Cardioprotective Actions of Heart Revival Suspension

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***Abstract*—**

Background: Cardiovascular diseases (CVD) are the major cause of death. Safe and alternative medicine can reduce the burden of CVD.

Objectives: Heart Revival (HR), an herbal formulation was assessed to find out its preventive and therapeutic role on CVD.

Materials and Methods: The radical scavenging action of HR was evaluated using DPPH radical. *In vitro* anti-platelet activity was measured using platelet Aggregometer. Lipid lowering action of HR was assessed in Triton WR1339 induced hypercholesterolaemic rats. Myocardial ischemic protective action was note in Isoproterenol induced rats. **Results:** HR possessed antioxidants. It dose dependently prevented ADP induced platelet aggregation. HR pre-treatment significantly lowered both cholesterol and triglycerides in blood. It also significantly protected the cardiac tissues from ischemic damage in rats as evident by lowering serum marker enzymes like lactate dehydrogenase (LDH), creatine kinase (CK), aspartate transaminase (AST) and alanine transaminase (AST). Moreover, HR pre-treatment significantly lowered lipid peroxides (LPO), but enhanced superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) in cardiac tissues. Histopathological studies also exhibited its tissue protective actions. All these findings were corroborated the cardiotonic action of HR.

Keywords—Cardiovascular diseases, myocardial infarction, lipids, platelet, ischemia, creatine kinase, herbs, rats

1. INTRODUCTION

Cardiovascular diseases (CVD) are disorders of heart blood vessels including coronary heart disease and cerebrovascular disease. In 2016, World Health Organization reported nearly 17.9 million deaths were due to CVD, representing 31% of all global deaths [1]. It includes death resulting from acute myocardial infarction (MI), stroke, heart failure, hemorrhage and sudden cardiac death [2]. The cause of heart attacks and strokes are usually the presence of a combination of risk factors, such as hypertension, diabetes, hypercholesterolaemia, physical inactivity, obesity and harmful use of alcohol and tobacco [3]. *The* risk for CVD is just 20% at the age of 24, 50% at age of 45 and 90% over the age of 80 [4]. Moreover, it has been postulated that reactive oxygen species (ROS) are directly involved in CVD. Key risk factors, such as hypertension, dyslipidemia, diabetes and smoking all are associated with marked increase in vascular ROS production [5].

It is widely accepted that strategies adapted to lower hypertension play a protective role by delaying atherosclerotic lesion formation [6]. Health care providers are concerned about hyperlipidemia, elevations of fasting total cholesterol concentration because of the well established association between lipid concentrations and the risk of CVD, the leading cause of death in USA [7-8]. Platelets play a key role in arterial thrombosis underlying CVD, including MI or

stroke and therefore, platelet function is the target of drug treatment for the prevention of thrombosis [9].

In modern medicines, β-blockers are commonly used to control hypertensions, inhibitors of HMG-CoA reductases for control hypercholesterolaemia or hyperlipidemia and prostaglandins inhibitors for the prevention of thrombosis, although all these medicines have serious adverse effects [10-13]. Hence, searching of new and safe medicines for the preventions and management of CVD is burning issue.

