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Evaluation of Effect of Chronic Unpredictable Mild Stress on Albino Rats WSR To Hematology Parameters

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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.

Keywords – *Manasik trass*, *Bhaya* (Anxiety), Hematology Parameters, CVDs

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]

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 [2] [3]. 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 sawdust bedding), continuous illumination. These stressors will be randomly scheduled over a week period and will be repeated maintain the aspect of unpredictability. Hematological parameters like Hb%, RBC count, WBC count, platelet count are PCV, measured to know the effect of stressors on hemopoietic

condition.

Review Of Literature:

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 ^[4] and Syndrome of MI is mentioned by Sushrut Uttartantra 43/131-132 [5] in the form of 'Hrit Shoola'. In the common etiology 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^[6]. 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 and alteration of normal hematological indices.

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 alteration of normal hematological indices.
- Alternate Hypothesis (H0)-

Ayurvedokta Bhaya (fear) does not acts as an aetiological factor for alteration of normal hematological indices.

Material & Methodology:

Study Design:

- Center of Study Dept of Roga Nidana & Vikrutvigyana, Government Ayurvedic College, Nanded And National Testing Centre, Pune
- Study Population And Sampling:
 Animal required for the Study
- Species/Common name -
- **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			
V 5	Chronic			
Disease Control	unpredictab <mark>le mild</mark>			
	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 will be 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 will be randomly scheduled over a one week period and

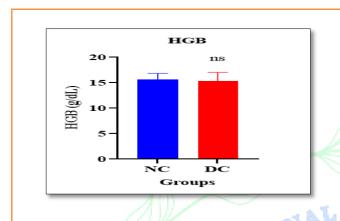
will be repeated to maintain the aspect of unpredictability. After confirmation of stress in animals, Hematological investigations were done.

Assessment Criteria:

- The normal reference range of HB% in rats is 10.4 to 16.5 g/dl. The level lower than the
 normal range is considered to be significant
 decrease.
- 2. The normal reference range of **PCV Value** in rats is **18 to 48%**.
- 3. The normal reference range of RBC Value in rats is 3.5 to 7.5 $10^{12}/L$ or 3.5 to 7.5 x $10^6/\mu L$.
- 4. The normal reference range of Platelet Value in rats is 107 to $61510^9/L$. or 1.7 to 5.57 x $10^6/\mu L$.
- 5. The normal reference range of WBC Value in rats is 4.44 to 14.8 10 L or 4400 to 14800/cumm.
- 6. The normal reference range of Neutrophil count in rats is 13 to 63% or 1.3 to 6.3 10⁹/L.
- 7. The normal reference range of Lymphocyte count in rats is 61 to 86 % or 6.1 to 8.6 10⁹/L.

Observation & Result: Table No. 1 – Hb% Values (G/Dl)

Die 110.1 Hb / V tildes (G/D1)						
	HGB					
Group	Animal No	Sex	Marking	g/dL		
	1	M	1 '	15.3		
	2	M	2	17.3		
NC	3	M	///3	15.8		
NC	4	F	1	15.8		
-	5	F	2	16.1		
	6	F	3	13.8		
MEAN	1/2			15.7		
SD				1.1		
	7	M	1	16.2		
	8	M	2	16.6		
DC	9	M	3	16.3		
DC	10	F	1	16.2		
	11	F	2	14.5		
	12	F	3	12.2		
MEAN				15.3		
SD				1.7		



Graph No.1: Hb% Values (G/Dl)

Table No. 2 – RBC Value (10¹²/L) :

	A				
1/\		. 72	RBC		
Group	Animal No	Sex	Marking	*10 ¹² /L	
NC	1 1	M	1	7.7	
- W/	2	M	2	8.4	
Y	3	M	3	8.2	
	4	F	1	7.7	
$\mathbb{N}I$	5 🥞	F	2	8.4	
NV a	6	F	3	7.1	
MEAN			1	7.9	
SD				0.5	
DC	7	M	1	8.7	
	8	M	2	8.6	
	9	M	3	8.5	
M IB	10	F	1	8.0	
	11	F	2	8.1	
$\langle MD \rangle$	12	F	3	6.2	
MEAN			J JGC	8.0	
SD				0.9	

