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Research Article
Chrysin Ameliorates the Lipid Profiles in N$^{\omega}$-nitro-l-arginine-methylester-induced Hypertensive Rats

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Abstract

Background: It is investigated to deduct the action of chrysin on the cardiovascular risk of N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rats. The L-NAME is a non-specific Nitric Oxide (NO) synthase inhibitor, commonly used for the induction of NO-deficient hypertension. **Materials and Methods:** The L-NAME (40 mg kg^{-1} b.wt.) was dissolved in drinking water and was given to rats at an interval of 24 h for 8 weeks. Chrysin were administered orally once in a day in the morning for 4 weeks. The compound was suspended in 2% dimethyl sulfoxide solution and fed by incubation. After 8th week morning the animals were sacrificed by cervical dislocation and done with lipid profiles parameters. **Results:** Administration of L-NAME significantly increased the mean arterial pressure and heart rate compared to control rats, while treatment with chrysin significantly reduced the mean arterial pressure and heart rate compared to hypertensive rats. When L-NAME-induced hypertensive rats compared with the control, an extend sign were seen in the factors such as the concentrations of plasma, tissue (liver and kidney) lipids, lipoproteins and hepatic marker enzymes and a decrement were noted in the concentration of high-density lipoprotein cholesterol. A recent of hyperlipidemia resulted from oral prescription of chrysin. **Conclusion:** Thus, chrysin gives protection against hyperlipidemic and hepatic damage in rats with L-NAME induced hypertension.

Key words: Cardiovascular diseases, nitric oxide, triglycerides, total cholesterol

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The Principal factor causing cardiovascular diseases (CVD) in universe is hypertension which spells for humanity and sickness[1]. It pretends 600 million more populations arising 13% of deaths in whole and it is estimated that approximately 29% of the mankind affected by 2025[2]. Endothelial dysfunction resulting from a reduction in Nitric Oxide (NO) bioavailability plays a key role in the pathogenesis of CVD. Chronic administration of NO synthase inhibitor, L-NAME, induces hypertension and endothelial dysfunction that reproduces many aspects of the pathological conditions related to the hypertension, one of the most important risk factors for CVD[3]. The L-NAME is a nonspecific inhibitor of all three NO synthase (NOS) isoforms (including neuronal nitric oxide synthase, inducible nitric oxide synthase and endothelial nitric oxide synthase) and causes an increase of Blood Pressure (BP) in a dose dependent manner when administered to the experimental animals[4]. The blockage of NOS by L-NAME seems to be involved in lipid metabolism alterations: Increases serum cholesterol levels in rats and impairs endothelium function in hypercholesterolemia rabbits[5] in which it also causes atherosclerosis[6]. The National Health and Nutrition Examination Survey have shown a strong linear relationship between systolic/diastolic blood pressures and Body Mass Index (BMI)[7].

The plant polyphenolic compounds namely flavonoids which constitutes flavanols, flavones and flavones of which chrysin (5,7-dihydroxy flavones structure shown in Fig. 1) is a natural flavones in flowers such as the blue passion flower (*Passiflora caerulea*) and the Indian trumpet flower, also in edible items such as mushroom[8], honey and propolis[9]. The properties of chrysin have been found it as antioxidant[10], anti-allergic[11], anti-inflammatory[12], anti-cancer[13], antiestrogenic[14]), anxiolytic[15] and antihypertension[16] one.

Chrysin is also said to have tyrosinase inhibitory activity[17] and moderate aromatase inhibitory activity[18]. It inhibits estradiol-induced DNA synthesis[19]. Numbers of reactions are being performed to improve its biological activity[20]. The C-iso prenylated hydrophobic derivatives of chrysin are potential P-glycoprotein modulators in tumor cells[21]. In recent report of our study chrysin has been found exert antihypertensive effects; reduce hepatic renal damages and endothelial dysfunction in L-NAME induced hypertensive rats[22]. But still chrysin lags on investigating its antihyperlipidimic activity on L-NAME induced hypertensive rats. In this proposed study it is important to investigate the preventive effects of chrysin on BP, plasma and tissue lipid profiles in L-NAME-induced hypertensive rats.