Some herbal derivates (aspirin, digoxin, codeine, morphine, reserpine, quinine, artemsinin, taxol, vincristine etc.) have become the mainstay of pharmacology [14]. Medicinal plants have been observed to possess numerous activities with regard to CVD *viz.* antiplatelet, hypolipidemic, anti-inflammatory and hypotensive actions [15-18]. On the basis of traditional and other scientific information, a new formulation, Heart Revival (suspension) has been developed with standardized extracts of *Terminalia arjuna* (stem bark), *Punica granatum* (seed), *Allium sativum* (bulb), *Tinospora cordifolia* (stem) and *Emblica officinalis* (fruit). *T. arjuna* has been reported for decreasing arterial blood pressure, lowering serum cholesterol and decreasing anginal frequency in human [19]. Allicin, active constituent of *A. sativum* has also been reported for antihypertensive, hypolipidaemic, hypocholesterolemic and anti-thrombotic effects [20-21]. *E. officinalis* reported for therapeutic roles on hyperlipidemia, hypertension, myocardial and endothelial function, cardiac specific antioxidants, and coagulation factors [22]. *P. granatum* seeds have polyphenolic compounds to decrease the infarct size in ischemic animals [23]. *T. cordifolia* have antioxidant, anticoagulant, platelet antiaggregatory and hypolipidaemic activities [24]. Nevertheless, all the ingredients present in Heart Revival have supported for helpfulness in the treatment of CVD, but till date, there is no valid statement of their synergistic action in combination and therefore, the present study was undertaken to confirm its therapeutic efficacies in the management of CVD in preclinical set up.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents: Fine chemicals like 1,1-diphenyl-2-picrylhydrazyl (DPPH), adenine dinucleotide phosphate (ADP), Isoproterenol and isooctylpolyoxyethylene phenol (Triton WR1339) were purchased from Sigma Aldrich (USA) and other common reagents were AR/GR grade. The biological kits of commercial grade were used in ready form.

2.2 Animals: Wistar rats were used following the recommended guidelines for the care and use of the animals [25]. Briefly, room temperature was maintained at 23±2°C, humidity between 40 and 60% and light cycle 12:12h. The animals were fed supplementary pallets for rodents and purified water *ad libitum*. The prior permission from Institutional Animal Ethic Committee has also been obtained.

2.3 Test Drug: Heart Revival (HR) was prepared and supplied by M/s Health Reactive, Kota, India. HR contained active extracts of *T. arjuna* (60 mg), *P. granatum* (60 mg), *A. sativum* (30 mg), *T. cordifolia* (30 mg) and *E. officinalis* (20 mg) in suspension form with natural base materials. HR was used in aqueous form for conducting the experiments.

2.4 Antioxidant Potentiality: The scavenging activity of HR was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals [26]. In brief, 2.5 ml of HR at different known concentrations (1-100 mg/ml) was mixed with 1 ml of 0.3 mM DPPH solution and allowed to stand in dark for 30 minutes. The absorbances were measured against blank at 518 nm (Pharmacia

Biotech Ultrospec 2000, USA) and IC_{50} value of HR was determined.

2.5 Platelet Aggregation: Citrated healthy human blood was centrifuged and the platelet rich plasma (PRP) was carefully removed. Platelet poor plasma (PPP) was obtained by centrifugation of the residual blood sample at high speed. The platelet count was adjusted to 2-3x10^5 per mm^3 by diluting PRP with normal saline. The test substance, HR was mixed with PRP at different concentrations (1-100 mg/ml) and incubated at 37°C for 30 min. Then, ADP (20 mM) was added in the incubation mixture and the aggregation was observed against PPP mixed with equal volume of supernatant of same test samples. The optical density due to platelet aggregation was recorded in CHRONOLOG Optical Aggregometer (Model 490, USA). The percent inhibition was compared to ADP control [27-28].

2.6 Lipid Lowering Action: Male Wistar rats (200-250 g) were segregated into following 3 groups of 6 animals each. Group I: received distilled water (0.2 ml/100 g body weight) orally as a vehicle for 7 days; Group II: received distilled water (0.2 ml/100 g body weight) orally as a vehicle for 7 days and at day 7, Triton WR1339 was injected intravenously (200 mg/kg); Group III: pre-treated with HR orally at the dose of 200 mg/kg once daily for seven days and at day 7, Triton WR1339 was administered intravenously (200 mg/kg) [29]. The therapeutic dose of HR was selected on the basis of pilot study. Forty four hours after injection, blood was collected from the left heart under deep anaesthesia (Ketamin HCl, 80 mg/kg, i.p). Total cholesterol and triglycerides in serum were determined spectrophotometrically [30].

