Cardioprotective effect of ethanol extract of *Acacia auriculiformis* in ischemic preconditioning

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ABSTRACT

Ischemic preconditioning (IPC) represents a powerful endogenous protective mechanism against ischemia and reperfusion (I-R) induced myocardial injury. The major mechanism of ischemia and reperfusion- induced injury is oxidative stress. Several reports have demonstrated the protective effects of antioxidants as these agents known to be prevent myocardial damage by interacting with reactive oxygen species. *Acacia auriculiformis* is an antioxidant plant rich in glucuronic acid, methylglucuronic acid, galactose, rhamnose, arabinose, tannins as well as triterpenoid saponins. Therefore, the present study is designed to investigate the effect of *Acacia auriculiformis* ethanolic extract (100 mg/kg and 200 mg/kg) on myocardial I-R injury and IPC. The magnitude of I-R injury assessed in terms of myocardial infarct size, lactate dehydrogenase and creatine kinase. *Acacia auriculiformis* (100mg/kg and 200mg/kg) ethanolic extract has been found to provide cardioprotection by significantly reducing I-R injury assessed in terms of infarct size, LDH and CK concentrations. However, *Acacia auriculiformis* (100 mg/kg and 200 mg/kg) did not modulated the protective effect offered by IPC on heart.

Key words: *Acacia auriculiformis*, Ischemic preconditioning, Ischemia reperfusion injury

INTRODUCTION

Cardiovascular diseases (CVD) are regarded a global burden on health care resources and is a prime cause of mortality worldwide mainly in developing countries. Ischemic heart disease is ranked on the top among various cardiovascular diseases and a great demand of effective treatment is required to prevent it [1,11]. Quick reperfusion is required of clogged blood vessels to protect the ischemic myocardium from sustained irreversible myocardial injury, however, paradoxal reperfusion is not without risk and associated with further cellular stress resulting in "ischemia-reperfusion (I-R) injury" [6]. The major mechanism of I-R injury during ischemia followed by reperfusion is oxidative stress [15]. Furthermore, oxidative stress increases the reactive oxygen species generation including hydroxyl radical ($OH^{\cdot}$), superoxide species (O^{2-}) and hydrogen ion (OH^{-}) and these leads to functional modifications of many cellular proteins and therefore contribute more damage to already injured myocardium [6]. Further, an experimental phenomenon known as "ischemic precondioning" a markedly protective intervention is reported to limit ischemic- reperfusion injury as well as other ischemic heart diseases in a consistent and reproductive manner [1]. When brief incident of ischemia

followed by reperfusion in myocardium have been provided to myocardium and it becomes transiently more resistant to the harmful effects of sustained ischemia, this paradoxal manifestation of myocardial adaption is termed as "ischemic preconditioning" (IPC) [18]. Several reports have demonstrated the protective effects of antioxidants as these agents known to be prevent myocardial damage by interacting with reactive oxygen species and thus preventing not only contractile dysfunction, apoptosis, necrotic cell death, arrhythmias but also change in gene expression caused by I-R injury [1, 3]. Many factors such as cardioprotective growth factors including fibroblast growth factor (FGF) [13], insulin like growth factors (IGF) [7], vascular endothelium growth factor (VEGF), transforming growth factor- β (TGF- β), urocortin [16] and cardiotropin releasing hormone family [9,12], nitric oxide and endocannabanoids have demonstrated to be liberated during short episodes of I-R and, therefore, protects the myocardial endothelium during ischemic preconditioning phenomenon.

Now-a-days researchers show more attention for developing new therapeutic strategies to sort the consequences of ischemic heart diseases in order to reduce the burden of the disease globally [5]. One such strategy is to develop the drugs with more antioxidant activity and less adverse effects [10].