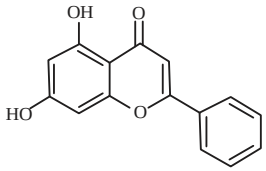

Fig. 1: Chemical structure of chrysin (5,7 dihydroxyflavone)

MATERIALS AND METHODS

Chemicals: Chrysin and L-NAME was shipped from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used in this study were of analytical grade and obtained from E-Merck or HIMEDIA, Mumbai, India.

Animals: Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee of Bharathidasan University (Registration No: 418/01/a/date 04.06.2001) and animals were intake of care in accordance with the Indian National Law on Animal Care and Use. Male Wistar rats (180-220 g) shipped from the Indian Institute of Science, Bangalore, India were housed in plastic cages with filter tops under controlled conditions of a 12 h light-dark cycle, 50% humidity and temperature of 28°C. The standard pellet diet (Lipton Lever Mumbai, India) and water *ad libitum* (BDU/IAEC63/09.04.2013) were consumed by all the rats.

Induction of L-NAME-induced hypertension: Dissolved in drinking water L -NAME (40 mg kg^{-1} b.wt.) and was given to rats at an interval of 24 h for 8 weeks. Mean arterial blood pressure (MAP) was measured using tail cuff method. The MAP measurements were performed at the time of 1-8 weeks.

Blood pressure measurements: Using tail-cuff method (IITC, model 31, Woodland Hills, CA, USA) the Mean Arterial Pressure (MAP) and Heart Rate (HR) were determined. The animals were placed in a heated chamber at an ambient temperature of 30-34°C for 15 min and from each animal one to nine BP values were recorded. The lowest three readings were averaged to obtain a mean BP. All recordings and data analyses were done using a computerized data acquisition system and software.

Study design: Animals were divided into four groups of 6 rats each and all were fed the standard pellet diet.

Rats in groups are given below:

Group I : Control
Group II : Control+chrysin (25 mg kg^{-1} b.wt.) after 4th week
Group III : L-NAME induced hypertension (40 mg kg^{-1} b.wt.)
Group IV : L-NAME induced hypertension+chrysin (25 mg kg^{-1} b.wt.)

Chrysin were administered orally once in a day in the morning for 4 weeks. The compound was suspended in 2% dimethyl sulfoxide solution and fed by intubation. After 8th week morning the animals were sacrificed by cervical dislocation. After the 8th week morning the animals were sacrificed by cervical dislocation. The blood was collected in clean dry test tubes and allowed to coagulate at ambient temperature for 30 min. Serum was separated by centrifugation at 175×g for 10 min. The blood, collected in a heparinized centrifuge tube, was centrifuged at 175×g for 10 min and the plasma was separated by aspiration. After the separation of plasma, the buffy coat, enriched in white cells, was removed and the remaining erythrocytes were washed three times with physiological saline. A known volume of erythrocyte was lysed with hypotonic phosphate buffer at pH 7.4. The hemolysate was separated by centrifugation at 290×g for 10 min and the supernatant was used for various estimations. The liver, heart and kidney were immediately removed and washed in ice-cold saline to remove the blood. The tissues were sliced and homogenized in 0.1 M tris-HCl buffer (pH 7.0). The homogenates were centrifuged at 48×g for 10 min at 0°C in a cold centrifuge. The supernatants were separated and used for the determination of various parameters.

Biochemical parameters and lipid profile markers: The cholesterol content was estimated by the method of Zlatkis *et al.*[23]. Triglycerides were estimated by the method of Foster and Dunn[24]. Free fatty acids were estimated by the method of Falholt *et al.*[25]. Phospholipids content was estimated by the method of Zilversmit *et al.*[26]. High density lipoprotein (HDL-C) as analyzed in the supernatant obtained after precipitation of plasma with phosphotungstic acid/Mg^{2+} by method of Nerurkar and Taskar[27]. Very Low Density Lipoprotein-Cholesterol (VLDL-C) and Low Density Lipoprotein- Cholesterol (LDL-C) fractions were calculated as follows: VLDL-C = TGs/5 and LDL-C = TC-(HDL-C+VLDL-C), respectively. The activities of serum aspartate aminotransferase (AST) and alaninie aminotransferase (ALT) were assayed by the method of Reitman and Frankel[28] and alkaline phosphatase (ALP) was assayed by the method of Kind and King[29], respectively.

