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# EVALUATION OF CARDIOVASCULAR STRESS OF THE FEMALE WORKERS ENGAGED IN POST HARVESTING TASKS

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## ABSTRACT

A large number of women in India are involved in agriculture, especially in post harvesting tasks. They face a lot of work related stresses during work. The present study was aimed to assess the cardiovascular stress of female workers in different post harvesting tasks and their causative factors. The study was conducted on 30 female workers from different villages of Paschim Medinipur District of West Bengal. The cardiovascular stress index (CSI) was determined from the resting and working heart rates. The work done for each post harvesting tasks was calculated. The highest value of work done was observed during storing of dried paddy grain. The working heart rates of workers for different post harvesting tasks were significantly different. The magnitude of CSI was found to be very high in cases of storing dried paddy and carrying of wet paddy to the oven. The relationship between the magnitude of CSI and work done was noted. It was concluded that the modification of workstation and change in work posture may reduce the cardiovascular stress.

**Key words :** Cardiovascular stress, Post harvesting tasks, female worker.

## INTRODUCTION

Agricultural work is the most primitive type of employment in the world. The women are the backbone of agricultural work force but worldwide their hard work has mostly been unpaid. They do the most tedious and back-breaking tasks in agriculture. About 70% of the Indian women are engaged in agricultural work either in their own fields or as hired labourers (Hasalkar et.al. 2004). In West Bengal about 46% of female populations are involved in agriculture. In Midnapore District (East and West) the participation of women workers is 61% (Census of India, 2001).

Agriculture is also an industry, with certain basic distinction. Primarily, agriculture is seasonal in nature; in addition, the vast unorganized working sectors are constantly confronted with adverse

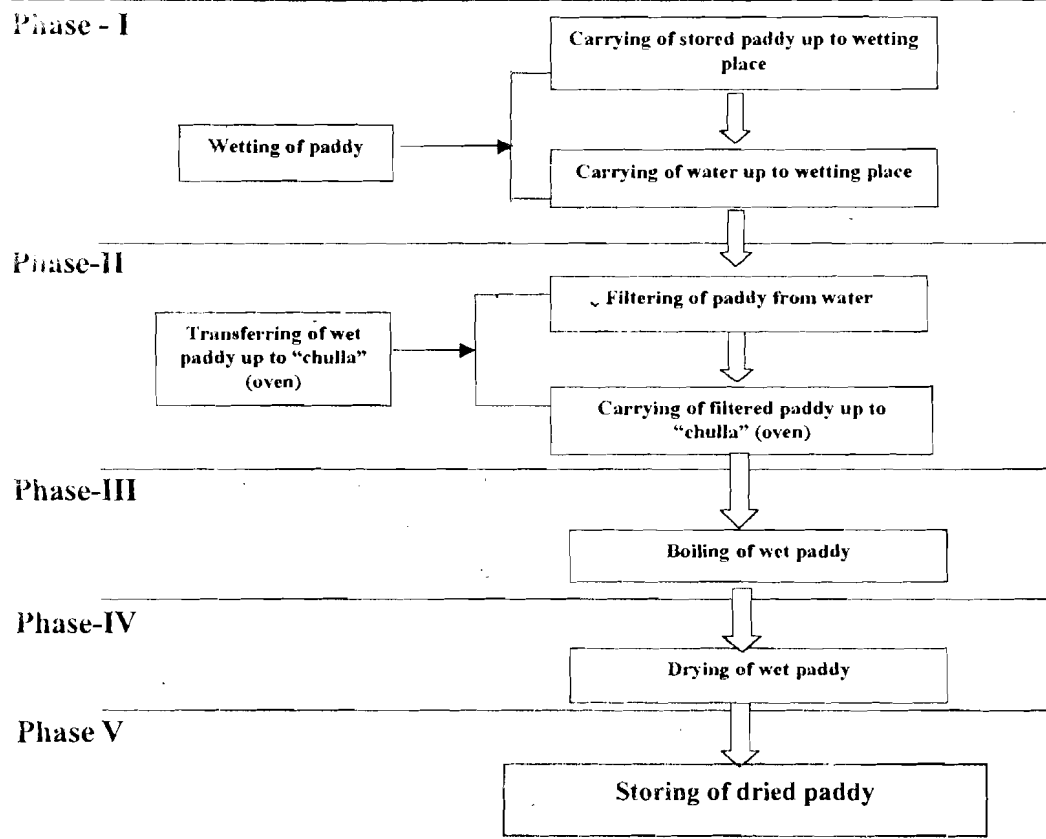
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work environment. The social costs due to ill health and under productivity of the sector are real and substantial.