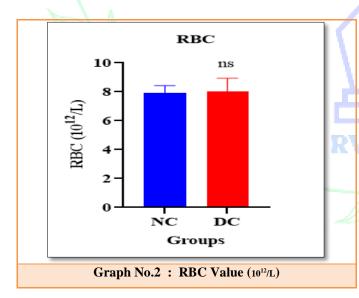


Table No. 3 – Haematocrit Values (%):

Group	Animal No	Sex	Marking	%	
NC	1	M	1	64.6	
	2	M	2	70.5	
7	3	M	3	67.0	
	4	F	1	64.7	
	5	F	2	68.1	
740	6	F	3	59.0	
MEAN	i Se			65.7	
SD				3.9	
DC	7	M	1	69.8	
1 /	8	M	2	68.8	
7 /	9	M	3	68.7	
_ /	10	F	1	69.6	
	11	F	2	63.8	-
	12	F	3	51.5	
MEAN				65.4	M
SD				7.1	

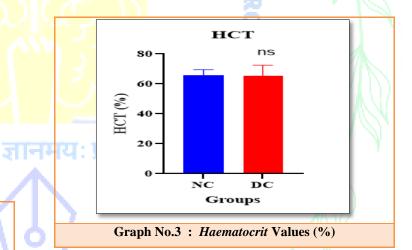
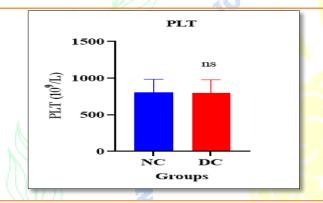


Table No. 4 – Platelet Values (109/L):

		PLT			
A	Group	Animal No	Sex	Marking	*10 ⁹ /L
	NC	1	M	1	1116.0
	7	2	M	2	726.0
1		3	M	3	804.0
		4	F	1	565.0
		5	F	2	838.0
		6	F	3	782.0
	MEAN				805.2
	SD				180.1

	PLT			
Group	Animal No	Sex	Marking	*10 ⁹ /L
DC	7	M	1	935.0
	8	M	2	846.0
	9	M	3	927.0
	10	F	1	671.0
	11	F	2 / //	919.0
	12	F	3	484.0
MEAN	411			797.0
SD				182.8



Graph No.4: Platelet Values (10°/L)

Table No. 5 - MID Value $(10^9/L)$:

			1	MID
Group	Animal No	Sex	Marking	*10 ⁹ /L
NC	1	M	1	1.6
	2	M	2	2.4
\ \	3	M	3	1.1
1	4	F	1	1.4
-	5	F	2	1.2
	6	F	3	1.8
MEAN				1.6
SD				0.5
DC	7	M	1	3.0
	8	M	2	1.3
	9	M	3	2.7
	10	F	1	0.5
	11	F	2	0.8
	12	F	3	1.1
MEAN				1.6
SD				1.0

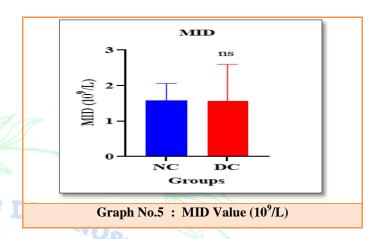


Table No. 6 – Total WBC Value (109/L):

	/ "			WBC
Group	Animal No	Sex	Marking	*10 ⁹ /L
NC	1	M	1 1 \	17.5
	2	M	2	17.5
ームベ	3	M	3	15.4
・、ノ)・	4	F	1	16.1
	5	F	2	16.3
Γ(,)	6	F	3	13.9
MEAN	\$1	- 10		16.1
SD				1.4
DC	7	M	1	31.9
	8	M	2	18.2
	9	M	3	21.3
T. 172	10	F	1	6.4
ाः प्रदा	11	F	2	10.5
	12	F	3	11.2
MEAN				16.6
SD				9.3
			7 W	

