Cardioprotective Potential of Gemmomodified Extract of *Terminalia arjuna* against Chemically Induced Myocardial Injury in Rabbits

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**ABSTRACT**

The objective of this study was to determine the comparative cardioprotective potential of bark and gemmomodified extract of *Terminalia arjuna*. Cardioprotective potential was evaluated against isoproterenol induced cardiotoxicity. Both ways of treatment, curative and preventive were studied. In preventive cardioprotective potential, rabbits were pretreated with both extracts of *T. arjuna* (200 mg/kg) for three weeks and then cardiotoxicity was induced with isoproterenol. In curative way of treatment isoproterenol was given for two days and then these cardio intoxicated rabbits were treated with plant extracts. The activities of cardiac marker enzymes (CK-MB, AST, ALT, and LDH) and antioxidants enzymes (SOD, CAT) were analyzed in serum and heart tissues of different groups of experimental rabbits. Isoproterenol significantly (P<0.001) increased the level of enzymes. The three week prior administration and curative treatment of *T. arjuna* extracts resorted the enzymes and antioxidants level equal to normal. HPLC analysis of the extracts confirmed the presence of important flavonol and phenolic acids in both extracts. It can be concluded that gemmomodified and bark extract of *T. arjuna* has strong cardioprotective potential.

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**INTRODUCTION**

Coronary heart diseases are leading cause of death in developed and industrialized countries and increasing alarmingly in developing and poor countries (Karthikeyan et al., 2007; Torabian et al., 2009; Subhashini et al., 2011; El-Sayed et al., 2011). The chemotherapeutic agents, which inhibit the free radical formation and can reduce the risk of heart diseases, have gained imperative value in the modern medicines. Herbal medicines having antioxidant properties may therefore, have protective role in cardiovascular diseases (Viswanatha et al., 2010).

There is increasing trend towards the application of herbal medicines to treat the cardiovascular diseases (Nandave et al., 2007; Hina et al., 2010; Ojha et al., 2011). Gemmotherapy is a subdivision of phytotherapy which utilizes glycerin macerates of fresh plants and other vegetative tissues in the growing stage. These tissues are rich in growth factors including phytohormones, auxins and gibberellins. The bioactive compounds are present in embryonic and growing tissues which start to disappear after plant maturation. The use of the buds, young leaf and rootlets makes it possible to obtain a more active medication than remedies that are prepared from the whole plant. Gemmotherapeutically treated remedies are safe, abundant in nutrients and impart positive effect on cure and recovery of human health.

Ancient physicians used the powdered bark of *T. arjuna* for alleviating the cardiovascular diseases (Gauthaman et al., 2001; Parveen et al., 2011; Oberoi et al., 2011) and wound healing. Many scientific studies proved its medicinal importance. Experimental studies revealed that its bark showed significant antioxidant, antidiabetic, antimutagenic (Jahan et al., 2011a), anthelmintic and antimicrobial activities (Ramya et al., 2008; Jahan et al., 2011b).

*T. arjuna* is a versatile traditional medicinal plant. Some studies are available on preventive cardioprotective effect of bark extract. But no significant study is available on cardioprotective potential of gemmomodified extract which is also a rich source of bioactive compounds. In literature, many studies about preventive cardioprotective
mode of medicinal plants are available but research reports about curative mode are rare. The present study was therefore planned to evaluate the cardioprotective potential of *T. arjuna* extracts through both (preventive and curative) mode of treatments and to identify its important flavonoid and phenolic contents by HPLC.

**MATERIALS AND METHODS**

**Extract preparation:** *T. arjuna* was collected from University of Agriculture, Faisalabad Pakistan. The bark powder (30 g) was refluxed with methanol. After completion of time extract was filtered and methanol was evaporated under reduced pressure. Paste of plant material (100 g), which was freshly harvested from plants during their growing stage was macerated with one liter mixture of glycerin and methanol in a ratio of 1:2 and shake vigorously. After one month macerate was filtered and solvent was removed with rotary evaporator and crude extract was stored.

**HPLC analysis of phenolic compounds:** The analytical HPLC system used for separation of flavonol was consisted of UV-visible detector (λ max 360 nm) Hypersil-ODS column (4.6 x 250 mm, I.d., 5-µm) at ambient temperature. The mobile phase consisted of solvent A (3% aq. trifluoroacetic acid) and solvent B (acetonitrile and methanol (80:20 v/v), was run with isocratic elution at flow rate of 1mL/min. The column and detector (λ max 280 nm) used for phenolic acid separation were similar to that used in flavonol analysis (Sultana and Anwar, 2008).

**Experimental protocol:** Cardioprotective potential of *T. arjuna* was evaluated in chemically induced myocardial infarction in animal model. Male albino rabbits weighing 1-1.5 kg were kept under standard conditions of environment. Animals were allowed free access to standard diet and water. Rabbits were kept for one week acclimatization period and then divided into six groups, each comprised of five animals (Table 1).