Statistical analysis: Data were analyzed by one-way analysis of variance followed by a Duncan's multiple range tests using a commercially available statistics software package (SPSS for Windows, version 11.0; SPSS Inc., Chicago, IL, USA). Results were presented as Mean±Standard Deviation (SD) values of $p<0.05$ were regarded as statistically significant.

RESULTS

Table 1 indicates the effect of chrysin on MAP and HR, in control rats and L-NAME induced hypertensive rats respectively for 4 weeks. The significant increase of MAP and HR has undertaken ($p<0.05$) in L-NAME induced hypertensive rats. The chrysin supplements decreased the MAP and HR ($p<0.05$). Table 2 shows concentrations of plasma lipids (TC, FFA, TGs and PLs) were increased in hypertensive rats as compared with the control rats. Treatment with chrysin significantly ($p<0.05$) reduced the concentrations of plasma lipids.

Effect of chrysin on plasma lipoproteins (LDL-C, VLDL-C and HDL-C) in control rats and L-NAME induced hypertensive rats are illustrated in Table 3. The raised levels of LDL-C and VLDL-C and reduced level of HDL-C were observed in

Table 1: Effect of chrysin on MAP and HR in control rats and L-NAME-induced hypertensive rats

Parameters tested	Control	Control+25 mg chrysin	L-NAME	L-NAME+25 mg chrysin
MAP (mm Hg)				
Initial	86.93±3.62	87.65±3.53	92.390±3.65	90.4±3.61
2 weeks	89.60±3.25^{a}	88.50±2.38^{a}	114.64±3.96^{b}	95.6±3.24^{c}
4 weeks	88.72±3.05^{a}	86.30±3.27^{a}	130.50±3.29^{b}	96.3±3.25^{c}
Heart rate (bPm)				
Initial	354.00±3.26	358.0±6.23	364.00±6.89	362±6.72
2 weeks	358.00±3.45^{a}	355.0±5.36^{a}	412.00±6.83^{b}	369±7.23^{c}
4 weeks	366.00±3.65^{a}	360.0±6.23^{a}	434.00±9.86^{b}	378±6.52^{c}

Values are expressed as Means±SD for six rats in each group. Values not sharing a common superscript differ significantly at $p<0.05$ (Duncan's multiple range test), SD: Standard deviation, MAP: Mean arterial pressure

Table 2: Effect of chrysin on plasma cholesterol, TGs, FFA and PLs in control rats and L-NAME-induced hypertensive rats

Parameters tested (mg dL^{-1})	Control	Control+25 mg chrysin	L-NAME	L-NAME+25 mg chrysin
TC	86.14±8.82[a]	84.23±7.49[a]	186.37±15.76[b]	90.27±8.74[c]
TGs	65.37±6.01[a]	63.76±6.87[a]	171.36±16.31[b]	70.13±6.89[c]
FFAs	60.46±5.21[a]	58.37±6.31[a]	119.31±11.18[b]	64.37±6.13[c]
PLs	118.8±11.3[a]	114.30±10.7[a]	163.00±11.08[b]	124.7±11.72[c]

Values are expressed as Means±SD for six rats in each group. Values not sharing a common superscript differ significantly at p<0.05 (Duncan's multiple range test), SD: Standard deviation, TC: Total cholesterol, TGs: Triglycerides, FFA: Free fatty acids, PLs: Phospholipids

Table 3: Effect of chrysin on plasma lipoproteins in control rats and L-NAME-induced hypertensive rats

Parameters tested (mg dL^{-1})	Control	Control+25 mg chrysin	L-NAME	L-NAME+25 mg chrysin
HDL-C	46.28±4.41[a]	46.99±4.28[a]	22.74±2.65[b]	45.72±4.42[c]
VLDL-C	17.22±1.76[a]	16.85±1.50[a]	37.27±3.15[b]	18.05±1.75[c]
LDL-C	22.64±2.65[a]	20.39±1.71[a]	126.36±9.96[b]	26.50±2.57[c]