Acacia auriculiformis, a multipurpose leguminous evergreen tree belonging to the family Mimosaceae and also known as Earpod Black Wattle, Australian Black Wattle, Australian Babul, Kasia and Auri [20]. *Acacia auriculiformis* has been reported to have high contents of glucuronic acid, methylglucuronic acid, galactose, rhamnose, arabinose, tannins as well as triterpenoid saponins [21]. The ethanolic extract of its funicles has been reported to have antifilarial activity on *Dirofilaria immitis* [2]. Moreover, saponins isolated from extract of its funicles showed antimicrobial and anthelmintic activity [14]. Furthermore, its bark has antimutagenic activity, chemoprotective activity and antiplasmodic activity [20,21] Its various extract have also been evaluated for its free radical scavenging and antioxidant activity [20]. Therefore, on the basis of antioxidant potential of *Acacia auriculiformis,* this study was planned to evaluate the protective effects of *Acacia auriculiformis* against myocardial I-R injury and ischemic preconditioning.

METHOD

Plant bark collection

Acacia auriculiformis bark was procured from local market of Lucknow and is authenticated from Guru Nanak Dev University Amritsar.

Extraction of plant

Acacia auriculiformis bark was shade dried. The crushed bark was loaded properly in soxhlet apparatus and continuous hot extraction was performed using ethanol as solvent until the extraction process completed. The extract was filtered out and the resultant extract was concentrated by evaporating the solvent completely. The resultant dry powder was weighed, practical amount obtained and percentage amount was calculated. The dried extract was stored properly in a desiccators till experimentation.

Qualitative examination of extract

The extract obtained was subjected to different qualitative chemical investigations for spotting various phytochemical constituents like alkanoids, flavanoids, glycosides, tannins, saponins, steroids and terpenoids etc.

Selection of animals and approval

In the present study, healthy wistar albino rats were employed. The rats were procured from NIPER, Mohali after approval from Institutional Animal Ethical Committee (Reg.no. 1407/a/11/CPCSEA). All animals were encased in group of six animals each in clean acrylic cages animals, maintained at temperature 21± 2º C under natural day and night cycle and fed with the commercial pelleted feed and water. All animals are acclimatized to environmental conditions for atleast 10 days prior to commencement of the experiment.

Table 1: Protocol Design

Groups	Dosing Schedule (n=6, p.o.)
I	Control
II	*A. auriculiformis* ethanolic extract (100mg/kg)
III	*A. auriculiformis* ethanolic extract (200 mg/kg)
IV	Ischemic Preconditioning group
V	*A. auriculiformis* ethanol extract (100mg/kg) and IPC
VI	*A. auriculiformis* ethanol extract (200mg/kg) and IPC

IPC: "Ischemic Preconditioning"

Isolated perfused rat heart

Animals were heparinised (500 IU, i.p.) nearly for 20 minutes prior the animals were sacrificed. Rat heart was quickly removed and fixed instantly on Langendorff"s apparatus. Further, heart is retrogradely perfused at a constant pressure of 80 mm of Hg with Kreb's Henseleit (KH) buffer (pH 7.4) maintained at 37^0 C. Flow rate was adjusted to 7-9 ml/minutes. The heart was enclosed in a double walled jacket to provide continuous temperature to heart, Global ischemia was employed in heart by blocking the inflow of KH solution for 30 minutes accompanied by reperfusion for 120 minutes. In case of preconditioned groups, brief four episodes of ischemia and reperfusion, each episode comprising of 5 minute occlusion and 5 minute reperfusion, were employed to produce IPC. Coronary flow rate measured and coronary effluent was collected for estimating lactate dehydregense (LDH) and creatine kinase (CK) immediately, 5 minutes and 30 minutes after reperfusion as per Ochei method. Infarct size assessment was performed by using 2, 4-dinitrophenylhydrazine method as mentioned in the study by Bhatti et al. 2008.