**Table 1:** Treatment protocol in different experimental groups of rabbits

<table>
<thead>
<tr>
<th>Groups</th>
<th>Detail</th>
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<tbody>
<tr>
<td>I</td>
<td>Normal controls; animals were given standard diet only</td>
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<tr>
<td>II</td>
<td>ISO control group; rabbits were injected with ISO (85 mg/Kg b.wt.) for two consecutive days</td>
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<tr>
<td>III</td>
<td>Baseline group: rabbits were treated with 200 mg/kg b.wt. <em>T. arjuna</em> extracts once daily by oral gavage for three weeks.</td>
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<tr>
<td>IV</td>
<td>Preventive group: rabbits were pretreated with <em>T. arjuna</em> 200 mg/kg b.wt. once daily by oral gavage for three weeks and then ISO (85 mg/kg) was injected for two days.</td>
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<tr>
<td>V</td>
<td>Curative groups: rabbits were injected with ISO (85 mg/kg) for two days to induce cardio toxicity. Then these cardio intoxicated rabbits were treated with 200 mg/kg b.wt of plant extracts (gemmomodified and bark) once daily for four days and blood samples were collected daily.</td>
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<tr>
<td>VI</td>
<td>Standard drug curative group: rabbits were injected with ISO (85 mg/kg) for two days to induce cardio toxicity. Then these cardio intoxicated rabbits were treated with combination of two standard drugs including Propranolol (5 mg) and gemfibrazole (50 mg) once daily for four days and blood samples were collected daily.</td>
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**Biochemical assessment:** At the end of experimental period the blood samples were taken and serum was separated for analysis of different enzymes related to myocardial infarction such as lactate dehydrogenase (LDH), creatine kinase-MB fraction (CK-MB), aspartate transaminase (AST), alanine transaminase (ALT). All the analyses were performed with commercially available kits using chemistry analyzer. Animals were slaughtered and the heart tissues were removed for the analysis of antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).

**Statistical Analysis:** Each sample was analyzed in triplicate and data were expressed as mean±SD. Data was analyzed using analysis of variance (ANOVA) in SPSS 15 software. Tukey Multiple Comparison test was used for comparison of means of different treatments (P<0.001).

**RESULTS**

**HPLC analysis of polyphenols:** HPLC analysis demonstrated that *T. arjuna* bark and gemmomodified extract contained the highest amount of myricetin followed by quercetin and kaempferol (Table 2). *T. arjuna* bark extract exhibited good concentration of phenolic acids. Bark extract contained highest amount of ferulic acid followed by p-coumaric acid, gallic acid, chlorogenic acid, caffeic acid and catechin. In gemmomodified extract ferulic acid, gallic acid, caffeic acid, chlorogenic acid and catechin were identified (Table 2).

**Preventive cardioprotective activity:** The present study demonstrated the cardioprotective potential of polyphenolic rich extract of *Terminalia arjuna* in terms of curative and preventive modes of treatments. Isoproterenol (ISO) significantly (P<0.001) increased the level of serum cardiac marker enzymes like CK-MB, LDH, AST, ALT in isoproterenol-induced group as compared to normal control group indicating myocardial infarction in rabbits. Prior administration of plant extracts (200 mg/kg b.wt.) significantly decreased the ISO-induced elevated level of cardiac marker enzymes (Table 3).

Group of rabbits treated with the bark extract brought ISO-induced elevated enzyme level close to the normal. Gemmomodified extract of *T. arjuna* also demonstrated significant (P<0.001) decline in enzyme level when compared to ISO control group. Treatment of *T. arjuna* bark extract showed greater effect in comparison of gemmomodified extract. This decline in enzyme level could be due to the action of *T. arjuna* extracts on...
maintaining membrane integrity therefore constrained the leakage of enzymes.

Myocardial antioxidant enzymes activities like SOD, CAT and peroxidase were significantly (P<0.001) lower in isoproterenol-treated group than in control group. Pretreatment with T. arjuna followed by ISO administration significantly (P<0.001) protected the rabbits against ISO-induced changes in SOD, CAT and GPx level. Treatment of rabbits with gommomodified extract of T. arjuna at the dose of 200 mg/kg b.wt. restored the antioxidant enzyme level near to the normal. T. arjuna bark extract also significantly restored antioxidant enzyme level as compared to ISO control group. However, this effect was slightly less than gommomodified extract. In baseline groups level of cardiac and antioxidant enzymes was normal.

**Curative cardioprotective activity:** Curative cardioprotective effect is important parameter for the evaluation of cardioprotective activity of medicinal plants. In curative cardioprotective activity, cardiotoxicity (ischemia and myocardial infarction) was induced in rabbits and then plant extract (200 mg/kg b.wt) was fed orally to ischemic/ myocardial infarcted rabbits once daily for four days.