Values are expressed as Means±SD for six rats in each group. Values not sharing a common superscript differ significantly at p<0.05 (Duncan's multiple range test), SD: Standard deviation, HDL-C: High density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol

Table 4: Effect of chrysin on the concentrations of cholesterol, TGs, FFAs and PLs in the liver and kidney of control rats and L-NAME-induced hypertensive rats

Parameters tested	Control	Control+25 mg chrysin	L-NAME	L-NAME+25 mg chrysin
Liver				
TC (mg g^{-1} tissue)	4.69±0.38[a]	4.62±0.41[a]	7.19±0.76[b]	4.74±0.45[c]
TGs (mg g^{-1} tissue)	3.82±0.33[a]	3.76±0.32[a]	7.93±0.64[b]	3.99±0.37[c]
FFAs (mg g^{-1} tissue)	8.13±0.62[a]	8.04±0.76[a]	19.25±1.18[b]	8.28±0.63[c]
PLs (g g^{-1} tissue)	21.08±1.94[a]	20.98±1.86[a]	58.87±5.61[b]	21.38±2.08[c]
Kidney				
TC (mg g^{-1} tissue)	3.64±0.31[a]	3.57±0.34[a]	6.82±0.62[b]	3.78±0.36[c]
TGs (mg g^{-1} tissue)	4.79±0.41[a]	4.72±0.42[a]	7.46±0.72[b]	5.11±0.50[c]
FFAs (mg g^{-1} tissue)	3.89±0.31[a]	3.79±0.35[a]	8.01±0.78[b]	4.08±0.41[c]
PLs (g g^{-1} tissue)	15.76±1.45[a]	15.62±1.41[a]	28.43±2.16[b]	16.08±1.58[c]

Values are expressed as Means±SD for six rats in each group. Values not sharing a common superscript differ significantly at p<0.05 (Duncan's multiple range test), SD: Standard deviation, TC: Total cholesterol, TGs: Triglycerides, FFA: Free fatty acids, PLs: Phospholipids

Table 5: Effect of chrysin on hepatic function indicators of control and experimental rats

Parameters tested (IU L^{-1})	Control	Control+25 mg chrysin	L-NAME	L-NAME+25 mg chrysin
AST	64.82±6.38[a]	64.04±6.22[a]	132.74±12.52[b]	66.21±6.48[c]
ALT	28.34±2.82[a]	27.98±2.72[a]	70.81±7.05[b]	30.05±2.98[c]
ALP	79.49±7.59[a]	79.02±7.76[a]	138.63±12.98[b]	81.02±8.08[c]

Values are Means±SD for six rats. Values not sharing a common superscript differ significantly at p<0.05 (Duncan's multiple range test), SD: Standard deviation, AST: Aspartate aminotransferase, ALT: Alaninie aminotransferase, ALP: Alkaline phosphatase

hypertensive rats as compared with control rats. Oral administration of chrysin significantly (p<0.05) shows a reduced levels of plasma lipoproteins LDL-C and VLDL-C and increased the level of HDL-C. Table 4 shows the impacts of chrysin in the levels of lipids (TC, FFA, TGs and PLs) in tissues (liver and kidney) of control rats and L-NAME induced hypertensive rats. The lipid concentrations of tissue were significantly increased in hypertensive rats as compared with the control rats. Chrysin effects significantly (p<0.05) reduced the concentrations of tissue lipids.

Table 5 shows the effect of chrysin on the activities of hepatic marker enzymes such as AST, ALT and ALP of control and L-NAME induced hypertensive rats. The elevated levels of hepatic markers and activities were observed in L-NAME-induced hypertensive rats. The activities of these enzymes in the serum of L-NAME-treated rats got reduced after the chrysin oral consumption.

