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Critical Evaluation of Anxiety As Aetiological factor for Development of Heart Disease In Albino Rats WSR To Lipid Profile And Cardiac Biochemistry and Biopsy

Dr.Subhash Waghe [1], Dr. Vijay R. Potdar [2]

[1]Professor & HOD – Dept. of Rognidana & Vikrutivigyna, Sardar Patel Ayurvedic Medical College & Hospital, Dongariya, Balaghat – 44 3318 (M.P.)

[2]Associate Professor - Dept. of Rognidana & Vikrutivigyna, Govt. Ayurvedic College, Nagpur.

Corresponding author: Dr.Subhash Waghe

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Abstract

During the past three decades the number of deaths due to CVDs has increased from 15.2% to 28.1% in India. There are many dietary and lifestyle factors are responsible for this rise. In the common aetiology of heart diseases stated by acharya Charaka, along with other causes, psychological causes like *Chinta* (worry), *Bhaya* (Fear/Anxiety), *manasik trass* (mental tension) are mentioned as factors responsible for heart disease. There is sharp increase in cases of anxiety and depression due to change lifestyle in present era. Hence, it is essential to evaluate the role of Ayurvedokta psychological factor such as *Bhaya* (Anxiety) in the development of heart disease. Chronic unpredictable mild stress (CUMS) is the most elegant model for evaluation of anxiety in the rats as this model possesses construct, predictive and face validity in rats. Hence, this model is used in the present study. In CUMS process, animals will be subjected chronically and unpredictably to a variety of low-grade stressors which resembles to those associated with anxiety like symptoms in humans and also cause cognition impairment. It is observed that CUMS had generated the anxiety in rats leading to alteration in normal cardiac physiology. Elevated triglyceride and elevation of low-density Cholesterol are the biomarkers used to know the risk of ischemic heart disease. Cardiac biochemical parameters like CPK-MB, SGOT are measured to know the effect of stressors on cardiac health. And accordingly, they are evaluated in both normal and disease control rats. Cardiac biopsy was also done at the end of the study for further evaluation. Significant changes were observed in disease control group indicating that fear lead to cardiac discomfort in experimental rats.

Keywords : Anxiety, *Bhaya*, *Chinta* ,CVD, Heart Disease

Introduction :

Nearly there are 3 million (30 lac) cases of Myocardial Infarction occurs every year (API Study) in India and 15 million (1.5 Cr.) cases across the globe every year. Out of this, 25% are under 40 age, 50% are under 50 age, 25% > 50 years of age. The death due to myocardial infarction is increasing in Indian population at an alarming rate and accounts for around 15-20% of all deaths. During the past three decades the number of deaths due to CVDs has increased from 15.2% to 28.1% in India. [1] The number of factors play role in the development of ischemic heart diseases but over consumption of oily fatty food and unhealthy lifestyle (*mithya ahar vihar*) with mental stress are the important basic factors enumerated by both the science.In Ayurveda it can be called as '*Hrit Aposhanaj Hrit Roga'* and the pathophysiology of MI is mentioned by Sushruta in Sutrasthana 15/32 and Syndrome of MI is mentioned by Sushrut Uttartantra 43/131-132 in the form of '*Hrit Shoola*'. In the common aetiology of heart diseases stated by acharya Charaka, along with other causes, psychological causes like *Chinta* (worry), *Bhaya* (Fear/Anxiety), *manasik trass* (mental tension) are mentioned as factors responsible for heart disease. There is sharp increase in cases of anxiety and depression due to change lifestyle in present era. Hence, it is essential to evaluate the role of Ayurvedokta psychological factor such as *Bhaya* (Anxiety) in the development of heart disease. Stress is an important factor having high impact on the psychological development which alters emotion, cognition and related behavioral outputs. Chronic unpredictable mild stress (CUMS) is the most elegant model for evaluation of anxiety as this model possesses construct, predictive and face validity in rats. In CUMS process, animals will be subjected chronically and unpredictably to a variety of low-grade stressors which resembles to those associated with anxiety like symptoms in humans and also cause cognition impairment. CUMS protocol will be performed in separate room but the normal animal left unchallenged. During the 7 weeks, animals were submitted to 6 different stressors: tilted cage (45°), food and water deprivation, restricted access to food, exposure to empty bottle, 24 h wet cage (200ml of water in 100g of sawdust bedding), continuous illumination. These stressors will be randomly scheduled over a week period and will be repeated to maintain the aspect of unpredictability. Cardiac biochemical parameters like CPK-MB, SGOT are measured to know the effect of stressors on cardiac health. Cardiac biopsy was also done at the end of the study for further evaluation.