2.7 Ischemia Protection: Male Wistar albino rats were grouped into 3 groups of six animals each: Group I: received distilled water (0.2 ml/100 g body weight) orally for 7 days; Group II: received distilled water (0.2 ml/100 g body weight) orally for 7 days, while Isoproterenol (ISO) was administered subcutaneously at the dose of 20 mg/100 g at day 5 and day 6; Group III: pre-treated with HR orally at the dose of 200 mg/kg once daily for seven days and ISO was administered as mentioned in Group II. 48h after the first injection of ISO, all animals were sacrificed under deep anaesthesia and blood was withdrawn from the left heart. Levels of serum lactate dehydrogenase (LDH), creatine kinase (CK), aspartate transaminase (AST) and alanine transaminase (AST) were estimated spectrophotometrically [23,31]. The heart was removed and washed in normal saline. Small portion of myocardium was homogenized in 0.15 M KCl in ice cold condition and centrifuged at 2500 g. The aliquots were taken for assessing lipid peroxides (LPO) [32], superoxide dismutase (SOD) [33], catalase (CAT) [34], reduced glutathione (GSH) [35] and protein [36]. Histological evaluation was done on lower portion of myocardium. The stained myocardium was examined under microscope at high magnification (400X).

2.8 Statistical Analysis: The data were expressed as mean ± SEM. The differences between groups were statistically analyzed by t-test using software (spss v20, IBM, USA). The significance level was less than 0.05.

3. RESULTS

3.1 Antioxidant Potentiality: HR showed promising DPPH scavenging activity. The IC_{50} value of HR was 5.3763 mg/ml (Table 1).

3.2 Platelet Aggregation: The results of platelet aggregation assays are shown in Table 2. The IC_{50} value of HR for the inhibition of ADP-induced platelet aggregation was found to be 10.2 mg/ml.

Table 1. DPPH scavenging activity	
HR (mg/ml)	Percent Inhibition
2.5	34.9±0.014
5.0	46.5±0.019
10.0	62.1±0.042
20.0	74.2±0.035
40.0	83.6±0.052
N=5; Mean ± SEM; IC_{50} value= 5.3763mg/ml	

Table 2. Platelet aggregation in human blood		
	Dose (mg/ml)	Platelet Aggregation inhibition (%)
ADP	20 nM	100% aggregation
ADP + HR	2.5	16.3±0.002*
ADP + HR	5.0	29.1±0.004*
ADP + HR	10.0	48.7±0.001*
ADP + HR	20	73.2±0.008*
N=5; Mean ± SEM; *p<0.05; IC_{50} value= 10.2 mg/ml		

Table 3. Triton induced hyperlipidemia in rats			
	Normal	Triton	Triton + HR
Total Cholesterol (mg/dl)	73.8±5.32	294.1±16.25(a)* [+298.5]	158.6±21.4(b)* [-46.0]
Triglycerides (mg/dl)	65.5±4.06	322.9±18.60(a)* [+392.9]	217.4±28.35(b)* [-32.6]
N=6; Mean ± SEM; *p<0.05; (a) normal vs. Triton; (b) Triton vs. Triton + HR; percent change in parenthesis			

3.3 Lipid Lowering Action: Total cholesterol and triglycerides were significantly increased in Triton WR1339 injected rats than normal control. Triton increased blood cholesterol 289.5% and triglycerides 392.9% within 2 days. HR (200 mg/kg) significantly lowered the blood cholesterol 46% and triglycerides 32.6% compared to Triton control (Table 3).

3.4 Ischemia Protection: All serum enzymes were markedly enhanced, but reversed in myocardial endogenous antioxidants in ISO control rats than normal control. CK was elevated 245%, LDH 103.3%, AST 176.3% and ALT 150.8%. Moreover, LPO was enhanced to198.5%, but SOD, CAT and GSH were lowered to 71.1%, 74.4% and GSH 98.9% respectively in myocardium. HR pre-treatment, not only significantly down regulated the levels of serum CK (-50%), LDH (-44.3%), AST (-55.4%) and ALT (-48.9%) and tissue LPO (-44%) but also elevated SOD (154%), CAT (133%) and GSH (146.7%). Confluent necrosis of cardiac muscle fibre with infiltration of red blood cells leading to impairment of membrane structural and functional integrity was noted in ISO treated myocardium, whereas HR prevented the degeneration of myofibriller tissues and infiltration of leukocytes into myocardium (Fig. 1).