DISCUSSION

This study performed the investigation of the chrysin in L-NAME-induced hypertensive rats and its effects on lipid metabolism and marker enzymes. Recent results are in good agreement with other reports depicting that chronic administration of L-NAME cause's arterial hypertension in mice[30] and rats. Chronic inhibition of NO produces volume-dependent increase of BP and its physiological and pathological characteristics resemble essential hypertension[31]. In this present study MAP and HR were increased significantly in L-NAME induced hypertensive rats. The presence of high BP and hyperlipidemia is so common in hypertension in which many argument shows that the high BP itself may play a role in altering lipid metabolism, resulting in abnormalities[32]. Chrysin administration significantly decreased MAP and HR in L-NAME induced hypertensive rats.

The major site for the synthesis and metabolism of cholesterol, bile acids and phospholipids[33] is liver. Experimental animals whose NO levels were reduced by the administration of L-NAME showed increased TC and decreased HDL-C: In particular, the HDL-C and TC ratios were significantly different. It has been shown that HDL-C removes not only cholesterol but also oxidized lipids from peripheral tissue via reverse cholesterol transport, which is affected by LCAT activity[34]. It inhibits the oxidative modification of LDL-C[35] as per the recent studies. The close relationship between NO and cholesterol values found in this study in line with previous observations which reduced NO availability increases the incorporation of labeled precursors in cholesterol molecules[36]. An array of bioactive compounds yielded from the complex process LDL-C oxidation with different biological properties and the individual composition depends on the degree of LDL-C oxidation. Due to the defect in LDL-C receptor either through failure in its production or function, LDL-C concentration is increased in plasma HDL-C may be protective by reversing cholesterol transport, inhibiting the oxidation of LDL-C and by neutralizing the atherogeneic effects of oxidized LDL-C. The tremendous increase of LDL-C and VLDL-C may also cause a greater decrease of HDL-C as there is inverse relation between the concentration of VLDL-C and HDL-C. Here after the chrysin inoculation the results have showed reduced levels of plasma LDL-C, VLDL-C and increased HDL-C in L-NAME hypertensive rats. As per the clinical trials it is observed that a reduction in total LDL-C made a decent of coronary morbidity and mortality without affecting LDL-C particle size[37].

The PLs acts as vital components of biomembrane. These PLs and FFA have the quality for maintaining the cellular integrity, micro viscosity and survival[38]. Due to membrane damage caused by decreased plasma NO and increased lipid peroxidation, there were the observations of peaked levels of plasma PLs and FFA in L-NAME rats. Resuming with oxidative stress that occurs when the dynamic balance between pro-oxidant and antioxidant mechanism is impaired[39]. With the help of results of present study and previous findings, it is observed that treatment with chrysin significantly lowered the levels of plasma PLs, FFA, TC and TGs in L-NAME rats. Hence, these results provided the indication of antihyperlipidaemic activity of chrysin. The excessive/ectopic fat depositions in the liver could be due to increased fatty acid delivery from adipose tissue, increased synthesis of fatty acid via the *de novo* pathway, increased dietary fat, decreased mitochondrial β-oxidation, decreased clearance of VLDL-C particles, or all of these factors in combination[40]. An imbalance between the uptake, synthesis, oxidation and export of lipids results in excessive fat accumulation in the liver. The L-NAME is associated with accelerated lipid deposition[41]. An association between lipid abnormalities and the pathogenesis of renal disease was first suggested by Virchow[42] when he described extensive fatty metamorphosis in renal autopsy tissue obtained from patients with Bright's disease. Several reports have suggested that renal lipid accumulation, lipotoxicity is associated with the development of such renal injury[43]. In a previous study, it was demonstrated that a daily oral dose (25 mg kg^{-1}) of chrysin for 4 weeks reduced the elevated blood pressure and recover the renal damage in L-NAME induced hypertensive rats[44]. Accumulation of TGs is one of the risk factors of CVD. The mechanism of observed increase in TGs after hypertension may be due to elevated flux of fatty acids and impaired removal of VLDL from the plasma. Treatment with chrysin decreased the levels of total cholesterol, free fatty acids, triglycerides and lipoproteins in hypertensive rats.