Review Of Literature :

Circulatory System As Per Ayurveda :

As per Ayurveda the root of *Rasavaha strotas* (circulatory system) is mentioned as Heart and blood vessels. [3, 4] As per acharya Charaka, Vyan vayu circulates the blood in the entire body and it gets aggravated whenever there is obstruction to the flow of the blood in the circulation. [5]

Aetiology of Ischemic Heart Diseases As Per Ayurveda : As per acharya Charaka, psychological factors like excessive worry along with hyperlipidemic diet leads to the vitiation of circulatory system [6] As per acharya Charaka, excessive worries, fear, mental stress, chronic disease leads and trauma leads to heart diseases. [7]

As per acharya Sushruta, excessive consumption of incompatible diet, excessive diet, antagonistic diet leads to heart diseases.[8] As per the book Yogaratnakar, the smoking of tobacco leads to heart diseases. [9]

Atherosclerosis In Arteries As Per Ayurveda : In Ayurveda, *Dhamani -pratichaya* (Atherosclerosis) is defined as the excessive deposition of layer of fatty sticky unctuous material inside the lumen of arteries and it is the disease of Kapha origin. As per Ayurveda, *Dhamni Pratichay* is one of the diseases, caused exclusively by the vitiation of Kapha (*Kaphaj Nanatamaj Vyadhi*) [10, 11, 12] Hence, the factors, responsible for the vitiation of Kapha, also serves as the aetiological factors for the atherosclerosis in arteries (*Dhamni Pratichaya*). As per acharya Charaka, it is *Raspradoshaj Vikara* and it is due over nourishment. [13, 14] The function of pathologically increased *Kapha* is to cause coating, obstruction and hardness in the arterial lumen. [15]

Aetiology of Ischemic Heart Diseases As Per Ayurveda :

As per acharya Sushruta, due to consumption of high fatty and carbohydrate diet and lack of exercise, the arterial lumen gets obstructed with fat and area to be supplied, remain under perfused. [16]

As per acharya Sushruta, the vitiated plasma gets obstructed due to blockages in coronaries of the heart, and alters the normal functioning of the heart and also gives rise to Angina. [17] The angina if not treated soon, kills the patient instantly. [18]

Myocardial Infarction (MI) :

MI refers to the condition where there is imbalance between the myocardial oxygen demand and its supply due to the obstruction of blood supply in coronary arteries.[19] [20]

The commonest causes responsible for it are :

- Atherosclerosis in coronary artery
- Thrombosis

Investigations To Diagnose MI :

- **Lipid profile** – It may show dyslipidaemia (Increased LDL cholesterol and Triglycerides)
- **Cardiac Markers** - Serum Troponin and CPK-MB elevated.
- **ECG** shows ST-T changes. In rats ST segment is absent in waveforms.
- **Coronary Angiography** (CAG) shows coronary occlusions.
- **2-D Echocardiography** shows regional wall motion abnormalities. [20]