4. DISCUSSIONS

CVD is estimated to be the leading cause of cardiovascular morbidity and mortality worldwide. The Indian subcontinent, including India, Bangladesh, Pakistan, Sri Lanka and Nepal has among the highest rates of CVD [37]. Urbanization is characterized by a marked increase in the intake of energy-dense foods, a decrease in physical activity and a heightened level of psychological stress, all of which promote the development of dysglycemia, hypertension and dyslipidemia [2]. Considering the huge burden of CVD management, there has been a continuous attempt to develop drugs that may delay the development and halt the progress of the disease. In this regards, there are great potential for identifying outstanding herbal medicinal components. Indian traditional medicinal (Ayurvedic) remedies for CVD are usually mixed formulation containing cholesterol lowering herbs in combination with anti-hypertensive, anti-thrombotic, anti-inflammatory, immune modulatory, anxiolytic and antioxidant agents [38]. The test formulation, Heart Revival is prepared with active extracts of *Terminalia arjuna*, *Punica granatum*, *Allium sativum*, *Tinospora cordifolia* and *Emblica officinalis.* There are ample clinical evidences of their beneficial effects on risks of CVD.

Endothelial dysfunction, atherosclerosis, hypertension, heart failure, myocardial ischemia – all are occurred due to oxidative stress or more accurately due to over production of ROS [5,31]. Detoxification of ROS by antioxidants are therefore affords protection against such dysfunction. The individual ingredients of HR, namely, *T. arjuna*, *P. granatum*, *A. sativum*, *T. cordifolia* and *E. officinalis*

Table 4. Health Revival on Isoproterenol induced ischemia in rats

	Normal	Isoproterenol (ISO)	ISO + HR
Serum LDH (U/L)	174.3±8.09	354.4±16.27(a)* [+103.3]	197.4±12.38(b)* [-44.3]
Serum CK (U/L)	84.7±3.75	295.7±8.12(a)* [+245]	144.9±6.18(b)* [-50.9]
Serum AST (U/L)	38.4±1.18	106.1±2.06(a)* [176.3]	47.3±2.59(b)* [-55.4]
Serum ALT (U/L)	46.2±1.20	115.9±2.30(a)* [+150.8]	59.2±2.07(b)* [-48.9]
Tissue LPO (nM MDA/mg protein)	0.69±0.02	2.06±0.08(a)* [+198.5]	1.15±0.06(b)* [-44.1]
Tissue SOD (U/mg protein)	1.28±0.01	0.37±.04(a)* [-71.1]	0.94±0.05(b)* +154]
Tissue catalase (nM H_2O_2 / min / mg protein)	1.76±0.04	0.45±0.07(a)* [-74.4]	1.05±0.03(b)* [+133]
Tissue glutathione (µM/mg protein)	85.2±4.38	27.6±2.70(a)* [-98.9]	68.1±3.92(b)* [+146.7]

N=6; Mean ± SEM; *p<0.05; (a) normal vs. ISO; (b) ISO vs. HR; percent change in parenthesis

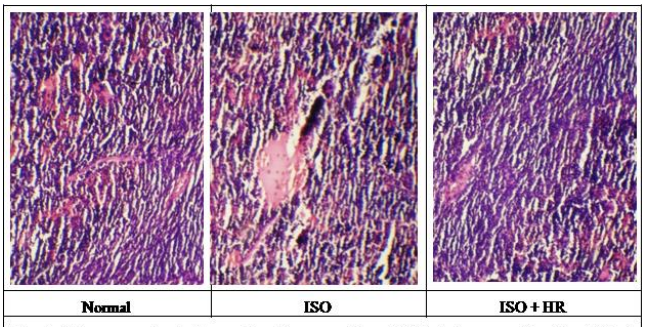

Fig. 1: Microscopic photographs of myocardium (H/E stain, magnification 400x)