RESULTS

Phytochemical screening: The screening of ethanolic extract of *A. auriculiformis* was performed for detection of different phytochemical constituents and presence of carbohydrates, flavanoids, saponins, tanins and phenols was observed (Table 2 and Fig. 1a, 1b)

Table 2: Antioxidant activity

DPPH scavenging assay			***NO radical scavenging assay***		
Conc. in mcg/ml	**SAA% Scavenging Activity**	**EEA% Scavenging Activity**	**Conc. in mcg/ml)**	**SAA % Scavenging Activity**	**EEA % Scavenging Activity**
10	86.157±0.504	80.850±1.974	50	38.263 ±2.90	33.387±6.168
20	87.870±0.344	83.690±0.661	100	47.947±1.033	46.103±3.134
30	90.060±1.115	89.067±0.215	200	54.743±2.919	59.943±0.654
40	91.575±0.194	89.567±0.203	400	59.963±2.772	61.670±0.774
50	92.503±0.605	90.510±0.312	800	59.827 ± 1.553	72.130±2.244

Table % age DPPH scavenging activity, % age NO radical scavenging activity.

Values are mean ± SEM, n=6. Conc., concentration; SAA, standard ascorbic acid; EEA, ethanolic extract of *Acacia auriculiformis.* ($p<0.05$ vs SAA)

Fig. 1 (a) **Antioxidant activity of ascorbic acid standard and ethanolic extract of *Acacia auriculiformis* by DPPH scavenging assay**	**Fig. 1 (b)** **Antioxidant activity of ascorbic acid standard and ethanolic extract of *Acacia auriculiformis* by NO radical scavenging assay.**

Effect *A. auriculiformis* ethanolic extract and IPC on I-R induced myocardial infarct size: A significant increase in myocardial infarct size was observed with global ischemia and reperfusion by weight and volume method. However, IPC has been observed to attenuate the I-R injury induced increase in myocardial infarct

size significantly. Furthermore, with *A. auriculiformis* ethanolic extract (100 mg/kg), the reduction in infarct size was observed significantly when compared with control group. Moreover, *A. auriculiformis* ethanolic extract (200 mg/kg), produced more significant attenuation of infarct size induced by I-R injury when compared with control group (Fig. 2, table 3).

Group	Volume (%age)	Weight (%age)
C	22.437 ± 1.790	30.317 ± 1.552
IPC	14.530± 0.810[a]	18.523± 0.897[a]
EAAD1	15.637± 1.190[a]	26.542 ± 1.887
EAAD2	12.965± 1.138[a]	17.337± 1.262[a]

Table 3. Values are mean ± SEM, n= 6, C, control; IPC, ischemic preconditioning; EAAD1, ethanolic extract of *Acacia auriculiformis* (100mg/kg); EAAD2, ethanolic extract of *Acacia auriculiformis* (200mg/kg). ([a]p< 0.05 vs C)

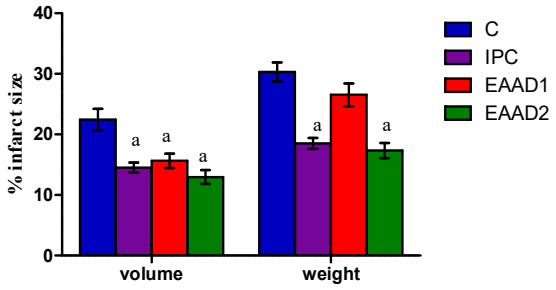

Fig. 2
Effect of EAA and IPC on I-R induced MI size.
Values are mean ± SEM (a=p<0.05 vs C)

Effect of *A. auriculiformis* ethanolic extract and IPC on I-R induced LDH release: A significant increase in LDH was observed with global ischemia for 30 minutes accompanied by reperfusion for 120 minutes. IPC and *A. auriculiformis* ethanlic extract at doses 100 mg/kg and 200 mg/kg have been observed to attenuate the I-R injury induced increase in LDH significantly when compared with control (Fig. 3, table 4).

Group	Basal (IU/L)	Immediate Reperfusion (IU/L)	30 min after Reperfusion (IU/L)
C	79.330±2.525	482.833±8.758	408.833±4.651
IPC	77.167±3.156	294.833±5.941[a]	250.167±3.842[a]
EAAD1	73.333±1.282	304.830±2.845[a]	280.167±1.905[a]
EAAD2	72.333±1.430	245.667±2.951[a]	183.000±3.916[a]

Table 4. Values are mean ± SEM, n=6. C, control; IPC, ischemic preconditioning; EAAD1, ethanolic extract of *Acacia auriculiformis* (100mg/kg); EAAD2, ethanolic extract of *Acacia auriculiformis* (200mg/kg). ([a]P<0.05 vs C).

