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Sub Chronic effect of High fat consumption on heart rate variability and Nitric oxide (NO) in experimental rats: Protective role of *Terminalia arjuna*

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ABSTRACT:

Background: In the present study Albino wister rats displayed with increase in body weight, sympathetic activity and decreased parasympathetic function following a high fat consumption for a sub chronic period. Possible alterations of cardiac autonomic functions and molecular behaviour of nitric oxide were observed in the presence of ethanolic extract of *Terminalia arjuna* (ETA). **Objectives:** The study was aimed to find out impact of high fat diet on cardiac health in terms with HRV also through NO pathways alterations in experimental rats and to assess the cardio protective efficacy of *Terminalia arjuna* on hyperlipidemic rats. **Methodology:** Bark of *Terminalia arjuna* was extracted with 99% ethanol. Rats were grouped into four groups (n=6); Control as group 1 (20% fat); group 2 fed with (20% fat+ ETA 100 mg/kg/b w); group 3 fed with (30% fat) and group 4 fed with (30% fat + ETA 100mg/kg/b w). Body weight and percentage of body weight gain were calculated. Electrophysiological analysis(HRV and sympatho-vagal balance) were done. Biochemical assay was done to assess serum Nitric oxide levels(NO). ANOVA, Pearson's correlation and multiple regression were done to analyze data. **Results:** Electrophysiological evaluation revealed altered sympatho-vagal balance in hyperlipidemic rats. Significant increase in sympathetic drive along with decrease in parasympathetic functions suggests cardiac autonomic dysfunction in rats fed with high fat. Subchronic supplementation of ethanolic extraction of *Terminalia arjuna* to hyperlipidemic rats showed significant beneficial effect on cardiac autonomic function. Significant negative correlation was observed between HRV and nitric oxide. **Conclusion:** *Terminalia arjuna* have exhibited its cardioprotective role in high fat fed rats by enhancing cardiac functions. Supplementation of ETA have shown important role in modulating autonomic control and in improving cardiovascular function. Probably polyphenolic compounds and flavonoids might have cardioprotective activity by rejuvenating action and free radical quenching actions.

KEYWORDS: Hyperlipidemic rats, Heart rate variability, *Terminalia arjuna* , Nitric oxide.

INTRODUCTION:

Cardiovascular diseases are on the increasing trend in worldwide and multiple factors are attributed to develop this alarming threat.[1] Hyperlipidemic diet mainly, more amount of saturated fatty acids compared to mono and poly unsaturated fatty acids may cause advent effect on cardiovascular health.[2] Overconsumption of such high fat diet can cause positive energy balance and lead to the overweight and/or obesity state.[3] Obesity associated with dyslipidemia may have adverse impact on cardiovascular autonomic functions. Cardiac autonomic dysfunctions includes increased heart rate, decreased parasympathetic control, sympathetic hyperactivity and reduced heart rate

variability.[4] Heart rate variability, indicates autonomic influence over the cardiac functioning. Cardiac rhythm is under minute to minute and circadian influence of the autonomic nervous system.[5] It has been reported that nitric oxide (NO) is an effective anti-hypertrophic and cardiac remodeling inhibitor. However, high fat diet increases fat mediated oxidative stress through lipid Peroxidation and reducing NO bioavailability.[6] Impairment of NO availability may present with central defect triggering many cardiac and vascular pathophysiological responses.[7]

In recent years, the keen interest in medicinal plants and phytochemicals has been enhanced due to their medicinal properties in cardiovascular diseases due to their several mechanisms like antioxidant activity.[8,9] *Terminalia arjuna* (Arjuna) is herbal tree of combretaceae family. Bark of *Terminalia arjuna* contains hypolipidemic agents and flavonoids such as arjunolic acid, arjun glycosides, arjunone with its rich antioxidative properties. It serves as a cardiac tonic.[10] Hence, we aimed to explore influence of high fat diet on cardiac health in terms with HRV also through NO pathways alterations in experimental rats. Furthermore, to find out the cardio protective efficacy of *Terminalia arjuna* on high fat fed rats.

