

## Pharmacological evaluation of cardioprotective potential of *Berberis orthobotrys* Bien.ex Aitch.

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**Abstract:** The resurgence of scrutiny in plant-based medicine is mainly due to the current widespread belief that “green medicine” is safe and more dependable than the expensive synthetic drugs. The current study was focused to evaluate the anti-myocardial ischemic potential of *Berberis orthobotrys* Bien ex Aitch against chemically induced myocardial ischemia in animal models. Myocardial ischemia was instigated in Sprague Dawley rats of either sex (250-450g) by administration of Isoproterenol (ISO) and doxorubicin (DOX) at doses of 25mg/kg b.w and 15mg/kg b.w. respectively. The protective effect of the plant extract was explored by pretreating a group of animals with aqueous methanolic extract of *Berberis orthobotrys* roots at a dose of 50mg/kg b.w. (orally) for 10 days in ISO-ischemic model while for doxorubicin ischemic model; the study was conducted for 14 days. The findings of the study revealed that serum levels of cardiac marker enzymes were significantly increased ( $p < 0.0001$ ) followed by the administration of Isoproterenol and doxorubicin whereas the pretreatment with aqueous methanolic plant extract had significantly ( $p < 0.0001$ ) prevented the rise in the same, as compared to both intoxicated groups. The statistical analysis of the study led to the conclusion that *Berberis orthobotrys* possesses cardio protective potential against chemically induced myocardial ischemia

**Keywords:** *Berberis orthobotrys*, doxorubicin, isoproterenol, myocardial ischemia.

### INTRODUCTION

Myocardial ischemia also called as Cardiac ischemia is a state of ailment in which heart muscle suffers from lack of oxygen due to inadequate blood flow (Kang *et al.*, 2007). It serves as focal cause of coronary heart disease, angina pectoris, myocardial infarction, heart failure and ultimately heart attack (Kang *et al.*, 2007). One of the leading causes of human death worldwide is myocardial ischemia caused infarction (Ishii *et al.*, 2009). Coronary heart disease deaths in Pakistan accounted for 111,367, or 9.87 percent of total deaths, according to WHO data released in May 2014. Pakistan ranks No. 63 for disease prevalence in the world due to the age adjusted death rate which is 110.65 per 100,000 of population (WHO, 2015). It was estimated that by 2020 the number of people who die from ischemic heart disease will increase by around 50% in states with established market economies and by over 100% in low- and middle-income nations (WHO.2007).

An undesirable souvenir of modern medicine for treatment and management of myocardial ischemia such as aspirin, nitroglycerin, beta blockers, calcium channel

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blockers, is that they cause number of side effects which results in other complications. This is considered as one of the causes of the resurgence of scrutiny in plant derived medicines which are considered safer, more dependable and cost effective in comparison to the synthetic drugs (Shinwari and Qaisar, 2011). Pakistan has been God gifted with rich natural resources, vast ecological zones and rich flora of more than 6000 plant species. There is a greater need to excel in ethano-research to tackle chronic infirmities such as cardiovascular diseases, given the economic insecurity and broken infrastructure of the country. Scientists are also eager to explore ethnomedicine for the treatment of myocardial ischemia as it is the most misinterpreted topic of the decade. *Berberis orthobotrys* (family: Berberidaceae) is a plant native to Pakistan, found primarily in Gilgit Baltistan, commonly known as Ishkeen. For the treatment of wounds, parasites, jaundice, liver disorders, kidney stones, diabetes, sore throat and uterine tumors, its roots and stem bark were used (Alamgeer *et al.*, 2014). The plant has an extensive history of traditional use in treatment of various cardiovascular ailments. The urge to determine cardioprotective potential of said plant also includes specifics that it has also been reported to have anti-hyperlipidemic and antihypertensive potential with a

marked cardiosuppressant effect (Alamgeer *et al.*, 2013; Alamgeer *et al.*, 2014) Bearing in mind, these particulars the current study was designed to evaluate the anti-myocardial ischemic potential of *Berberis orthobotrys* Bien ex Aitch against isoproterenol and doxorubicin induced myocardial ischemia in animal models.

## MATERIALS AND METHODS

### **Chemicals, Drugs and Reagents Used**

The following chemicals and drugs were used for the executing the present research study; Methanol (RCI Labscan Limited, Bangkok, Thailand), Isoproterenol Hcl (Sigma Aldrich chemie GmbH), Doxorubicin Hcl (Zhejiang Hisun Pharmaceuticals Co.Ltd,China), Normal Saline and Water for injection. For the purpose of biochemical analysis of various cardiac markers, commercial diagnostic kits for CK-MB, LDH, and AST were used (Randox Diagnostic kits, Randox laboratories.UK).