The liver and kidney actively detoxify and handle endogenous and exogenous chemicals, making them vulnerable to injury. Disruption of liver tissue architecture and vacuolation under hypertension and NO deficiency is an indication of hepatic fatty infiltration and hepatocellular injury[45]. The AST is present in the cytoplasm as well as the mitochondrion, ALT is a cytoplasmic enzyme found in very high concentration in the liver and ALP is excreted by the liver via bile. The AST, ALT and ALP are the major hepatic marker enzymes. The elevation of hepatic markers in the serum is the result of leakage from damaged cells and therefore reflects the hepatocyte damage[46]. The activities and the levels of AST, ALT and ALP of hepatic markers were elevated in L-NAME-induced hypertensive rats. Injury to the hepatocytes alters their transport function and membrane permeability, leading to leakage of enzymes from liver cells[47] this leakage causes increased activity of the enzymes ALT, AST and ALP in serum[48]. Oral administration of chrysin significantly reduced the activities of these hepatic enzymes.

CONCLUSION

The results clearly notified that treatment with chrysin significantly reduced the BP and diminishes lipid profile in L-NAME-induced hypertensive rats. Our previous findings clearly demonstrated that chrysin increased plasma NO level in L-NAME induced hypertensive rats. For that, protective role of chrysin reduced hyperlipidemia related to the risk of L-NAME induced hypertensive rats. Furthermore, it could restore the abnormal metabolism of lipids in L-NAME induced hypertensive rats. Investigation is warranted to define the mechanism by which chrysin protects.