MATERIALS AND METHODS:

Extraction and phytochemistry of bark of *Terminalia arjuna*:

Bark of *Terminalia arjuna* was procured from the local market of city. Scientific authentication was done in the Botany department. Extraction process was conducted with Soxhlet apparatus by using 300gms of *Terminalia arjuna* dry powder and 99% of ethanol. The entire procedure was done for 24 hours and temperature was maintained at 22-24°C. Evaporated solvent was collected in a perfect vacuum with 19% of semisolid yield with respect to the dried powder.[11] Preliminary phytochemical analysis of freshly prepared ethanolic extract of *Terminalia arjuna* (ETA) was achieved by using standard protocols.[12,13]

This extracted solvent was stored in the form of stock solution in the refrigerator for further reference. Solvent was labelled with Voucher specimen no. BMPTA/09 and was preserved in research laboratory for further use.

Dietary protocol:

The control and high fat diet (HFD) was prepared for 1000gms, according to the standard dietary regimen protocol. Control diet was made up of protein (casein 18%), carbohydrate (Amylum 60%), fat (vegetable oil 20%), vitamin and minerals (2%). Subsequently, HFD was made by adding 10% extra fat (fat; 30%) and reducing 10% carbohydrate (carbohydrate; 50%) from the control diet. Protein (casein 18%), vitamin and minerals (2%) were kept constant as in control diet. Dietary protocol was maintained on pair feeding.[14]

Study Protocol:

Total 24 number of healthy rats (albino Wistar) of weight approximately 180-220gm's were obtained from Institutional Animal House. Rats were made acclimatized laboratory environment at 22-24°C at 12 hrs. day and night cycle.[15] Food and water were made available to them.

Rats have been grouped into four, each group containing six rats; group 1named as control, fed with control diet (20% fat); group 2 = control diet+ ETA 100mg/kg/b w; group 3 = high fat diet (30% fat) and group 4 = high fat diet (30% fat) + ETA 100mg/kg/b w.[10] Dietary and ETA interventions were followed for 21 days.

Ethics statement:

Animal care and experimental protocol were followed according to the Institutional Animal Ethics Committee (IAEC; Ref No268/11 dated 01/06/2011). Guidelines stated by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of INDIA were followed. Animals were sacrificed by cervical dislocation and incinerated electrically.[16]

Experimental Protocol:

***Gravimetry*:**

Body weight of all rats was recorded on first and last day of intervention using digital weighing Machine (Practum 1102-10IN, Sartorius Lab Instruments, and Germany). Percent of body weight gain was calculated. Retro-orbital puncture was done to take blood in 10% EDTA tubes on overnight fasting after the completion of 21 days of interventions.[17] Centrifugation process was done at x 2300 G for 10 mins and serum was collected.

Heart rate variability:

Each rat of all groups was anesthetized with an intra-peritoneal injection of 60mg/kg ketamine and 6 mg/kg xylazine. In dorsal recumbence ECG was recorded on day1 and 22nd day using BioPac instrument (BioPac MP 45:PC windows based; Biopac Student Lab 4.1 software). Bipolar electrodes were inserted into the upper and lower limbs of the rats. HRV was analyzed by using Kubois HRV analysis software version 2 produced by Physics department, university of Kuopio, Finland. HRV was determined by calculating 600 RR intervals. Following HRV parameters were recorded; heart rate (HR) in bpm, RR intervals duration(ms), Low frequency power (LF; low frequency power/ baroreceptor activity/ sympathetic activity), high frequency power (HF; corresponding to parasympathetic activity) and LF/HF (low frequency and high frequency power ratio)

Biochemical assay:

Estimation of Nitrosative stress marker Nitric oxide (NO) Levels[18]

Nitrate, the stable product of nitric oxide is reduced to nitrite by cadmium reduction principle. The nitrite produced is determined by diazotization with sulphanilamide and coupling to N-naphthylethylenediamine. The intensity of the pink colored complex is measured at 540nm by using spectrophotometer (Schimadzu UV 800, Schimadzu Corporation, Zapan).