have been known for their antioxidant properties. In this study, HR exhibited strong antioxidant and ROS protective action and thereby it may be useful in the preventive management of CVD. Medicinal plants with antiplatelet and anticoagulant activities had been reviewed. In this study, HR dose dependently inhibited human platelet aggregation induced by ADP. Prostaglandins (PGs), especially PGI_2 from the vascular endothelium and TXA_2 from the platelets, have a very important function in modulating platelet aggregation [9,13]. The enzyme involved in the synthesis of both the PGs is cyclooxygenase (COX) and inhibition of COX by the plant derived products may be the solution [15,31]. The anti-platelet effects of Arjunolic acid from *T. arjuna* and Allicin of *A. sativum* have been well documented [39-40]. HR has the ability to reduce platelet aggregation induced by ADP and therefore, it may it has the therapeutic role in the hypertensive patients.

Hyperlipidemia is characterized by elevated serum total cholesterol and low density and very low density lipoprotein cholesterol and triglycerides and consider to leading cause in development of arthrosclerosis and ischemic heart disease [7]. Currently available hypolipidemics drugs have been associated with number of side effects, like hyperuricemia, diarrhoea, nausea, gastric irritation, flushing, dry skin and abnormal liver function [12,30]. Injections of Triton WR1339 lead to a rapid increase in cholesterol and triglycerides in the blood and therefore is used for screening of hypolipidemics drugs. In this study, total cholesterol and triglycerides levels were significantly increased in Triton WR1339 injected animals whilst, HR pre-treatment reversed these conditions. Earlier reports marked that *T. arjuna* and *A. sativum* have promising lipid lowering actions [19,21]. It has been presumed that the hypolipidemic effect of HR may be due to an increased catabolism of cholesterol into bile acids.

Myocardial infarction (MI), the most deadly sequel among ischemic heart diseases is invariably followed by several biochemical alterations such as lipid peroxidation, free radical damage, hyperglycaemia, hyperlipidemia, hypertension etc. leading to qualitative and quantitative alterations of myocardium [3]. The damage of myocardial cell induced by cycles of ischemia and reperfusion may be due, in part to the generation of toxic ROS such as superoxide radical, hydrogen peroxide and hydroxyl radicals [5]. Isoproterenolol (ISO), systemic catecholamine and β-

adrenergic agonist, has been found to cause a severe stress in the myocardium resulting in infarct like necrosis of the heart muscle and generates free radicals to stimulate lipid peroxidation, which is causative factor for irreversible damage to the myocardial membrane in experimental myocardial infarction [29]. The serum enzymes AST, ALT, CK, LDH and endogenous antioxidants SOD, CAT, GSH and lipid peroxides are usually measure to detect cardiac injury during MI. Earlier studies reported that *T. arjuna*, *A. sativum*, *E. officinalis*, *P. granatum* and *T. cordifolia* have protective role in ISO-induced MI in animals [19,21-24]. In this study, HR pre-treatment significantly attenuated ISO-induced elevations of serum marker enzymes CK (-50%), LDH (-44.3%), AST (-55.4%) and ALT (-48.9%), while, alternatively elevated the endogenous antioxidants. Histopathological observations revealed that Heart Revival prevented the degeneration of myofibriller tissues and infiltration of leukocytes in the myocardial infarction. Actually, the present results indicated that the rat pre-treated with Heart Revival was significantly protected from myocardial damage caused by generation of free radicals. Pharmacological augmentation of endogenous myocardial antioxidants has been identified as a promising therapeutic approach in disease associated with increased oxidative stress.

From considering the above observations, it is therefore concluded that the therapeutic efficacy of Heart Revival may be due to its free radical quenching, antioxidant, antiplatelet, antihyperlipidemic and cardiotonic properties and may be helpful in the management of cardiovascular disease such as hypertension, hyperlipidemia, thrombotic disorders and myocardial ischemia.

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REFERENCES

[1] World Health Organization. Report: Global health estimates 2015: Deaths by cause, age, sex, by country and by region, 2000‑2015, Geneva: Switzerland, 2016.

