. (2003). Managing clumps of *Dendrocalamus asper* in Bukidnon, the Philippines. Australian Centre for International Agricultural Research Proceedings 129, 37-45.

ISSN: 2094-5906

Feranil, A., Duazo, P., Kuzawa, C., & Adair, L. (2011). Coconut oil predicts a beneficial lipid profile in pre-menopausal women in the Philippines. *Asia Pacific Journal of Clinical Nutrition,* 20(2), 190–195.

Giknis, M., and Clifford, C. (2008). Clinical laboratory parameters for Charles River laboratories: WI (Han). *Charles River*,1-14.

Goyal, A.K, Middha, S. K., and Sen, A. (2010). Evaluation of the DPPH radical scavenging activity, total phenols and anti-oxidant activities in Indian wild *Bambusa vulgaris* "Vittata" methanolic leaf extract. *Journal of Natural Pharmaceuticals*, 1(1), 40-45

Haleagrahara, N., Varkkey, J., and Chakaravarthi, S. (2011). Cardioprotective effects of glycyrrhizic acid against isoproterenol-induced myocardial ischemia in rats. *International Journal of Molecular Sciences*, 12, 7100-7113. doi:10.3390/ijms12107100

Kuppusamy, A., Varadharajan, S., Josey, C., John, H., Sherin, D., Chirakkan, D., et al. (2010). Cardioprotective Effect of Erythrinastricta leaves on isoproterenol-induced myocardial infarction in rat. *Bangladesh Journal Pharmacology*, 5, 1-4. DOI: 10.3329/bjp.v5i1.3809

Li, H., Xie, Y., Yang, Q., Wang, W., Zhang, B., Wang, J., Cao, W., and Bi, L. (2012). Cardioprotective effect of paeonol and danshensu combination on isoproterenol-induced myocardial injury in rats. PLOS ONE, 7(11), e48872.doi:10.1371/journal. pone.0048872

Liu, Z., Jiang, N., Ren, H., and Ma, Y. (2011). Changes of cell wall polysaccharides of mosobamboos of four different ages. *The Journal of the American Bamboo Society,* 24(1), 7-13.

Madetoja, J., Madetoja, M., Mäkinen, J., Riuttala, E. & Jokinen, J. (2009). Blood sampling from the tail vein, in comparison with two other techniques, causes less stress to mice. *Scandinavian Journal of Laboratory Animal Science* 36(2).

Malab, S., Batin, C., Malab, B., Alipon, M., and Midmore, D. (2009). Improving productivity of previously unmanaged *Bambusa blumeana* plantation for culms and shoots in Ilocos Norte, the Philippines.In *'Silvicultural Management of Bamboo in the Philippines and Australia for Shoots and Timber*', ed. by D.J. Midmore. Australian Centre for International Agricultural Research Proceedings 129, 24-35.

Momin, F., Kalai B., Shikalgar, T., Naikwade, N. (2011). Cardioprotective effect of methanolic extract of *Ixora coccinea* Linn.leaves on doxorubicin- induced cardiac toxicity in rats. *Indian Journal of Pharmacology*, 44(2), 178-173.doi 10.4103/0253-7613.93844.

ISSN: 2094-5906

Nikolić, M., Nikić, D., and Petrović, B. (2008). Fruit and vegetable intake and the risk for developing coronary heart disease. *Central Europe Journal Public Health*, 16 (1), 17–20.

Panda, V. & Naik, S. (2009). Evaluation of cardioprotective activity of *Ginkgo biloba* and *Ocimum sanctum* in rodents. *Alternative Medicine Review*. 14(2), 161-171.

Parasuraman, S., Raveendran, R., Kesavan, R. (2010). Blood sample collection in small laboratory animals. *Journal of Pharamacology and Pharmacotherapeutics*, 1(2), 87-93. doi 10.4103/0976-500X.72350.