Statistical analysis:
Data represented with Mean±SD. Significance of inter group differences was done by One Way ANOVA and Post Hoc Tukey's multiple comparison tests were done. P≤0.05 was taken as statistically significant. All the parametrical analysis was done using SPSS 16.0 (SPSS Inc., Chicago, USA).

RESULTS:

Table 1: Ethanolic extract of *Terminalia arjuna* (ETA) on Gravimetry in rats fed with high fat diet.

Parameter	Group 1	Group 2	Group 3	Group 4	ANOVA	
					F Value	p value
1st day body weight (gms)	205±18	200±14	214±8.4	215±5.4		
22nd day Body weight (gms)	226±6.6	222±16	252±26 [a, b]	236±10 [c]	5.138	0.016*
% of body weight gain	10±1.2	10.5±3	16±2.9[a,b]	10.2±3.09	4.392	0.026*

Values are in mean ±SD. ANOVA with Post Hoc Tukey's multiple comparison test. Superscript a, b, c tells significant difference among groups. a shows comparison with group 1, b shows comparison with group 2, c shows comparison with group 3.(*p ≤0.05). Group 1= control (fat 20%), group 2= control diet (fat 20%) and ETA, group 3= HFD (fat 30%) and group 4= HFD (fat 30%) and ETA.

Observations from table 1 values show significant increase in body weight and percent body in rats fed with high fat diet (group 3; fat 30%) compared to control (20 % fat) and group2 (control diet+ ETA) on 22nd day. However, there was significant decrease in both final body weight and % of body weight gain in rats supplemented with high fat diet and ETA (group 4;fat 30%).

Table 2: Ethanolic extract of *Terminalia arjuna* (ETA) on Heart rate variability in rats fed with high fat diet.

Parameter	Group 1	Group 2	Group 3	Group 4	ANOVA	
					F Value	p value
HR (bpm)	132±5.1	138±3	260±16 [a, b]	162±4.7 [c]	124	0.00*
Sympathetic Function (LF)	0.35±0.03	0.35±0.03	0.61±0.08[a, b]	0.36±0.03 [c]	19	0.01*
Parasympathetic function (HF)	0.67±0.03	0.64±0.04	0.4±0.04[a,b]	0.62±0.02 [c]	32.3	0.000*
Sympatho-vagal balance(LF/HF)	0.53±0.06	0.54±0.09	1.5±0.35 [a, b]	0.57±0.06 [c]	20.6	0.000

Values are in mean ±SD. ANOVA with Post Hoc Tukey's multiple comparison test. Superscript a, b, c tells significant difference among groups. a shows comparison with group 1, b shows comparison with group 2, c shows comparison with group 3.(*p ≤0.05). Group 1= control (fat 20%), group 2= control diet (fat 20%) and ETA, group 3= HFD(fat 30%) and group 4= HFD(fat 30%) and ETA.

From table 2, we have observed significant higher values of heart rate in group 3 rats (fat 30%) than control rats and group 2 rats. Further, we observed significant decrease of heart rate in ETA supplemented high fat fed rats (group 4). We observed significant increase in sympathetic (LF) and sympatho-vagal balance (LF/HF) in rats fed with high fat diet, supporting an increase in sympathetic drive in rats. Furthermore increase in sympathetic drive along with decrease in parasympathetic function is suggesting cardiac autonomic dysfunction in rats fed with high fat. Eventually, we have observed cardio protective role of ETA in hyperlipidemic rats (fat 30%).

Figure 1: Ethanolic extract of *Terminalia arjuna* (ETA) on Nitric oxide (NO) in rats fed with high fat fed rats

Figure 1 depicts, significant decrease in Nitric oxide levels in rats fed with high fat diet, which indicates high fat diet may have acute impact on vascular tone by decreasing endothelial dependent vasodilation. Ethanolic extract of *Terminalia arjuna* supplementation to the high fat fed rats(group4) have shown remarkable improvements in the NO level as compared to rats fed with high fat fed rats (group 3).

Figure 2: Correlation between NO and LF/HF (Sympatho-vagal balance) Correlation between NO and LF/HF have been depicted in fig: 2. We have observed significant negative correlation (n=24, r= -0.776, p<0.05*) between NO and LF/HF among all groups of rats.