CPK -MB is creatine phospho kinase myocardial bound enzyme primarily found in heart muscle. It is used to detect the heart muscle damage in conditions such as myocardial infarction. It is released in blood circulation when heart muscle is damaged.Serum Glutamic Oxaloacetic Transferase is an enzyme found in the liver and heart tissue. It's rise in blood indicates either liver or heart muscle injury.Inflammatory biomarker C-Reactive is a protein produced in the liver in response to the inflammation and it is used to assess the presence of inflammation in the body. HS-CRP is more specific to the cardiac tissue injury.Triglyceride is the stored form of fat which is used to derive energy. Elevated triglyceride level indicates an increased risk for ischemic heart disease. High density lipoprotein is a good cholesterol which helps to prevent the building of bad low density cholesterol in the circulation. HDL picks up the excess LDL cholesterol and send it to liver where it

is broken down and eliminated. Low levels of HDL cholesterol indicated increased risk for ischemic heart disease.

Research Question :

Whether Ayurvedokta *Bhaya* (fear) acts as a aetiological factor for development of heart disease

Hypothesis :

- **Null Hypothesis (H1) :** Ayurvedokta *Bhaya* (fear) acts as a aetiological factor for development of heart disease
- **Alternate Hypothesis (H0) :** Ayurvedokta *Bhaya* (fear) does not acts as an aetiological factor for development of heart disease.

Aims & Objectives :

- **Primary Objectives :**The present study, aims to study the aetiological factor ***Bhaya* (Fear/Anxiety)** as the cause for the development of heart disease.
- **Other Objectives :**To study the aetiopathogenesis of myocardial infarction from Ayurvedic point of view.

Material & Methodology :

7.1 Study Design

Center of Study – Dept of Roga Nidana & Vikrutvigyana, Government Ayurvedic College, Nanded And National Testing Centre, Pune

Duration of Study – Total study 18 months after approval of synopsis.

Study Population And Sampling :

Animal required for the Study

Species/Common name - Albino Rat

Weight - 200-250 g

Gender – Male and Female

Number to be used - 12

Groups :

Animals will be divided into 2 groups.

Groups (n = 6)	Treatment
Normal Control	No treatment
Disease Control	Chronic unpredictable mild stress induction

Data Collection & Instruments :

The animals will be subjected chronically and unpredictably to a variety of **low-grade stressors** which resembles to those associated with anxiety like symptoms in humans and also cause cognition impairment. CUMS protocol will be performed in separate room. During the 7 weeks, animals are submitted to 6 different stressors:

1. Tilted cage (45°),
2. Tail Clamping for 3 minutes,
3. Cold swimming for 5 minutes at 4°C
4. Exposure to empty bottle,
5. 24 h wet cage,
6. continuous illumination.

These stressors are randomly scheduled over a one week period and are repeated to maintain the aspect of unpredictability. After confirmation of stress in animals, ECG was done using the power Lab data acquisition apparatus on 0, 28th and 49th Day. Rats were anaesthetized with Ketamine before taking the ECG.

Assessment Criteria :

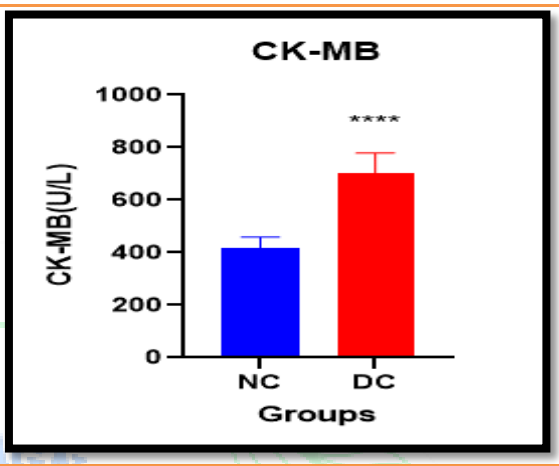
1. The normal reference range of CPK-MB in rats is 5 to 25 IU/L. Level. The level 5 times higher than the normal range is considered significant rise.