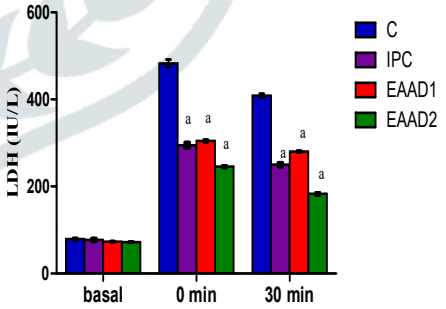

Fig. 3
Effect of EAA and IPC on I-R induced LDH release.
Values are mean ± SEM (a=p<0.05 vs C)

Effect of *A. auriculiformis* ethanolic extract and IPC on ischemia and reperfusion induced CK release: A significant increase in CK was observed with global ischemia of 30 minutes accompanied by reperfusion for 120 minutes. IPC and *A. auriculiformis* ethanolic extract (100 mg/kg and 200 mg/kg) has been observed

to attenuate the I-R injury induced increase in CK significantly when compared with control (Fig. 4 and table 5).

Group	Basal (IU/L)	5 min after reperfusion (IU/L)
C	29.825 ± 2.032	64.707 ± 1.611
IPC	28.163 ± 1.502	55.792 ± 1.695[a]
EAAD1	29.707 ± 0.655	55.682 ± 0.941[a]
EAAD2	24.930 ± 1.073	47.310 ± 2.562[a]

Table 5. Values are mean ± SEM, n=6. C, control; IPC, ischemic preconditioning; EAAD1, ethanolic extract of *Acacia auriculiformis* (100mg/kg); EAAD2, ethanolic extract of *Acacia auriculiformis* (200mg/kg). ([a]p<0.05 vs C)

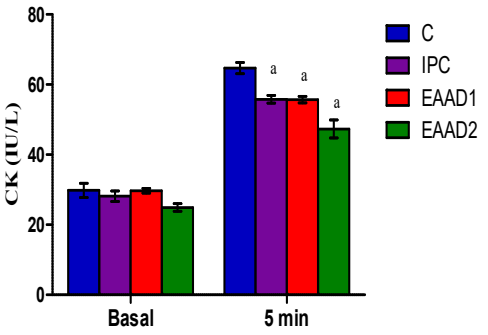

Fig. 4
Effect of EAA and IPC on I-R induced CK release.
Values are mean ± SEM (a=p<0.05 vs C)

Effect of *Acacia auriculiformis* ethanolic extract on cardioprotective effect of ischemic preconditioning: *Acacia auriculiformis* (100 mg/kg or 200 mg/kg) ethanolic extract did not showed an increase in IPC mediated decrease in MI size (Fig. 5 and table 6), LDH release (Fig. 6 and table 7) and CK release (Fig. 7 and table 8).

Group	Volume (%age)	Weight (%age)
IPC	14.530±0.810	18.523±0.897
EAAD1+IPC	13.968±0.977	17.972±1.526
EAAD2+IPC	12.035±0.336	15.59±1.776

Table 6. Values are mean ± SEM, n=6. IPC, ischemic preconditioning; EAAD1+ IPC, ischemic preconditioned group with infusion of *Acacia auriculiformis* (100 mg/kg) ethanolic extract; EAAD2 + IPC, ischemic preconditioned group with infusion of *Acacia auriculiformis* (200 mg/kg) ethanolic extract. (p<0.05 vs IPC)

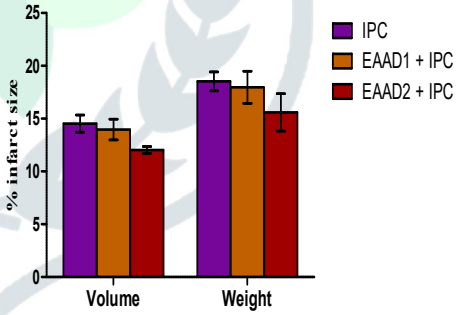

Fig. 5
Effect of EAA on IPC-mediated MI size.
Values are mean ± SEM (p<0.05 vs IPC)