Table 3: Model Summary; multiple linear regressions using NO as predictors for LF/ HF in all groups of rats.

A regression was done to estimate LF/ HF ratio to NO among all rats. NO variable shows significant impact on LF/ HF.

Table 3.1: Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	0.776[a]	0.602	0.584	0.30489

Superscript 'a 'indicates predictors at constant: NO

Table 3.2 :ANOVA

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	3.091	1	3.091	33.255	.000[a]
Residual	2.045	22			
Total	5.136	23			

'a ' is predictors at constant i.e. NO

LF/HF (R; dependent variable) with value of 0.776 shows significant level of prediction. R2 depicts coefficient of determination. From [Table 3.1], R2 with value 0.602 tells that independent variable (NO) explain 60.5% of the variability of dependent variable(LF/HF).
Table 3.2 indicates that independent variables significantly predict dependent variable $F(1, 22) = 3.091$ $p<0.05$*. The regression model is suitable for the data to indicate relation. Regression equation can be formed, LF/HF = 2.917 – 0.368 X NO.

DISCUSSION:

The main findings of the present study were that rats displayed with increase in body weight, sympathetic activity and decreased parasympathetic function following a high fat consumption for a sub chronic period. Results also indicate possible alteration of cardiac autonomic functions and molecular behaviour of nitric oxide in the presence of ethanolic extract of *Terminalia arjuna*.

Obesity and autonomic nervous system are interrelated such as, 10% increase in body weight is linked with decrease in parasympathetic tone and rise in heart rate.[19] Recent prospective studies have shown that intake of a high-fat diet may cause changes in neuropeptide levels within autonomic nuclei in the hypothalamus and brainstem which leads to the development of obesity-associated sympathetic hyperactivity and autonomic imbalance.[20,21]

Our findings suggest that a subchronic period, 3 weeks dietary intake of high levels of saturated fat is associated with sympatho-vagal imbalance, reduced HRV and transient impairments of endothelial dependent vascular functions due to the acute postprandial lipemia.[22]

Thus high fat diet may contribute to have acute effects on vascular tones mediated by reduction in endothelium dependent vasodilatation. Furthermore, it may lead to endothelial dysfunction and hemodynamic stress.[23] In addition exaggerated HRV is an acute change in cardiovascular function that occurs in response to stress. It may cause significant increase in total peripheral vasoconstriction which is sufficient to induce transient impairment in arterial vasoactivity, thus potentially impairing the adaptability of the vascular system to physiological demands during stress. Increased and prolonged cardiovascular responses to stress can create severe strain on the arteries and myocardium.[24] These cardiovascular responses to stress may be important predictors of future development of cardiovascular diseases like hypertension, atherosclerosis, and coronary artery diseases.[25,26]

It is well established that, physiologically endothelial cells maintain vascular homeostasis through complex interactions which involve nitric oxide. The pathophysiology of several cardiovascular diseases including atherosclerosis has been related in part to endothelial dysfunction. There are many risk factors those involve to atherogenesis like immune system inactivation, chronic inflammation, reactive oxygen species, C-reactive protein and nitric oxide/endothelin imbalance. These risk factors may perform in synergy with each other or in a "vicious cycle" to intensify endothelial dysfunction.[27] Reactive oxygen species act an important role in the endothelium to happen endothelial dysfunction. Reactive oxygen species distort physiological bioactivity of nitric oxide by decreasing the activity of nitric oxide synthase and by shortening the half-life of nitric oxide.[28] Furthermore, a decrease in levels of nitric oxide bioavailability leads to an imbalance between nitric oxide and endothelin. Nitric oxide and endothelin have contrast effect on the vasculature: vasodilation and vasoconstriction, respectively. Imbalance of these two compounds in the vasculature may cause endothelial dysfunction via upregulation of adhesion molecules, inflammation and vasoconstriction.[29] Studies on endothelial dysfunction have explained about the uncoupling of NO from endothelial nitric oxide synthase (eNOS/NOS 3),and reactive oxygen species superoxide anion reacts with nitric oxide to form peroxynitrite which damages endothelial cells. Eventually these events may end with advert impact on autonomic functions.[1]