2. The normal reference range of SGOT in rats is 5 to 40 IU/L. Level. The level higher than the normal range will be considered significant rise.
3. The normal reference range of CRP in rats is 300 to 600 mg/ml. Level. The level higher than the normal range is considered significant rise.
4. The normal reference range of Triglyceride in rats is 25 to 145 mg/dl. Level. The level higher than the normal range is considered significant rise.
5. The normal reference range of HDL cholesterol in rats is 35 to 55 mg/dl. Level. The level higher than the normal range is considered significant rise. [21]

Observation & Result:

Table No. 1 – CPK-MB Values

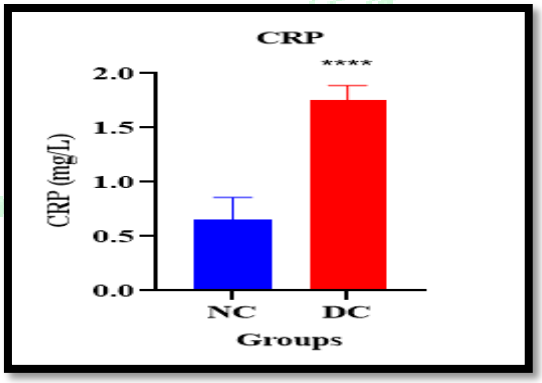
Date:07/Feb/2024				CKMB
Group	Animal No	Sex	Marking	U/L
NC	1	M	1	429.8
	2	M	2	373.0
	3	M	3	410.0
	4	F	1	488.7
	5	F	2	421.4
	6	F	3	370.1
MEAN				415.5
SD				43.6
DC	7	M	1	665.0
	8	M	2	706.7
	9	M	3	645.1
	10	F	1	651.4
	11	F	2	710.8
	12	F	3	845.8
MEAN				704.1
SD				74.7



Graph 1: CPK-MB Values

Table No. 2 – CRP Values

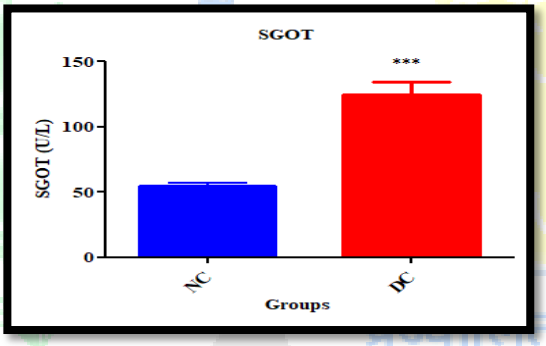
Date:07/Feb/2024				CRP
Group	Animal No	Sex	Marking	mg/L
NC	1	M	1	0.7
	2	M	2	0.9
	3	M	3	0.4
	4	F	1	0.7
	5	F	2	0.4
	6	F	3	0.8
MEAN				0.6
SD				0.2
DC	7	M	1	1.6
	8	M	2	1.7
	9	M	3	1.9
	10	F	1	1.9
	11	F	2	1.6
	12	F	3	1.8
MEAN				1.8
SD				0.1



Graph 2: CRP Values

Table No. 3 – SGOT Values

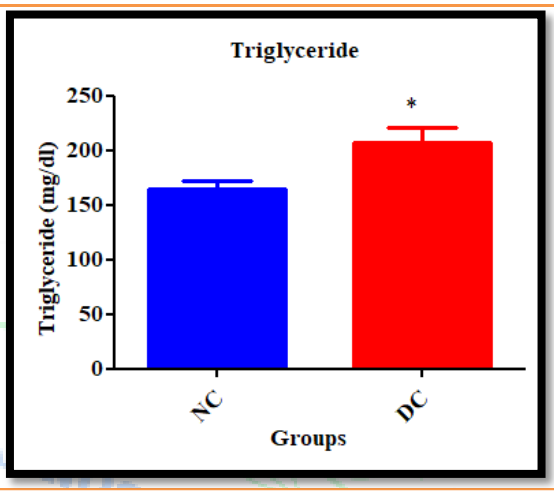
Date:07/Feb/2024				SGOT
Group	Animal No	Sex	Marking	U/L
NC	1	M	1	54.0
	2	M	2	55.1
	3	M	3	52.4
	4	F	1	48.6
	5	F	2	51.5
	6	F	3	65.4
MEAN				54.5
SD				5.8
DC	7	M	1	104.8
	8	M	2	172.2
	9	M	3	123.3
	10	F	1	104.4
	11	F	2	112.4
	12	F	3	128.6
MEAN				124.3
SD				25.4