### **Experimental Animals Used**

Young and healthy Sprague Dawley rats (either sex), weighing between 250-450g were used. The animals were housed under controlled room temperature (25±10°C) and received human care according to the guidelines of National Institute of Health (NIH) and the protocol was approved from animal ethical committee of the department.

### **Plant Material**

The plant collection was done from district Gilgit (Shikiyote), Pakistan during the month of June, 2015. It was then identified and authenticated by Dr. Shair Wali Khan, Assistant Professor Botany, Karakorum International University Gilgit Baltistan, Pakistan. A voucher (No. BO-15-12) had been deposited in the herbarium of Department of Pharmacy, University of Sargodha. Aqueous-methanolic (30:70) root extract of *Berberis orthobotrys* was prepared by following extraction method as detailed by Alamgeer *et al.*, 2013.

### **Evaluation of cardioprotective potential of *Berberis orthobotrys* crude extract against isoproterenol induced myocardial ischemia**

Cardioprotective effect of *Berberis orthobotrys* was explored in Sprague Dawley rats, by following the protocol given by Rai *et al.* (2010) with trivial adjustments. The animals were divided in to four groups (n=5) as normal control group that received only normal saline; ISO intoxicated group, baseline group received only plant extract at dose of 50mg/kg and BO treated group with animals pretreated with plant extract for 10 days followed by intoxication with ISO. The plant extract and Isoproterenol were freshly prepared throughout the experimental protocol in normal saline and were administered through oral route using animal feeding tube

(16G). Myocardial ischemia was induced by subcutaneous injection of isoproterenol hydrochloride (ISO, 25 mg kg-1b.w) for last 2 consecutive days of the protocol in ISO intoxicated and BO treated group (Rai *et al.*, 2010). The Cardioprotective effect of the plant extract was evaluated by comparing level of cardiac markers of myocardial ischemia in serum of treated group with Isoproterenol intoxicated group of experimental animals.

### **Evaluation of cardio protective effect of *Berberis orthobotrys* crude extract against doxorubicin induced myocardial ischemia**

Cardio protective effect of *Berberis orthobotrys* crude extract against doxorubicin induced myocardial injury was assessed in Sprague Dawley rats, following the procedure detailed by El-Syed *et al.* (2011). The animals were divided in four group (n=5) as normal control group given only normal saline, DOXO –intoxicated group, baseline group received only plant extract at dose of 50mg/kg and BO treated group with animals pretreated with plant extract for 14 days followed by intoxication with DOXO. Myocardial ischemia was induced by single IP injection of doxorubicin Hcl (15mg/kg b.w) to the experimental animals at the end of protocol for DOXO intoxicated and BO treated group. Plant extract and normal saline were administered as explained above. Heart to body weight ratio of the experimental animals was also measured. The Cardioprotective effect of the plant extract was assessed by comparing level of cardiac markers of myocardial ischemia in serum of treated group with DOXO- intoxicated group of experimental animals.

## STATISTICAL ANALYSIS

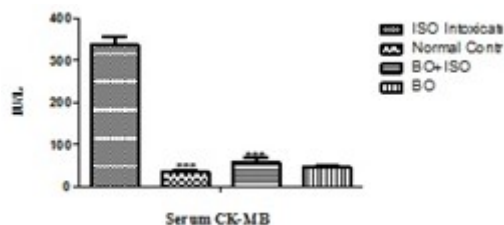
The data was presented as Mean± S.E.M. The significance of difference between various experimental groups was estimated by one-way analysis of variance followed by application of the Dunnett's Multiple Comparisons test. A *P* value of less than 0.05 was considered to be significant. The statistical analysis was carried out by use of GraphPad Prism software Version 5.00 (GraphPad, San Diego, CA, USA).

## RESULTS

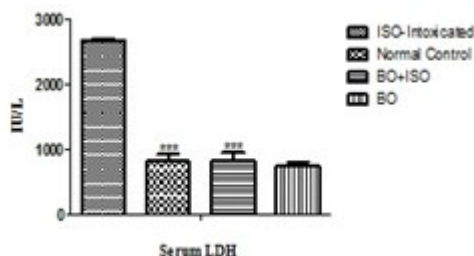
### **Evaluation of cardioprotective potential of *Berberis orthobotrys* crude extract against isoproterenol induced myocardial ischemia**

The results of present study showed that the subcutaneous administration of Isoproterenol at dose of 25mg/kg b.w. significantly elevated ( $p<0.0001$ ) the level of cardiac marker enzymes (CK-MB, LDH and AST) in serum of Isoproterenol intoxicated rats when compared to normal control group. Whereas pretreatment with *Berberis orthobotrys* extract for 10 days has shown a significant prevention of rise ( $p<0.0001$ ) in mean levels of cardiac enzymes when compared with ISO-intoxicated group.