Cardiotoxicity was induced in experimental rabbits by injection of isoproterenol at the dose of 85 mg/kg b.wt. Isoproterenol significantly increased (P<0.001) the level of diagnostic cardiac markers in serum of the rabbits as compared to the rabbits of normal control group indicating cardiac insufficiency, ischemia and myocardial infarction (Table 4).

Treatment of myocardial infarcted rabbits with T. arjuna extracts significantly (P<0.001) reduced the ISO-induced rise in cardiac marker enzymes after four treatments (one dose daily). Both bark and gommomodified extracts of T. arjuna (200 mg/kg b.wt) decreased the ISO-induced elevated enzyme level close to normal. These results are comparable with the effect of standard drug. Combination of two standard drugs Propranolol and gemfibrozole is used to compare the activity of medicinal plants.

**DISCUSSION**

The present study demonstrated both important curative and preventive modes of cardioprotective activity. It explained the cardioprotective potential of bark and gommomodified extract of T. arjuna in widely used ISO-induced model of myocardial infarction in rabbits. ISO, a synthetic catechol amine, is β-adrenergic receptor agonist. In high dose, it has ability to destruct myocardium and cause cardiotoxicity due to cytosolic Ca2+ overload. As a result of this myocardium destruction, cytosolic enzymes (CK-MB, LDH, AST, and ALT) are secreted into blood and serve as diagnostic markers of cardiotoxicity. Pathophysiological changes including cell necrosis, contractile failure, ventricular arrhythmias and subcellular changes after ISO administration (85 mg/kg) are comparable to those taking place in human myocardial ischemia/infarction (Nandave 2012).
ISO treated rabbits showed significant increase in the levels of diagnostic marker enzymes including CK-MB, LDH, AST, and ALT at the dose of 85 mg/kg. The high levels of enzymes are an indicator of the severity of ISO-induced myocardial cell necrosis. The myocardial cell necrosis can be due to increase in lipid peroxidation. These finding are in agreement with earlier reports (Karthikeyan et al., 2007).

Activities of antioxidant enzymes including SOD, CAT, and peroxidase were significantly lower in cardiac tissues of ISO treated group. Limited activity of endogenous antioxidant enzymes might be due to less availability of their substrates. These enzymes provide protection from peroxidative damage in oxidative stress as antioxidant enzyme consumption is increased due to high lipid peroxidation. Production of highly reactive free radical species inhibited the activities of antioxidant enzymes (Karthikeyan et al., 2007).

T. arjuna is very important medicinal plant and results of this study has exposed that both bark and gemmmodified extracts protected the myocardial tissues of experimental animals from ISO-induced myocardial changes by regulating the levels of serum diagnostic marker enzymes and activities of antioxidant enzymes. Preventive (prior administration for 3 weeks) and curative treatment of T. arjuna bark and gemmmodified extract significantly protected from ISO induced myocardial infarction. It had strong restoration effect on antioxidant enzymes (SOD, CAT peroxidase). Recent studies suggested that free radical formation, and oxidative stress associated with occurrence of deficiency in antioxidant enzymes might be the major causative factor for the development of heart failure after myocardial infarction (Karthikeyan et al., 2007; Ojha et al., 2011).

The cardioprotective potential of T. arjuna is attributed to the presence of potent antioxidant compounds and their free radical scavenging activity. Cardioprotective potential of T. arjuna extracts in terms of curative and preventive mode can be correlated with polyphenolic fraction and antioxidant activity.

HPLC analysis revealed the presence of most important flavonol (quercetin, myricetin, kaempferol), flavanole (catechin) and phenolic acids (gallic acid, P. coumaric, ferulic acid) in significantly higher concentrations. Many studies supported the cardioprotective effect of plant polyphenols (Alyane et al., 2008). Flavonoids can show their cardioprotective potential by various mechanisms. The intake of flavonoids may stop endothelial dysfunction by increasing the vasorelaxation process leading to reduction of arterial pressure (Narayana et al., 2001; Kurosawa et al., 2005).

Some studies are reported about cardioprotective effect of T. arjuna bark (Jahan et al., 2011a). But no pervious data is available on cardioprotective effect of gummomodified extracts (young buds and shoots). It is first time reported in this study that gummomodified extract showed cardioprotective effect close to bark extract. It could be used as new cardioprotective agent. Other aspect of this study (curative effect or post-myocardial infarction treatment) of plant is also first time reported in this study and no previous reference was available on this aspect. Both gummomodified and methanolic bark extracts offered a great potential to cure the myocardial infarction induced by consecutive doses of ISO.

Antioxidant constituents of T. arjuna is mainly responsible for its superlative cardioprotective activity. Bark of T. arjuna has also reported rich in co-enzyme Q which is highly effective to prevent heart disease. All these facts confirmed strong cardioprotective potential of gummomodified and bark extracts of T. arjuna.

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