[2] K.A. Hicks, H.M. James Hung, K.W. Mahaffey, R. Mehran, S.E. Nissen and N.L. Stockbridge et al., Standardized definitions for cardiovascular and stroke endpoint events in Clinical trials, Draft Definitions for CDISC August 20, 2014.

[3] D.C. Goff Jr, D.M Lloyd-Jones, G. Bennett, S. Coady, R.B D'Agostino and R. Gibbons et al., 2013 ACC/AHA Guideline on the assessment of cardiovascular risk: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines, Circulation, 2014, 129:S49–S73.

[4] American Heart Association, Cardiovascular disease: A costly burden for America - projection through 2035, Report No. 1/17DS11775, 2011.

[5] S. Vijaya Lakshmi, G. Padmaja, P. Kupppusamy and V.K. Kutala, Oxidative stress in cardiovascular disease, Indian J. Exp. Biol., 2009, 46: 421-440.

[6] A.I. Tropeano, N. Saleh, N. Hawajri, I. Macquin-Mavier and P. Maison, Do all antihypertensive drugs improve carotid intima-media thickness? A network meta-analysis of randomized controlled trials, Fundam. Clin. Pharmacol., 2011, 25:395-404.

[7] R.H. Nelson, Hyperlipidemia as a risk factor for cardiovascular disease, Prim. Care., 2013, 40:195-211.

[8] N.G. Børge and A. Varbo. Triglycerides and cardiovascular disease, Lancet, 2014, 384: 626-635.

[9] S.G. Chu, R.C. Becker, P.B Berger, D.L. Bhatt, J.W. Eikelboom and B. Konkle et al., Mean platelet volume as a predictor of cardiovascular risk: a systematic review and meta-analysis, J. Thromb. Haemost., 2010, 8:148-156.

[10] A. Milane, J. Abdallah, K. Roy, G. Khazen, M. Ghassibe-Sabbagh and A. K. Salloum et al., Association of hypertension with coronary artery disease onset in the Lebanese population, Springer Plus, 2014, 3:533.

[11] Endo A, The discovery and development of HMG-CoA reductases inhibitors, J. Lipid Res., 1992, 33:1569-82.

[12] N. Sattar, D. Preiss , H.M. Murray, P. Welsh, B.M. Buckley and A.J. de Craen, et al., Statins and risk of incident diabetes: a collaborative meta-analysis of randomized statin trials, The Lancet, 2010, 375:735-742.

[13] C. Patroo, F. Bachmann, C. Baigent, C. Bode, C. Raffaele and B. Charbonnier et al., European Society of Cardiology Expert consensus documents on the use of antiplatelet agents, The task force on the use of antiplatelet agents with atherosclerotic disease of the European Society of Cardiology, Eur. Heart J., 2004, 25:166-181.

[14] K. Kohil, M. Gupta, S. Tejwani and D. Robertson, Contemporary perspectives on clinical pharmacotherapeutics. Elsevier, Noida, 2006.

[15] K. Kim and Kwang-Il Park, A review of antiplatelet activity of traditional medicinal herbs on integrative medicine studies, Evidence-Based Complementary and Alternative Medicine, 2019, Article ID 7125162, 18 pages https://doi.org/10.1155/2019/7125162

[16] P. Kumar, U. Bhandari and S. Jamadagni, Fenugreek seed extract inhibit fat accumulation and ameliorates dyslipidemia in high fat

diet-induced obese rats, BioMed Research International, 2014, Article ID 606021, 11 pages http://dx.doi.org/10.1155/2014/60602.

[17] D.K Das, Natural products and healthy heart, J. Cardiovasc. Pharmacol., 2009, 54: 366-368.

[18] K. Magyar, R. Halmosi, A. Palfi, G. Feher, L. Czopf and A. Fulopb, Cardioprotection by resveratrol: A human clinical trial in patients with stable coronary artery disease, Clin. Hemorheol Microcirc., 2012, 50:179-187.

[19] R. Chander, K. Singh, A.K. Khanna, S.M. Kaul, A. Puri and R. Saxena *et al.*, Antidyslipidemic and antioxidant activities of different fractions of *Terminalia arjuna* stem bark. Indian J. Clin. Biochem., 2004, 19:141-148.