Agricultural tasks in India, particularly rice cultivation, are carried out mainly through manual efforts. Though the agricultural workforce is by far the major work force in the third world countries, its work organization has not received much attention.

Post harvesting tasks are essential for the preparation of raw food materials. Among all tasks post harvesting tasks are mainly performed by women. The rice cultivation is the most popular agricultural activity in India. In rice cultivation, post harvesting tasks consists of several steps including threshing, winnowing, storing the paddy grain, preparation for making parboiled rice etc. For making parboiled rice a sequential work pattern is followed to produce boiled and dry grain (paddy), from which rice is made finally. The sequence of their work pattern is given in Fig.1.



**Fig.1: Flow chart of the post harvesting tasks in rice cultivation**

Workers are compelled to adopt different awkward postures during performing various post harvesting activities. Postural stress of the agricultural workers was evaluated (Van Dieen et.al. 1997). In addition, they are exposed to extreme environmental stress (Mc Neil MB et.al. 1999; Budd, 1995; Johnston et.al. 2000). It is also reported that the physically uncomfortable jobs in plant cultivation are traditionally assigned to women (Stoffert and Timme, 1988).

A few studies had been conducted on the physiological response of the Indian agricultural workers. Nag and Dutta (1980) had shown the circulo-respiratory efficiency of some agricultural works. The physiological reactions of the female workers in different agricultural operations were determined and the leisure time activities of those workers were also reported (Nag and Chatterjee, 1981). A number of research groups (Nag and Dutta, 1980; Persson and Kilbom, 1981; Malgeryd, 1988) showed that static work stress also affected the heart beat frequency while performing agricultural tasks.

However, no comprehensive study on post harvesting tasks had been performed in case of rice cultivation. The present investigation was aimed to evaluate the cardiovascular stress of female worker while performing different post harvesting tasks on a comparative basis.

## MATERIALS AND METHODS

### **Selection of site and subjects:**

The study was conducted in different villages of Paschim Medinipur District of West Bengal. Thirty female workers, who were engaged in post harvesting tasks, were selected by random sampling for this study. Their age was into the range of 20 years to 45 years.

### **Evaluation of physiological stress:**

#### ***Measurement of heart rate :***

The resting heart rate of subject was measured by 30 beats time recording method with the help of a stop watch and a stethoscope. Before the measurement of resting heart rate, the subject was asked to take rest for 15 minutes under sitting condition.

The working heart rate of the women workers were recorded by 10 beats time recording method. The working heart rates were taken with the help of stop watch throughout the working period with an interval of half an hour in different tasks of post-harvesting tasks. The mean working heart rate was calculated taking all the heart rate values recorded during work in each activity separately.

### *Measurement of cardiovascular stress index :*

Cardiovascular stress index (CSI) is good parameter for measuring the level of stress imposed on the human body due to work. It is determined by the following formula (Trites et.al. 1993).

$$\text{CSI} = \frac{100 * (\text{Heart rate during work} - \text{Resting heart rate})}{\text{Max. Heart rate} - \text{Resting heart rate}}$$

$$[\text{Max. Heart Rate} = 220 - \text{Age in years}]$$

### *Measurement of workload :*

in different phases of the work the workers had to handle different amount of loads (weight). The amount of load carried in a day was estimated and the total distance covered by them is also estimated. Weight is measured with the help of a weighing machine and a tape was used to measure the distance. Then work done by them was calculated by multiplying the distance covered and the load handled. The amount of load carried plus the body weight was taken as total load.

### *Statistical analysis :*

To test the significance of difference of different parameters, the students t- test was performed (Das and Das, 2004). The ANOVA study and post hock analysis were made by the use of STATISTICA software.

## **RESULT AND DISCUSSION**

There was a wide variation in work done by the workers in different phases of post harvesting tasks. Table 1 showed that work done was the highest in storing phase. In this phase they were required to gather the dried paddy grains and to shift it to the storing place. Work done during carrying of water and carrying of wet paddy up to the oven (chulla) was also high. But incase of drying phase the work done was the lowest among all the phases of post harvesting tasks. In this phase, there was no requirement of carrying load and the body movement was also very less.