Patel, J., Setty S., and Chakraborty, M., (2012). Evaluation of cardioprotective activity of *Medoharvati* by isoproterenol-induced myocardial damage in rats. *International Research Journal of Pharmacy*, 3(8), 241-247.

Peng, C., Liang, Y., Wang, X., Xie, H., Tianyou Zhang, T. and Ito, Y. (2009). Preparative isolation and purification of flavonoids from the Chinese medicinal herb *Belamcanda* by high-speed counter current chromatography. *Journal of Liquid Chromatography Related Technology*. 32(16), 2451–2461. doi:10.1080/10826070903188245.

Perez, V.J., Caviteno M.K., Malabanan, G., Manalo, C. and Muli, E., (2011), Hepatoprotective activity of crude leaf extract of *Ipomea batatas* Linn.against acetaminophen and alcohol induced liver damage on Sprague-Dawley rats, *Lyceum of the Philippines University-Batangas.*

Petrich, E., Schanne, O. and Zumino, A. (1996). Electrophysiological responses to ischemia and reperfusion. *Myocardial Ischemia: Mechanisms, Reperfusion, Protection*. 76. 115-133. doi:10.1007/978-3-0348-8988-9_8.

Prabhu, S., Jainu, M., Sabitha, K., and Devi, S. (2006). Cardioprotective effect of mangiferin on isoproterenol induced myocardial infarction in rats. *Indian Journal of Experimental Biology*. 44. 209-215.

Reddy, K., and Yusuf, S. (1998). Emerging epidemic of cardiovascular disease in developing countries. *Circulation*.97(6).596-601.

Robiel E., Merih T., and Abraha, B. (2011).Phytochemical screening of *Ziziphus spina* Christi and *Piper nigrum*. *Jamonline*, 1(1).41–47.

Singhal, P., Satya, S., and Sudhakar, P. (2011). Antioxidant and pharmaceutical potential of bamboo leaves. *The Journal of the American Bamboo Society*, 24(1), 19-28.

Stalikas, C.D (2007). Extraction, separation, and detection methods for phenolic acid and flavonoids. *Journal of Separation Science*, 30, 3268-3295.

ISSN: 2094-5906

Sun J., Yue Y-D., Tang, F., Guo, X-F. (2010). Flavonoids from the leaves of *Bambusa per variabilis* McClure. *Journal of Clinical ChemistrySociety*, 55(3), 363-365.

Wang, J., Yue, Y., Tang, F., and Sun J., (2012). TLC screening for antioxidant activity of extract from fifteen bamboo species and identification of antioxidant flavone glycosides from leaves of *Bambusa textilis* McClure. *Molecule*. 17.1229712311.doi: 10.3390/molecules17101229.

Wang, M., Baker, L., Tsai, B.M., Meldrum, K.K., and Meldrum, D.R., (2005). Sex differences in the myocardial inflammatory response to ischemia/reperfusion injury. *American Journal of Physiology*, 288, E321–E326.

Wong, S.M., Chiu, P.Y., Leung, H.Y., Zhou L., Zuo, Z., Lam, P., et al. (2011). Myocardial post-conditioning with Danshen-Gegen decoction protects against isoproterenol-induced myocardial injury via a PKCε/mKATP mediated pathway in rats. *Chinese Medicine*, 6:7, 1-10.

Zhang, J., Knapton, A., Lipshultz, S., Weaver, J. and Herman, E. (2008). Isoproterenol-induced cardiotoxicity in sprague-dawleyrats:correlation of reversible and irreversible myocardialinjury with release of cardiac troponin T and roles of iNOS in myocardial injury. *Toxicologic Pathology*, 36:277-288.doi: 10.1177/ 0192623307313010.

Zhang, Y., Wu, X. and Yu, Z. (2010). Composition containing total triterpenoidsapogenins extracted from bamboo, and the preparation method and use thereof. *United States Patent* 7,811,997 B2

ISSN: 2094-5906