Graph 3: SGOT Values

Table No. 4 – Triglyceride Values

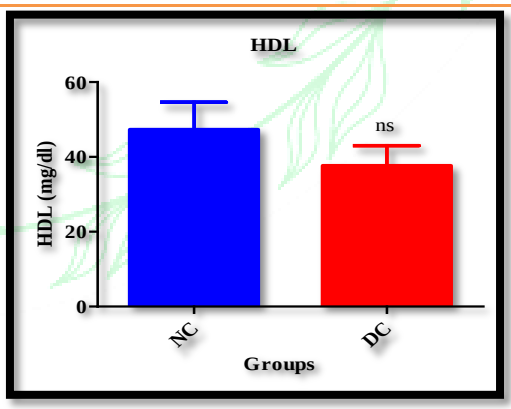
Date:07/Feb/2024				TGL
Group	Animal No	Sex	Marking	mg/dl
NC	1	M	1	170.7
	2	M	2	186.2
	3	M	3	140.9
	4	F	1	164.5
	5	F	2	183.1
	6	F	3	142.2
MEAN				164.6
SD				19.5
DC	7	M	1	182.0
	8	M	2	206.5
	9	M	3	157.8
	10	F	1	239.3
	11	F	2	242.5
	12	F	3	214.1
MEAN				207.0
SD				32.9



Graph 4: Triglyceride Values

Table No. 5 – HDL Values

Date:07/Feb/2024				HDL
Group	Animal No	Sex	Marking	mg/dl
NC	1	M	1	34.2
	2	M	2	28.5
	3	M	3	32.1
	4	F	1	70.4
	5	F	2	61.1
	6	F	3	57.8
MEAN				47.4
SD				17.8
DC	7	M	1	18.5
	8	M	2	35.7
	9	M	3	30.6
	10	F	1	39.0
	11	F	2	44.7
	12	F	3	57.4
MEAN				37.7
SD				13.2



Graph 5: HDL Values

Sr. N.	Group /Slide Code	Histopathological observations 1.Heart	Overall Pathological Grade
1	NC-1M	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart tissue.	NAD
2	NC-2F	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart tissue.	NAD
3	DC_3M	Mild degenerative changes in the cardiac muscle fibers. Multifocal areas of congestion and occasional foci of interstitial hemorrhages in pericardium and myocardium region.	Mild (+2)
4	DC-4F	Mild degenerative changes in the cardiac muscle fibers with focal areas of congestion and occasional foci of interstitial hemorrhages in pericardium and myocardium region.	Minimal (+1) to Mild (+2)