REFERENCES

1. Mittal, B.V. and A.K. Singh, 2010. Hypertension in the developing world: Challenges and opportunities. Am. J. Kidney Dis., 55: 590-598.
2. Vasdev, S., V.D. Gill and P.K. Singal, 2006. Modulation of oxidative stress-induced changes in hypertension and atherosclerosis by antioxidants. Exp. Clin. Cardiol., 11: 206-216.
3. Ribeiro, M.O., E. Antunes, G. de Nucci, S.M. Lovisolo and R. Zatz, 1992. Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. Hypertension, 20: 298-303.
4. Bernatova, I., O. Pechanova and F. Kristek, 1999. Mechanism of structural remodelling of the rat aorta during long-term N^G-nitro-L-arginine methyl ester treatment. Jpn. J. Pharmacol., 81: 99-106.
5. Cayatte, A.J., J.J. Palacino, K. Horten and R.A. Cohen, 1994. Chronic inhibition of nitric oxide production accelerates neointima formation and impairs endothelial function in hypercholesterolemic rabbits. Arterioscler. Thromb. Vascular Biol., 14: 753-759.
6. Naruse, K., K. Shimizu, M. Muramatsu, Y. Toki and Y. Miyazaki *et al*., 1994. Long-term inhibition of NO synthesis promotes atherosclerosis in the hypercholesterolemic rabbit thoracic aorta. PGH2 does not contribute to impaired endothelium-dependent relaxation. Arterioscler. Thromb. Vascular Biol., 14: 746-752.
7. Aneja, A., F. El-Atat, S.I. McFarlane and J.R. Sowers, 2004. Hypertension and obesity. Recent. Prog. Horm. Res., 59: 169-205.
8. Jayakumar, T., P.A. Thomas and P. Geraldine, 2009. *In-vitro* antioxidant activities of an ethanolic extract of the oyster mushroom, *Pleurotus ostreatus*. Innov. Food Sci. Emerg., 10: 228-234.
9. Williams, C.A., J.B. Harborne, M. Newman, J. Greenham and J. Eagles, 1997. Chrysin and other leaf exudate flavonoids in the genus *Pelargonium*. Phytochemistry, 46: 1349-1353.
10. Veerappan, R. and S. Rajagopal, 2015. Chrysin enhances antioxidants and oxidative stress in L-NAME-induced hypertensive rats. Int. J. Nutr. Pharmacol. Neurol. Dis., 5: 20-27.
11. Pearce, F.L., A.D. Befus and J. Bienenstock, 1984. Mucosal mast cells. III. Effect of quercetin and other flavonoids on antigen-induced histamine secretion from rat intestinal mast cells. J. Allergy Clin. Immunol., 73: 819-823.
12. Fishkin, R.J. and J.T. Winslow, 1997. Endotoxin-induced reduction of social investigation by mice: Interaction with amphetamine and anti-inflammatory drugs. Psychopharmacology, 132: 335-341.
13. Habtemariam, S., 1997. Flavonoids as inhibitors or enhancers of the cytotoxicity of tumor necrosis factor-α in L-929 tumor cells. J. Nat. Prod., 60: 775-778.
14. Kao, Y.C., C. Zhou, M. Sherman, C.A. Laughton and S. Chen, 1998. Molecular basis of the inhibition of human aromatase (estrogen synthetase) by flavone and isoflavone phytoestrogens: A site-directed mutagenesis study. Environ. Health Perspect., 106: 85-92.
15. Wolfman, C., H. Viola, A. Paladini, F. Dajas and J.H. Medina, 1994. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coerulea*. Pharmacol. Biochem. Behav., 47: 1-4.
16. Malarvili, T. and R. Veerappan, 2014. Effects of chrysin on free radicals and enzymatic antioxidants in Nω-nitro-l-arginine methyl Ester: Induced hypertensive rats. Int. J. Nutr. Pharmacol. Neurol. Dis., 4: 112-117.
17. Kubo, I., I. Kinsy-Hori, S.K. Chaudari, Y. Kubo, Y. Sandoz and T. Ogura, 2000. Flavonols from *Heterotheca inuloides*: Tyrosinase inhibitory activity and structural criteria. Bioorg. Med. Chem., 8: 1749-1755.
18. Joshi, S.C., L. Strauss, S. Makela and R. Santti, 1999. Inhibition of 17β-estradiol formation by isoflavonoids and flavonoids in cultured JEG-3 cells: Search for aromatase-targeting dietary compounds. J. Med. Food, 2: 235-238.
19. Wang, C. and M.S. Kurzer, 1998. Effects of phytoestrogens on DNA synthesis in MCF 7 cells in the presence of estradiol or growth factors. Nutr. Cancer, 31: 90-100.
20. Rice-Evans, C.A. and L. Packer, 1997. Flavonoids in Health and Disease. Marcel Dekker, New York, USA., pp: 179-197.
21. Larget, R., B. Lockhartb, P. Renardc and M.A. Largeron, 2000. A convenient extension of the Wessely-Moser rearrangement for the synthesis of substituted alkylaminoflavones as neuroprotective agents *in vitro*. Bioorg. Med. Chem. Lett., 10: 835-838.
22. Ramanathan, V. and M. Thekkumalai, 2014. Role of chrysin on hepatic and renal activities of Nω-nitro-l-arginine-methylester induced hypertensive rats. Int. J. Nutr. Pharmacol. Neurol. Dis., 4: 58-63.
23. Zlatkis, K., B. Zak and A.J. Boyle, 1953. A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med., 41: 486-492.
24. Foster, L.B. and R.T. Dunn, 1973. Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. Clin. Chim. Acta, 19: 338-340.
25. Falholt, K., B. Lund and W. Falholt, 1973. An easy colorimetric micromethod for routine determination of free fatty acids in plasma. Clinica Chimica Acta, 46: 105-111.
26. Zilversmit, D.B., A.K. Davis, B. Memphis and N. Tenn, 1950. Estimation of phospholipids in biological fluids. J. Lab. Clin. Med., 35: 155-160.
27. Nerurkar, S.V. and S.P. Taskar, 1985. Lipoprotein fractionation by precipitation (a comparison of two methods). J. Postgrad. Med., 31: 89-94.
28. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.