[20] H. Amagase, Clarifying the real bioactive constituents of garlic. J. Nutrition, 2005, 136:S716-725.

[21] A. Adhikari, R. Indu, T.K. Sur, D. Banerjee and A.K. Das, Is garlic safe remedy: An overlook herb drug interaction, Am. J. Phytomed. Clin. Ther., 2015, 3:622-632.

[22] S. Ojha, M. Golechha, S. Kumari and D.S. Arya, Protective effect of *Emblica officinalis* (amla) on isoproterenol-induced cardiotoxicity in rats, Toxicol. Ind. Health, 2012, 28:399- 411.

[23] R.N. Jadeja, M.C. Thounaojam, D.K. Patel, R.V. Devkar and A.V. Ramachandran, Pomegranate (*Punica granatum* L) juice supplementation attenuates isoproterenol induced cardiac necrosis in rats, Cariovascular Toxicol., 2010, 10:174-180.

[24] D. Singh and P.K. Chaudhuri,. Chemistry and pharmacology of *Tinospora cordifolia*., Natural Product Communications, 2017,12:299-308.

[25] National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academies Press (US), 2011.

[26] T.K. Sur, A. Hazra, A.K and Hazra, D. Bhattacharyya, Antioxidant and hepatoprotective properties of Indian Sunderban mangrove *Bruguiera gymnorrhiza* L. leave, J. Basic Clin. Pharm., 2016, 7:75-79.

[27] G.V.R. Born, Adenosin dipohphate as a mediator of platelet aggregation in vivo: an editoral view, Circulation, 1985, 72:741-746.

[28] T.K. Sur, T.K. Biswas, L. Ali and B. Mukherjee, Anti-inflammatory and anti-platelet aggregation activities of human placental extract, Acta Pharmacol. Sin., 2003, 24:187-92.

[29] H. Hyashi, S. Niinobe, Y. Mutsumoto and T. Suga, Effects of Triton WR-1339 of lipoprotein lipolytic activity and lipid content of rat liver lysosomes, J. Biochem., 1981, 89: 573-579.

[30] R. Indu, A. Adhikari, P. Basak, D. Banerjee, T.K. Sur, Evaluation of drug-drug interaction on concomitant administration of anti-diabetes and hypolipidemics in *in vivo* models, Int. J. Biosci., 2019, 14:317-327.

[31] T.K. Sur, B. Auddy and D. Bhattacharyya, Effects of Body Revival (herbal formulation) on human platelet aggregation and myocardial ischemia in rats, J. Chinese Integrative Med., 2011, 9:746-751.

[32] J.A. Burge and S.D. Aust, Microsomal lipid peroxidation, Methods Enzymol., 1988, 52:302-310.

[33] S. Kaul and N.K. Kapoor, Reversal changes of lipid peroxide, xanthine oxidase and superoxide dismutase by Cardioprotective drugs in isoproterenol induced myocardial necrosis in rats. Indian J. Exp. Biol., 1989, 27: 625-627.

[34] A.K. Sinha, Colorimetric assay of catalase, Anal. Biochem., 1972, 47:389-394.

[35] M.S, Moron, J.W. Depierre and B. Mannervik, Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver, Biochem. Biophys. Acta., 1979, 582:67-78.

[36] O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem., 1951,193: 265-275.

[37] E.J. Benjamin, S.S. Virani, C.W. Callaway, A.M. Chamberlain, A.R. Chang and S. Cheng et al. On behalf of the American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics, 2018 update: a report from the American Heart Association.

[38] Mishra LC. Scientific basis of Ayurvedic therapies. CRC Press, New York, 2004.

[39] S. Dwivedi and D. Chopra, Revisiting *Terminalia arjuna* – An ancient cardiovascular drug, J. Tradit Complement Med., 2014, 4:224-231.

[40] Ackermann RT, Garlic shows promise for improving some cardiovascular risk factors, Arch. Intern. Med., 2001, 161:813-824.