Group	Basal (IU/L)	Immediate Reperfusion (IU/L)	30min after reperfusion (IU/L)
IPC	77.167± 3.156	294.833± 5.941	250.167± 3.842
EAAD1+ IPC	77.167± 1.956	286.167± 1.249	244.000± 1.693
EAAD2+ IPC	77.333± 2.472	282.667± 1.229	242.500± 1.232

Table 7. Values are mean ± SEM, n=6. IPC,ischemic preconditioning; EAAD1+ IPC, ischemic preconditioned group with infusion of *Acacia auriculiformis* (100 mg/kg) ethanolic extract; EAAD2 + IPC, ischemic preconditioned group with infusion of *Acacia auriculiformis* (200 mg/kg) ethanolic extract. ($p<0.05$ vs IPC).

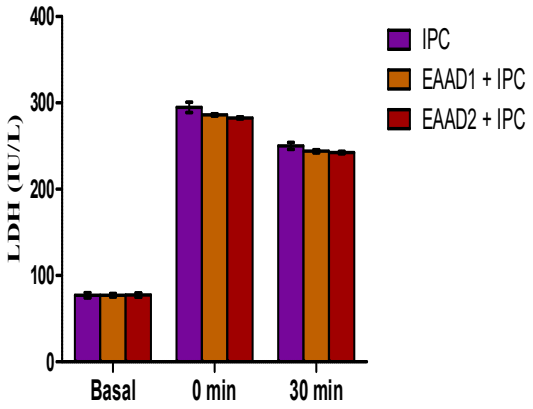

Fig. 6
Effect of EAA on IPC-mediated LDH release."
Values are mean ± SEM ($p<0.05$ vs IPC)

Group	Basal (IU/L)	5 min after reperfusion (IU/L)
IPC	28.163 ± 1.502	55.792 ± 1.125
EAAD1+IPC	28.217 ± 1.715	54.642 ± 1.482
EAAD2+IPC	28.498 ± 1.307	54.017 ± 1.420

Table 8. Values are mean ± SEM, n=6. IPC, ischemic preconditioning; EAAD1+ IPC, ischemic preconditioned group with infusion of *Acacia auriculiformis* (100 mg/kg) ethanolic extract; EAAD2 + IPC, ischemic preconditioned group with infusion of *Acacia auriculiformis* (200 mg/kg) ethanolic extract. ($p<0.05$ vs IPC).

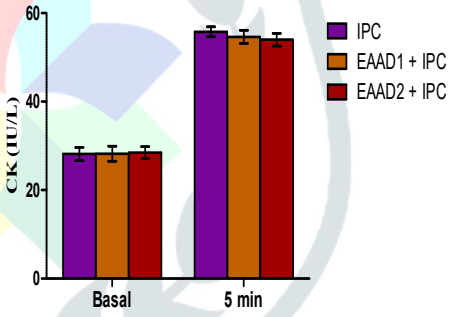

Fig. 7
Effect of EAA on IPC-mediated CK release.
Values are mean ± SEM ($p<0.05$ vs IPC)

DISCUSSION

Oxidative stress leads to generation of diverse free radicals has been responsible for I-R induced myocardial injury [6]. Diverse variety of plants having antioxidant activity has been reported to attenuate myocardial injury caused by I-R by preventing the formation of free radicals [3]. *Acacia auriculiformis*, a multipurpose leguminous evergreen tree related to Mimosaceae family and also known as Earpod, Black

Wattle, Australian Black Wattle, Australian Babul, Kasia and Auri [6]. *Acacia auriculiformis* has been reported to have high contents of glucuronic acid, methylglucuronic acid, galactose, rhamnose, arabinose, tannins as well as triterpenoid saponins [19]. The ethanolic extract of its funicles has been reported to have antifilarial activity on *Dirofilaria immitis* [2]. Moreover, saponins isolated from extract of its funicles showed antimicrobial and anthelmintic activity [14]. Its bark also has antimutagenic activity, chemoprotective activity and antiplasmodic activity [20,21]. Furthermore, antioxidant activity of its extracts has also been evaluated [19]. Therefore, on the basis of antioxidant potential of *Acacia auriculiformis,* this study was planned to find the protective role of *Acacia auriculiformis* against I-R injury and ischemic preconditioning.