Experimental study reported that the sympathetic nervous system is critically influenced by the most relevant factors regulating vascular function like NO, reactive oxygen species (ROS), endothelin, and the renin-angiotensin system. It is well documented that oxidative stress simultaneously affects the ANS and vascular function.[30,31]

Modulatory effects of neural mechanisms on the sinus node has been well understood by spectral analysis of HRV.[32] LF,HF and LF/HF are the quantitative marker of cardiac autonomic functions.[33] The frequency domain analysis of HRV spectrum in the present study showed significant incline in high fat diet compared to control rats but eventually have improved after *Terminalia arjuna* therapy. LF:HF ratio have taken as an index of sympatho-vagal balance was significantly increased in high fat fed rats but have observed improvement after *Terminalia arjuna* supplementation.[34]

Terminalia arjuna (Arjuna) as a medicinal plant contains many essential phytoconstituents which exhibit definitive physiological actions on the human body. These bioactive compounds are tannins, saponins, arjunic acid, flavonoids (arjunone, arjunolone, luteolin), gallic acid, ellagic acid, terpenoids, and phenols.[10] Ethanolic extract of *Terminalia arjuna* exhibits its beneficial effects by enhancing cardiac muscle functioning and improving pumping actions of the heart. It serves as a cardiac tonic. Many studies have reported that saponin glycosides may produce inotropic effects on cardiac muscles whereas could be responsible for the inotropic effects of *Terminalia arjuna*, whereas the flavonoids spare free radical antioxidant activity and enhance vascular strengthening.[35,36] *Terminalia arjuna* bark extract has an effective prophylactic and therapeutic effect mainly through endogenous antioxidant enzyme activities.[37] Till now very few experiments exist to report causal relations between cardiac autonomic dysfunction and *Terminalia arjuna* through no pathways alterations. Despite of few limitations, we have reported cardio protective actions of Ethanolic extract of *Terminalia arjuna* (ETA) in high fat fed rats by modulating cardiac autonomic functions. Eventually studies are required to identify the active biological compounds of *Terminalia arjuna* extract and to relate underlying mechanisms.

Figure 3: Postulated mechanism of actions of ethanolic extract of *Terminalia arjuna* on high fat induced cardiac autonomic functions

CONCLUSION:

10% additional fat for subchronic period to the albino wister rats developed sympathovagal imbalance. Also was observed reduced bioavailability of NO in hyperlipidemic rats. Possibly, high fat diet may contribute to have acute effects on vascular tones mediated by reduction in endothelium dependent vasodilatation. Impairment of NO availability presented with central defect triggering many cardiac and vascular pathophysiological responses. The efficacy of *Terminalia arjuna* as a cardioprotective agent against high fat induced albino rats was observed in the present study. It may be due to the fact that ethanolic extract of *Terminalia arjuna* possess many biological active compounds like tannins, saponins, arjunic acid and flavonoids. Its role in improving the autonomic control plays significant role in enhancing cardiac function. Probably polyphenolic compounds and flavonoids might have cardioprotective activity by rejuvenating action and free radical quenching actions.

REFERENCES:

1. Das KK, Chadchan KS, Reddy RC, Biradar MS, Kanthe PS, Patil BS, Ambekar JG, Bagoji IB, Das S. Effects of some indigenous plants of North Karnataka (India) on cardiovascular and glucose regulatory systems in alloxan-induced diabetic rats. Cardiovascular and Hematological Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Cardiovascular and Hematological Agents). 2017 Apr 1;15(1):49-61.
2. Priya Singh, Yogesh Shiv hare, UK Patil. Assessment of Hypolipidemic Potential of Chenopodium album Linn on Triton Induced Hyperlipidemic Rats. Research J. Pharm. and Tech. 3(1): Jan.-Mar. 2010; Page 187-192.
3. Medai E, Lima Leopoldo AP, Pererira Junior PP, Leopold AS, Campos D HS, Raimundo JM,et al. Could high fat diet rich in unsaturated fatty acids impair the cardiovascular system?. Can J Cardiol. Descember 2010;26(10): 542-48.
4. Kanthe PS, Patil BS, Bagali SC, Reddy RC, Aithala MR, Das KK. Protective effects of ethanolic extract of Emblica officinalis (amla) on cardiovascular pathophysiology of rats, fed with high fat diet. Journal of clinical and diagnostic research: JCDR. 2017 Sep;11(9):CC05.
5. Rono DK. Effect of variable high fat diets on heart rate variability and selected modifiable cardiovascular risk factors.2013; University of Nairobi.
6. Xia N, Horke S, Habermeier A, Closs EI, Reifenberg G, Gericke A, Mikhed Y, Münzel T, Daiber A, Förstermann U, Li H. Uncoupling of endothelial nitric oxide synthase in perivascular adipose tissue of diet-induced obese mice. Arteriosclerosis, thrombosis, and vascular biology. 2016 Jan;36(1):78-85.
7. Nascimento TB, Baptista RD, Pereira PC, Campos DH, Leopoldo AS, Leopoldo AP, Oliveira Júnior SA, Padovani CR, Cicogna AC, Cordellini S. Vascular alterations in high-fat diet-obese rats: role of endothelial L-arginine/NO pathway. Arquivos brasileiros de cardiologia. 2011 Jul;97(1):40-5.
8. Preeti Tiwari . Evaluation of Antihyperlipidemic Potential of Amritarishta Prepared by Traditional and Modern Methods in Hyperlipidemic Rats. Research J. Pharmacognosy and Phytochemistry 2013; 5(6): 315-319 .
9. Kumar V, Kshemada K, Ajith KG, Binil RS, Deora N, Sanjay G, Jaleel A, Muraleedharan TS, Anandan EM, Mony RS, Valiathan MS. Amalaki rasayana, a traditional Indian drug enhances cardiac mitochondrial and contractile functions and improves cardiac function in rats with hypertrophy. Scientific reports. 2017 Aug 17;7(1):1-7.
10. Desai SD, Patil BS, Kanthe PS, Potekar RM. Effect of ethanolic extract of Terminalia arjuna on liver functions and histopathology of liver in albino rats fed with hyperlipidemic diet. Int J Pharm PharmSci. 2015;7(10):302-6.
11. Kanthe PS, Patil BS, Aithala MR, Das KK. Effect of ethanolic extract of Emblica officinalis (amla) on glucose homeostasis in rats fed with high fat diet. J. Krishna Inst. Med. Sci. Univ. 2017 Jul 1;6(3):31-7.
12. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. International Pharmaceutica Sciencia 2011; 1:103-4.
13. T. Sundarrajan, Vijey Aanandhi M. Hypolipidemic Activity of Amaranthus Tristis Linn in Triton WR-1339 Induced Hyperlipidemic Rats. Research J. Pharm. and Tech. 2017; 10(1): 171-173.
14. Das KK, Dasgupta S. Effect of nickel sulfate on testicular steroidogenesis in rats during protein restriction. Environ Health Perspect 2012; 110 (9):923-26.
15. Srivastava Vaibhav, Dubey Subodh, Singh Vigyan. Hypolipidemic activity of stem bark of Ailanthus excelsa Roxb in Triton WR 1339 induced hyperlipidemic rats. Research J. Pharm. and Tech. 2019; 12(3): 1338-1342.