29. Kind, P.R. and E.J. King, 1954. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J. Clin. Pathol., 7: 322-326.
30. Mattson, D.L., 1998. Long-term measurement of arterial blood pressure in conscious mice. Am. J. Physiol., 274: R564-R570.
31. Attia, D.M., A.M.G. Verhagen, E.S.G. Stroes, E.E. van Faassen and H.J. Grone *et al*., 2001. Vitamin E alleviates renal injury, but not hypertension, during chronic nitric oxide synthase inhibition in rats. J. Am. Soc. Nephrol., 12: 2585-2593.
32. Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.
33. Chiang, J.Y.L., 2002. Bile acid regulation of gene expression: Roles of nuclear hormone receptors. Endocr. Rev., 23: 443-463.
34. Fossati, P. and L. Prencipe, 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem., 28: 2077-2080.
35. Matsuda, Y., K. Hirata, N. Inoue, M. Suematsu, S. Kawashima, H. Akita and M. Yokoyama, 1993. High density lipoprotein reverses inhibitory effect of oxidized low density lipoprotein on endothelium-dependent arterial relaxation. Circulat. Res., 72: 1103-1109.
36. Senna, S.M., R.B. Moraes, M.F.R. Bravo, R.R. Oliveira and G.C. Miotto *et al*., 1998. Effects of prostaglandins and nitric oxide on rat macrophage lipid metabolism in culture: Implications for arterial wall-leukocyte interplay in atherosclerosis. IUBMB Life, 46: 1007-1018.
37. Jeppesen, J.O., H.O. Hein, P. Suadicani and F. Gyntelberg, 1998. Triglyceride concentration and ischemic heart disease: An eight-year follow-up in the Copenhagen male study. Circulation, 97: 1029-1036.
38. Iacono, J.M., M.W. Marshall, R.M. Dougherty, M.A. Wheeler, J.F. Mackin and J.J. Canary, 1975. Reduction in blood pressure associated with high polyunsaturated fat diets that reduce blood cholesterol in man. Prev. Med., 4: 426-443.
39. Leiba, A., A. Vald, E. Peleg, A. Shamiss and E. Grossman, 2005. Does dietary recall adequately assess sodium, potassium and calcium intake in hypertensive patients? Nutrition, 21: 462-466.
40. Postic, C. and J. Girard, 2008. Contribution of *de novo* fatty acid synthesis to hepatic steatosis and insulin resistance: Lessons from genetically engineered mice. J. Clin. Invest., 118: 829-838.
41. Kumai, T., S. Oonuma, N. Matsumoto, Y. Takeba and R. Taniguchi *et al*., 2004. Anti-lipid deposition effect of HMG-CoA reductase inhibitor, pitavastatin, in a rat model of hypertension and hypercholesterolemia. Life Sci., 74: 2129-2142.
42. Virchow, R., 1860. A More Precise Account of Fatty Metamorphosis. In: Cellular Pathology: As Based Upon Physiological and Pathological Histology, Virchow, R.L.K. (Ed.). R.M. De Witt, New York, USA., pp: 324-366.
43. Jiang, T., Z. Wang, G. Proctor, S. Moskowitz and S.E. Liebman *et al*., 2005. Diet-induced obesity in C57BL/6J mice causes increased renal lipid accumulation and glomerulosclerosis via a sterol regulatory element-binding protein-1c-dependent pathway. J. Biol. Chem., 280: 32317-32325.
44. Veerappan, R.M., T. Malarvili and G. Archunan, 2014. Effects on chrysin on lipid and xenobiotic metabolizing enzymes in l-NAME-induced hypertension. Int. J. Nutr. Pharmacol. Neurol. Dis., 4: 17-22.
45. Hoetzel, A., A. Welle, R. Schmidt, T. Loop and M. Humar *et al*., 2008. Nitric oxide-deficiency regulates hepatic heme oxygenase-1. Nitric Oxide., 18: 61-69.
46. Loria, P., A. Lonardo, L. Carulli, A.M. Verrone and M. Ricchi *et al*., 2005. Review article: The metabolic syndrome and non-alcoholic fatty liver disease. Aliment. Pharmacol. Ther., 22: 31-36.
47. Zimmerman, H.J. and L.B. Seef, 1970. Enzymes in Hepatic Disease. In: Diagnostic Enzymology, Goodly, E.I., (Ed.). Lea and Febiger, Philadelphia, pp: 1-38.
48. Yadav, N.P. and V.K. Dixit, 2003. Hepatoprotective activity of leaves of *Kalanchoe pinnata* Pers. J. Ethnopharmacol., 86: 197-202.