The four short intervals of ischemia accompanied by reperfusion have revealed the protection of myocardial endothelium from I- R induced myocardial damage in many previous studies [1,5,8]. Likewise, in this study, with brief incident of ischemia accompanying reperfusion, a significant attenuation of liberation of enzymes LDH and CK and reduction in MI size was observed. This cardio protective outcome of IPC may be executed through the release of various cardioprotecive mediators like nitric oxide [11], bradykinin and catecholamines and varying type of growth factors [5].

Further, in this study the efforts have been made to assess the role of *Acacia auriculiformis* ethanolic extract on I-R induced myocardial damage and effect of *Acacia auriculiformis* ethanolic extract on protective effect produced by IPC. *Acacia auriculiformis* has already demonstrated to exhibit antioxidant and free radical scavenging activity [20,21]. Therefore, taking into consideration the antioxidant potential of *Acacia auriculiformis* the recent study has been designed. Global ischemia accompanied by reperfusion for 120 minutes results in enhanced oxidative stress associated with increase in various reactive oxygen species generation which may be responsible for the myocardial injury,

In this study, global I-R injury reported to enhance myocardial infarct size, LDH and CK release and this increase was observed markedly in control group. IPC has been observed to attenuate the I-R injury induced increase in MI size, LDH and CK levels significantly when compared with control The results of this study has suggested that *Acacia auriculiformis* (100mg/kg and 200mg/kg) ethanolic extract provides cardioprotection by significantly reducing ischemia and reperfusion induced injury assessed in terms of infarct size, LDH and CK concentrations when compared with control. However, *Acacia auriculiformis* (100 mg/kg and 200 mg/kg) did not modulated the protective effect offered by IPC on heart.

On the basis of findings in this study, it may be deduced that *Acacia auriculiformis* may provides cardioprotection by inhibition of generation of free radicals. However, the précised mechanism needs to be investigated.

REFERENCES

[1] Anvari MA, Imani A, Faghihi M, Karimian SM, Moghimian M, Khansari M (2012). The administration of oxytocin during early reperfusion, dose-dependently protects the isolated male rat heart against ischemia/reperfusion injury. Eur J Pharmacol 682: 137-141.

[2] Chakraborty T, Sinhababu SP, Sukul NC (1995). Antifilarial Effect of a Plant Acacia auriculiformis on Canine Dirofilariasis. Trop Med 37: 35-37.

[3] Cuzzocrea S, Riley DP, Caputi AP, Salvemini D (2001). Antioxidant Therapy: A New Pharmacological Approach in Shock, Inflammation and Ischemia/Reperfusion Injury. Pharmacol Rev 53: 135-159.

[4] Davidson SM, Rybka AE, Townsend PA (2009). The powerful cardioprotective effects of urocortin and the corticotropin releasing hormone (CRH) family. Biochem Pharmacol 77: 141-150.

[5] Detillieux KA, Cattini PA, Kardimi E (2004). Beyond angiogenesis: the cardioprotective potential of fibroblast growth factor-2. Can J Physiol Pharmacol 82: 1044-1053.

[6] Dhalla NS, Elmoselhi AB, Hata T, Makino N (2000). Status of myocardial antioxidants in ischemia–reperfusion injury. Cardiovasc Res 47: 446-456.

[7] Aguirre, G.A., Del Estal, I.M., de Cortázar Larrea, M.I.C. and Guerra, J.L.G., 2018. Partial IGF-1 deficiency is sufficient to reduce heart contractibility, angiotensin II sensibility, and alter gene expression of structural and functional cardiac proteins.