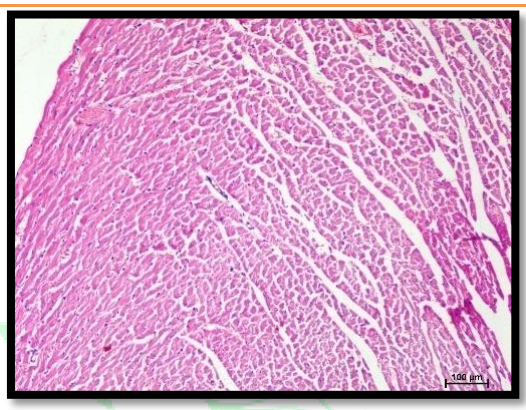


Image 1: Cardiac Biopsy – NCM

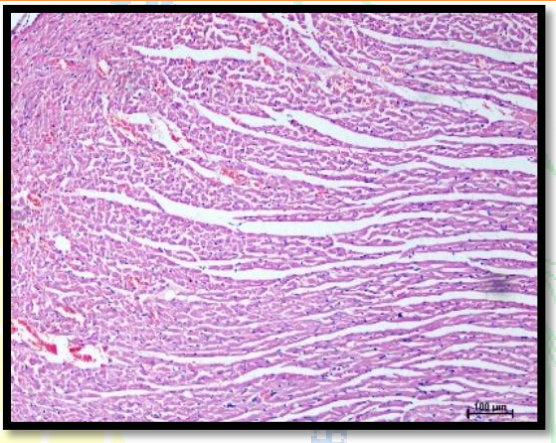


Image 2: Cardiac Biopsy – DCM

Discussion :

The mean CPK-MB observed in normal control is 415.5 U/L whereas in Disease control, it is 704.1 U/L. Statistically significant increase in CPK-MB is observed. CPK -MB is creatine phospho kinase myocardial bound enzyme primarily found in heart muscle. It is used to detect the heart muscle damage in conditions such as myocardial infarction. It is released in blood circulation when heart muscle is damaged. The normal reference range of CPK-MB in rats is 5 to 25 IU/L. Level. The level 5 times higher than the normal range will be considered significant rise.The mean CRP observed in normal control is 0.6 mg/L whereas in Disease control, it is 1.8. mg/L. Statistically significant increase in inflammatory biomarker C-Reactive Protein is observed. CRP is a protein produced in the liver in

response t the inflammation and it is used to assess the presence of inflammation in the body. HS-CRP is more specific to the cardiac tissue injury. The normal reference range of CRP in rats is 300 to 600 mg/ml. Level. The level higher than the normal range will be considered significant rise.

The mean SGOT observed in normal control is 54.5 U/L whereas in Disease control, it is 124.3 U/L. Statistically significant increase in SGOT is observed. Serum Glutamic Oxaloacetic Transferase is an enzyme found in the liver and heart tissue. It's rise in blood indicates either liver or heart muscle injury. The normal reference range of SGOT in rats is 5 to 40 IU/L. Level. The level higher than the normal range will be considered significant rise.

The mean triglyceride observed in normal control is 164.6 mg/dl whereas in Disease control, it is 207.0 mg/dl. Statistically significant increase in triglyceride is observed. Triglyceride is the stored form of fat which is used to derive energy. Elevated triglyceride level indicate an increased risk for ischemic heart disease. The normal reference range of Triglyceride in rats is 25 to 145 mg/dl. Level. The level higher than the normal range will be considered significant rise.The mean high density cholesterol observed in normal control is 47.4 mg/dl whereas in Disease control, it is 37.7 mg/dl. Statistically significant decrease in protective high density cholesterol is observed. High density lipoprotein is a good cholesterol which helps to prevent the building of bad low density cholesterol in the circulation. HDL picks up the excess LDL cholesterol and send it to liver where it is broken down and eliminated. Low levels of HDL cholesterol indicated increased risk for ischemic heart disease. The normal reference range of HDL cholesterol in rats is 35 to 55 mg/dl. Level. The level higher than the normal range will be considered significant rise.In cardiac biopsy, mild degenerative changes in the cardiac muscle fibers with focal areas of congestion and occasional foci of interstitial hemorrhages in pericardium and myocardium region were seen. This again suggests the cardiac damage induced by the stressors.

Summary & Conclusion :

1. In the DC group, levels of CK-MB, CRP, SGPT, SGOT, Triglycerides were significantly elevated as compared to the NC group.
2. No significant difference was observed in HDL, Total ides (Cl) in the DC group as compared to NC.
3. Histopathology of heart tissue showed no abnormalities in NC group. While in DC group Mild (+2) degenerative changes in the cardiac muscle fibers. Multifocal areas of congestion and occasional foci of interstitial hemorrhages in pericardium and myocardium region were observed
4. On the basis of the ECG parameters like RR interval and QT interval results obtained, it can be concluded that fear can acts as an etiological factor for the development of heart disease in rats.

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