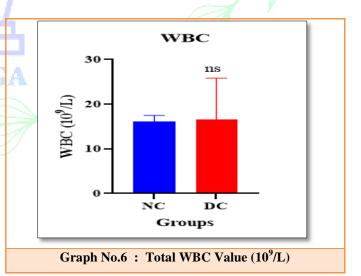
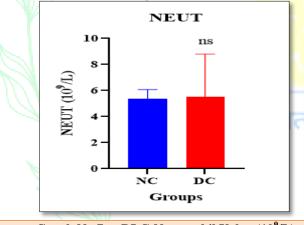


Table No. 7 – DLC-Neutrophil Value (109/L):

				NEUT
Group	Animal No	Sex	Marking	*10 ⁹ /L
NC	1	M	1	4.8
	2	M	2	6.3
	3	M	3/	4.8
	4	F	1	5.3
	5	F	2	6.2
	6	F	3	4.8
MEAN			, Q	5.4
SD				0.7
DC	7	M	1	11.0
	8	M	2	6.5
	9	M	3	6.8
	10	F	1	2.0
W.	/ 11 🍣	F	2	3.2
	12	F	3	3.6
MEAN	R		ح/	5.5
SD				3.3

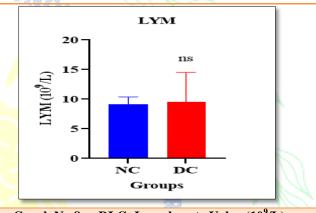


Graph No.7: DLC-Neutrophil Value (10⁹/L)

Table No. 8- DLC- Lymphocyte Value (109/L):

	LYM					
Group	Animal No	Sex	Marking	*10 ⁹ /L		
NC	1	M	1	11.1		
	2	M	2	8.8		
	3	M	3	9.5		
	4	F	1	9.4		
	5	F	2	8.9		
	6	F	3	7.3		
MEAN				9.2		
SD				1.2		

Group	Animal No	Sex	Marking	*10 ⁹ /L		
DC	7	M	1	17.9		
	8	M	2	10.4		
	9	M	3	11.8		
	10	F	1	3.9		
	11	F	2	6.5		
16	12	F	3	6.5		
MEAN			×.	9.5		
SD				5.0		



Graph No.8: DLC- Lymphocyte Value (10⁹/L)

Discussion:

The mean Hb% observed in normal control is 15.7 g/dl whereas in Disease control, it is 15.3 g/dl. Statistically not much significant decrease in Hb% is observed. This Hb% count is in the normal range. The mean RBC count observed in normal control is 7.9 10¹²/L whereas in Disease control, it is 8 10¹²/L. No much variation in the values of RBC count was observed in NC and DC group. This RBC count is in the normal range.

The mean *Haematocrit /* **PCV** observed in normal control is 65.7% whereas in Disease control, it is 65.4 %. No much variation in the values of PCV count was observed in NC and DC group.

The mean **Platelet value** observed in normal control is 805.2 **10**⁹/**L** whereas in Disease control, it is 797 **10**⁹/**L**. No much variation in the values of

Platelet count was observed in NC and DC group.

The mean **MID value** observed in normal control is 1.6 **10**⁹/**L** whereas in Disease control, it is 1.6 **10**⁹/**L**. No variation in the values of Platelet count was observed in NC and DC group.

The mean **WBC** value observed in normal control is 16.1 10⁹/L whereas in Disease control, it is 16.6 10⁹/L. No variation in the values of WBC count was observed in NC and DC group.

The mean **Neutrophil count** observed in normal control is 5.4 **10**9/L whereas in Disease control, it is 5.5 **10**9/L. No variation in the values of Neutrophil count was observed in NC and DC group.

The mean **Lymphocyte count** observed in normal control is 9.5 **10**9/**L** whereas in Disease control, it is 9.5 **10**9/**L**. No variation in the values of Lymphocyte count was observed in NC and DC group.

Summary & Conclusion:

There is no much variation in the values of blood indices were noticed in NC and DC group. Hence, it can be concluded that *Bhaya* (fear) may not act as an etiological factor for the alteration of hematological parameters in rats.

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Declaration:

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