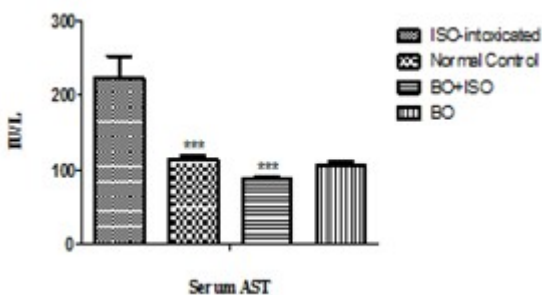
Interestingly, mean levels of cardiac marker enzymes of baseline group are in parallel to that of normal control group (figs. 1 and 3).



**Fig. 1:** Effect of *Berberis orthobotrys* crude extract on serum CK-MB, LDH and AST level against ISO induced myocardial ischemia in rats. Results are expressed as Mean ± S.E.M for 5 rats. ISO-intoxicated group is compared to Normal control and BO treated group (\*\* $p < 0.0001$ ).



**Fig. 2:** Effect of *Berberis orthobotrys* crude extract on serum CK-MB, LDH and AST level against ISO induced myocardial ischemia in rats. Results are expressed as Mean ± S.E.M for 5 rats. ISO-intoxicated group is compared to Normal control and BO treated group (\*\* $p < 0.0001$ ).



**Fig. 3:** Effect of *Berberis orthobotrys* crude extract on serum CK-MB, LDH and AST level against ISO induced myocardial ischemia in rats. Results are expressed as Mean ± S.E.M for 5 rats. ISO-intoxicated group is compared to Normal control and BO treated group (\*\* $p < 0.0001$ ).

**B. Evaluation of Cardioprotective effect of *Berberis orthobotrys* crude extract against doxorubicin induced myocardial ischemia**

The results statistics of this experiment clearly depicted that, single IP injection of doxorubicin Hcl augmented a significant increase in mean serum level of cardiac marker enzymes ( $p < 0.01$ - $p < 0.0001$ ) when compared with normal

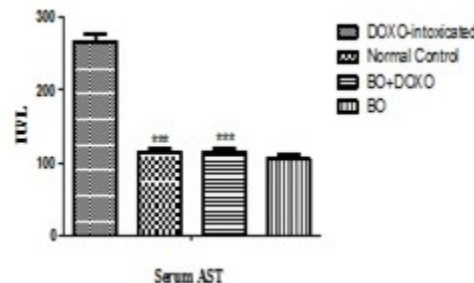
control group. However, pretreatment with *Berberis orthobotrys* aqueous methanolic extract for 14 days has shown to significantly impede the rise in mean serum levels of CK-MB, LDH and AST ( $p < 0.01$ - $p < 0.001$ ) when compared to DOXO-intoxicated group (figs. 4 and 6).



**Fig. 4:** Effect of *Berberis orthobotrys* crude extract on serum CK-MB, LDH and AST level against doxorubicin induced myocardial ischemia in rats. Results are expressed as Mean ± S.E.M for 5 rats. DOXO-intoxicated group is compared to Normal control and BO treated group (\*\* $p < 0.0001$ ).



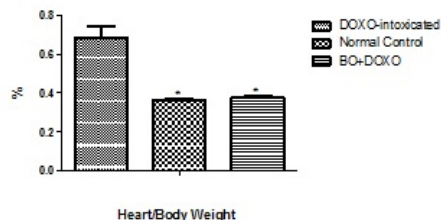
**Fig. 5:** Effect of *Berberis orthobotrys* crude extract on serum CK-MB, LDH and AST level against doxorubicin induced myocardial ischemia in rats. Results are expressed as Mean ± S.E.M for 5 rats. DOXO-intoxicated group is compared to Normal control and BO treated group (\*\* $p < 0.001$ ).



**Fig. 6:** Effect of *Berberis orthobotrys* crude extract on serum CK-MB, LDH and AST level against doxorubicin induced myocardial ischemia in rats. Results are expressed as Mean ± S.E.M for 5 rats. DOXO-intoxicated group is compared to Normal control and BO treated group (\*\* $p < 0.0001$ ).

Equally, mean levels of above mentioned marker enzymes of baseline group are in parallel to that of normal control group. Intoxication of experimental animals of DOXO-intoxicated group with doxorubicin Hcl (15mg/kg b.w.) have also caused a significant increase ( $p < 0.05$ ) in heart

to body ratio ( $0.650 \pm 0.150$ ) when compared to normal control group ( $0.364 \pm 0.011$ ). Whereas pretreatment of the animals with plant extract had shown to cause significant preservation ( $p < 0.05$ ) of Heart to body weight ratio ( $0.379 \pm 0.015$ ) in comparison to intoxicated group (fig. 7).