16. Preeti Tiwari. Antihyperlipidemic Potential of Balarishta Prepared by Traditional and Modern Methods in High Fat Diet Induced Hyperlipidemic rats. Asian J. Res. Pharm. Sci. 4(1): Jan.-Mar. 2014; Page 07-11.
17. T. Sundarrajan, Vijey Aanandhi M.. Hypolipidemic activity of Alternanthera ficoidea Linn in Triton wr-1339 induced hyperlipidemic rats.Research J. Pharm. and Tech 2018; 11(2):687-689.
18. Moshage H, Kok B, Huizenga JR, Jansen PL. Nitrite and nitrate determinations in plasma: a critical evaluation. Clinical chemistry. 1995 Jun 1;41(6):892-6.
19. Poirier P, Hernandez TL, Weil KM, Shepard TJ, Eckel RH. Impact of diet induced weight loss on the cardiac autonomic nervous system in severe obesity. Obesity 2003 Sep1;11(9):1040-7.
20. Lim K, Barzel B, Burke SL, Armitage JA, Head GA. Origin of aberrant blood pressure and sympathetic regulation in diet-induced obesity. Hypertension. 2016 Aug;68(2):491-500.
21. Viktor V. Gorelik, Valery N. Vlasov. Assessment of Regulatory Systems Functional Reserves of the Handball Players at Exercises in the Training Process Dynamics. Research J. Pharm. and Tech. 2017; 10(3): 875-881.
22. Sauder KA, Johnston ER, Skulas-Ray AC, Campbell TS, West SG. Effect of meal content on heart rate variability and cardiovascular reactivity to mental stress. Psychophysiology. 2012 Apr;49(4):470-7.
23. Rajendran P, Rengarajan T, Thangavel J, Nishigaki Y, Sakthisekaran D, Sethi G, Nishigaki I. The vascular endothelium and human diseases. International journal of biological sciences. 2013;9(10):1057.
24. Jakulj F, Zernicke K, Bacon SL, Van Wielingen LE, Key BL, West SG, Campbell TS. A high-fat meal increases cardiovascular reactivity to psychological stress in healthy young adults. The Journal of nutrition. 2007 Apr 1;137(4):935-9.
25. Kanthe PS, Patil BS, Khodnapur JP, Bagali SC, Mullur L, Aithala M. Comparative study of rate pressure product in obese women with nonobese women. Int J Biomed Adv Res. 2012;3:580-3.
26. Preeti Tiwari and Rakesh K. Patel Evaluation of Antihyperlipidemic Potential of Drakshasava Prepared by Traditional and Modern Methods in Hyperlipidemic Rats. Research J. Pharmacology and Pharmacodynamics. 2013; 5(2): 92-97.
27. Rajendran P, Rengarajan T, Thangavel J, Nishigaki Y, Sakthisekaran D, Sethi G, Nishigaki I. The vascular endothelium and human diseases. International journal of biological sciences. 2013;9(10):1057.
28. Patil BS, Kanthe PS, Reddy CR, Das KK. Emblica officinalis (Amla) Ameliorates High-Fat Diet Induced Alteration of Cardiovascular Pathophysiology. Cardiovascular and Hematological Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Cardiovascular and Hematological Agents). 2019 May 1;17(1):52-63.
29. Sandoo A, van Zanten JJ, Metsios GS, Carroll D, Kitas GD. The endothelium and its role in regulating vascular tone. The open cardiovascular medicine journal. 2010;4:302.
30. Amiya E, Watanabe M, Komuro I. The relationship between vascular function and the autonomic nervous system. Annals of Vascular Diseases. 2014;7(2):109-19.
31. A. Julius, K. Renugadevi, V. Hemavathy. Effect of Oxidative Stress in Essential Hypertension. Research J. Pharm. and Tech. 7(12): Dec. 2014; Page 1400-1403.
32. Khaliq F, Parveen A, Fahim M. Effect of Terminalia arjuna on heart rate variability in diabetic rats. Int J Curr Res Chem Pharma Sci. 2014;1:44-7.
33. Kirti Rawal, Gaurav Sethi, B S Saini, and Indu Saini. Impact of the Menstrual Cycle on Heart Rate Variability. Research J. Pharm. and Tech 2019; 12(8):3854-3856.
34. Acharya UR, Joseph KP, Kannathal N, Lim CM, Suri JS. Heart rate variability: a review. Medical and biological engineering and computing. 2006 Dec 1;44(12):1031-51.
35. Miller AL. Botanical Influences on Cardiovascular Disease. Alternative Medicine Review 1998; 3(6): 422-31.
36. Yadav RN, Rathore K. A new Cardinolide from the roots of Terminalia Arjuna. Fitoterpia 2001; 72:459-61.
37. Parveen A, Babbar R, Agarwal S, Kotwani A, Fahim M. Mechanistic clues in the cardioprotective effect of Terminalia arjuna bark extract in isoproterenol-induced chronic heart failure in rats. Cardiovascular toxicology. 2011 Mar 1;11(1):48-57.

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