[8] Eisen A, Fisman EZ, Rubenfire M, Freimark D, McKechnie R, Tenenbaum A, Motro M, Adler Y (2004). Ischemic preconditioning: nearly two decades of research. A comprehensive review. Atherosclerosis 172: 201-210.

[9] Hausenloy DJ, Yellon DM (2009). Cardioprotective growth factors. Cardiovasc Res 83: 179-194.

[10] Krishnaiah D, Sarbatly R, Nithyanandam R (2011). A review of the antioxidant potential of medicinal plant species. Food Bioprod Process 89: 217-233.

[11] Kumphune S (2011). P38 MAPK activation in myocardial ischaemia. IJPBS 2: 107-121.

Lai HC, Liu TJ, Ting CT, Sharma PM, Wang PH (2003). Insulin-like growth factor-1 prevents loss of electrochemical gradient in cardiac muscle mitochondria via activation of PI 3 kinase/Akt pathway. Mol Cell Endocrinol 205: 99-106.

[12] Lawrence KM, Townsend PA, Davidson SM, Carroll CJ, Eaton S, Hubank M, Knight RA, Stephanou A, Latchman DS (2004). The cardioprotective effect of urocortin during ischaemia/reperfusion involves the prevention of mitochondrial damage. Biochem Biophys Res Commun 321: 479-486.

[13] Liao S, Bodmer JR, Azhar M, Newman G, Coffin JD, Doetschman T, Schultz JEJ (2010). The influence of FGF2 high molecular weight (HMW) isoforms in the development of cardiac ischemia–reperfusion injury. J Mol Cell Cardiol 48: 1245–1254.

[14] Mandal P, Babu SPS (2005). Antimicrobial activity of saponins from Acacia auriculiformis. Fitoterapia 76: 462-465.

[15] McCord JM (2000). The Evolution of Free Radicals and Oxidative Stress. Am J Med 108: 652-659.

[16] Nakase TT, Takahashi K (2010). Cardioprotective activity of urocortin by preventing caspase-independent, non-apoptotic death in cultured neonatal rat cardiomyocytes exposed to ischemia Biochem Biophys Res Commun 402: 216–221.

[17] Okokon, Jude E, Jackson O, Opara KN, Emmanuel E (2010). In vivo antimalarial activity of ethanolic leaf extract of *Acacia auriculiformis*. International Journal of Drug Development and Research 2 (3): 482-487.

[18] Quarrie R, Lee DS, Steinbaugh BSG, Cramer B, Eardahl WBS, Pfeiffer DR, Zwier JL, Crestanello JA (2012). Ischemic preconditioning preserves mitochondrial membrane potential and limits reactive oxygen species production. J Surg Res 30: 1-10.

[19] Singh NN, Ramji DP (2006). The role of transforming growth factor-b in atherosclerosis. Cytokine and Growth Factor Rev 17: 487-499.

[20] Singh R, Singh S, Kumar S, Arora S, Arora S (2006). Free radical-scavenging activity of acetone extract/fractions of Acacia auriculiformis A. Cunn. Food Chem 103: 1403-1410.

[21] Singh R, Singh S, Kumar S, Arora S, Arora S (2007). Evaluation of antioxidant potential of ethyl acetate extract/fractions of Acacia auriculiformis A. Cunn. Food Chem Toxicol 45: 1216-1223.

[22] Aguirre, G.A., Del Estal, I.M., de Cortázar Larrea, M.I.C. and Guerra, J.L.G., 2018. Partial IGF-1 deficiency is sufficient to reduce heart contractibility, angiotensin II sensibility, and alter gene expression of structural and functional cardiac proteins.

[23] Bhatti R, Singh K, Ishar MP, Singh J. (2008). The effect of *Allium sativum* on ischemic preconditioning and ischemia reperfusion induced cardiac injury. Indian J Pharmacol 40(6): 261-265.

[24] Ochei J, Kolhatkar A (2006). Medical Laboratory Science-Theory and Practice. India: McGraw Hills : 168-170.