**Fig. 7:** Effect of *Berberis orthobotrys* crude extract on heart/Body weight ratio of rats against Doxorubicin induced myocardial ischemia. Values are expressed as Mean $\pm$ S.E.M for 5 rats. DOXO-intoxicated group is compared to Normal control and BO treated group (\* $p < 0.05$ ; significant)

## DISCUSSION

The current research study was conducted to elucidate the cardioprotective effect of *Berberis orthobotrys Bien ex Aitch* against chemically induced myocardial ischemia. The results of the present study have clearly illuminated the anti-myocardial ischemic potential of plant crude extract. Administration of Isoproterenol significantly increased the levels of cardiac marker enzymes such as CK-MB, AST and LDH (figs. 1-3), which was strongly in accordance with previous related studies (Velavan *et al.*, 2008; Asdaq and Chakraborty, 2010). The myocardial cells are rich in cardiac enzymes like creatinine kinase, lactate dehydrogenase, aspartate transaminase etc. Isoproterenol is reported to cause myocardial ischemia by various known mechanisms such as: exertion of a positive inotropic effect on heart thereby increasing the oxygen demand of the heart, resulting in prolonged ischemia and glucose deprivation (Haleagrahara *et al.*, 2011) along with excessive production of free radicals resulting from the oxidative metabolism of catecholamines. As a result of this excessive free radical formation deterioration of function and integrity of the myocardial membranes occur (Velavan *et al.*, 2008) causing the cells to be weakened, which culminates in increasing the permeability of the cell membranes, allowing cardiac enzymes to leak into the bloodstream. As findings of current clearly depicted that pretreatment of the experimental animals with aqueous methanolic extract of *Berberis orthobotrys* has showed a significant prevention of increase in the levels of cardiac markers enzymes followed by the administration of ISO (figs. 1-3). These results clearly suggest the cardio protective activity of the plant extract in terms of protection against myocardial ischemia. This effect of the plant can be attributed to its cardio suppressant /negative chronotropic effect as reported by Alamgeer *et*

*al.* in 2013. The plant has also been reported to have antihypertensive activity (Alamgeer *et al.*, 2013) owing to its antioxidant property. Any plant having the potential to slow the heart rate and reduce the work load of heart will possibly combat the stress on the myocardial cell resulting due to lack of oxygen.

In our study the cardioprotective potential was also determined against doxorubicin induced myocardial ischemia. The results of the study gave a much defined picture of myocardial ischemia followed by IP administration of Doxorubicin. The serum level of CK-MB, LDH and AST were markedly increased, confirming the model based induction (figs. 4-6), in line with previous studies (Wakade *et al.*, 2008; Garba and Ubom, 2005). As a consequence of doxorubicin administration, oxidative stress and mitochondrial dysfunction are associated with disease and toxic processes. Doxorubicin administration has been also seen to cause a relative increase in heart to body weight ratio of the experimental animals (fig. 7) which are parallel to the findings of many other studies (Koti *et al.*, 2009). This increase in heart weight can result from the inflammatory process in the areas of cell injury or necrosis, resulting in inflammatory cell infiltration and increased interstitial fluid level (Gandhi *et al.*, 2013). Findings of this experiment also show a marked protection against myocardial ischemia after pretreatment with plant extract as evident by much lower serum levels of CK-MB, LDH and AST, when compared to DOXO-intoxicated group (figs. 4-6). The extent of protection offered by *Berberis orthobotrys* crude extract might be linked to reduced oxidative stress causing possible stabilization of myocardial cell membranes leading to fewer leakages of cardiac enzymes. The heart to body weight ratio of the treated animals also came out to be near to the control group further authenticating a protective effect of the extract (fig. 7). Following the previously stated idea in view point of phytochemistry, degree of cardioprotection presented by plant extract can be linked to possible presence of phytochemical berberine in the plant crude extract, as plant being a member of Berberidaceae family, which is reported to be rich in above stated alkaloid (Dezfuli *et al.*, 2014). In a study carried out by Zhang *et al.* (2014) states that berberine possesses cardioprotective effect and the alkaloid has also been reported for its antioxidant, antihypertensive, vasorelaxant (Ko 2000) and antihyperlipidemic effect (Zhao *et al.*, 2011) providing further support to our findings and hypothesis about the presence of said phytochemical.

## CONCLUSION

From the above narrated statistics of our conducted research it can be concluded that aqueous methanolic extract of roots of *Berberis orthobotrys* Bien ex Aitch bears a remarkable cardioprotective potential in terms of

protection against chemically induced myocardial ischemia. Even so, considering all the above findings still a complete phytochemical analysis for identification of active principle(s) responsible for cardioprotective effect and elucidation of possible mechanism(s) of action of antimyocardial ischemic effect is still needs to be outlined.

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