**Table 1 : Work done by the women workers in different phases of post harvesting tasks (Mean ± SD).**

Sl.No	Post Harvesting Tasks	Work-done (kgm)
1.	Carrying of paddy for wetting	360.0 ± 5.67
2.	Carrying of water	2840.0 ± 12.32
3.	Carrying of wet paddy up to oven (chulla)	4270.5 ± 9.03
4.	Drying	278.13 ± 3.41
5.	Storing	5488.0 ± 10.05

The working heart rate of the female workers in different phase of post harvesting tasks has been presented in Table 2. It has been noted that the heart rate increased significantly from the resting level in different phases of the post harvesting tasks. Among the different phases the mean working heart rate was the highest in storing phase. Besides this, the heart rate in water carrying phase and wet paddy carrying phase were also high. But in case of boiling phase the working heart rate was the lowest among all the phases. The heart rate of female workers in different phases of making parboiled rice is significantly different from their resting heart rate.

According to the classification of physical work in terms of heart rate (Christensen, 1964) the task for carrying of paddy for wetting, filtering, boiling and drying might be considered as the moderate work. But carrying of water, carrying of wet paddy up to oven (chulla) and storing activity belonged to the heavy work category.

**Table 2 : Resting and working heart rate and work pulse of women workers in different phases of post harvesting tasks (Mean  $\pm$  SD).**

Sl.No	Post Harvesting Tasks	Mean working heart rate (b/min)	Work Pulse (b/min)
1.	Resting	77.50 $\pm$ 4.98	
2.	Carrying of paddy for wetting	108.29 $\pm$ 14.71*	30.79
3.	Carrying of water	124.20 $\pm$ 11.19*	46.7
4.	Filtering	103.00 $\pm$ 16.39*	25.5
5.	Carrying of wet paddy up to oven (chulla)	124.35 $\pm$ 15.64*	46.85
6.	Boiling	97.08 $\pm$ 9.04*	19.58
7.	Drying	108.31 $\pm$ 9.52*	30.81
8.	Storing	129.0 $\pm$ 12.63*	51.5

\* $p < 0.001$  w.r.t. resting heart rate

The mean working heart rates of different phases of work were statistically analyzed and are presented in Table 3. From the ANOVA study, it has been noted that there was a significant difference of working heart rate among all the phases of post harvesting tasks.

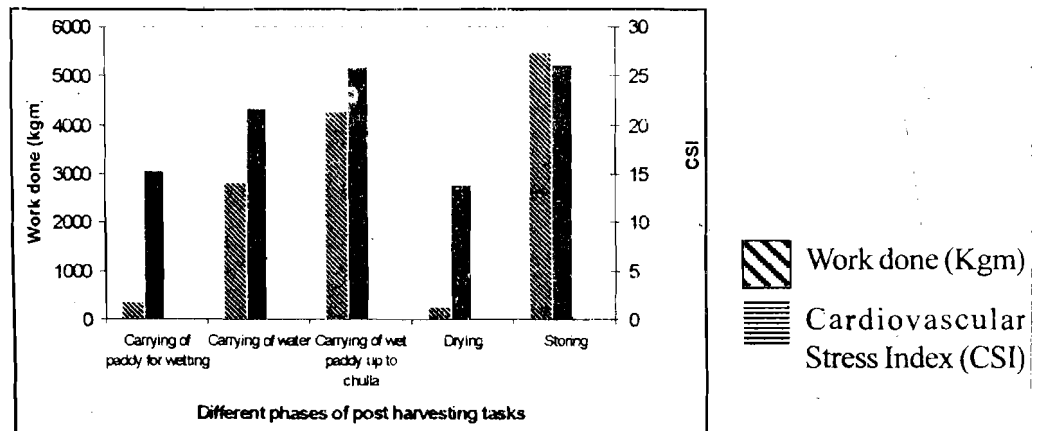
**Table 3: Analysis of variance of working heart rate among different phases of tasks.**

Source of variance	SS	df	SW	F
Between group	18124	6	3020.69	51.338
Within group	7825.6	133	58.839	
Total	25950	139		

**Table 4: Post hoc analysis of working heart rate between different phases of tasks.**

	Mean	Carrying of paddy	Carrying of water	Filtering up to oven	Wet paddy	Boiling	Drying
Carrying of paddy	108.30	MD	MD	MD	MD	MD	MD
		S	S	S	S	S	S
Carrying of water	124.20	15.90					
		P<0.001					
Filtering	103.0	5.30	21.2				
		NS	P<0.001				
Wet paddy up to oven	124.35	16.05	0.15	21.35			
		P<0.001	NS	P<0.001			
Boiling	97.08	11.22	27.12	5.92	27.27		
		P<0.001	P<0.001	NS	P<0.001		
Drying	108.32	0.02	15.88	5.32	16.03	11.24	
		NS	P<0.001	NS	P<0.001	P<0.001	
Storing	129.0	10.7	4.80	26.0	4.65	31.92	20.68
		P<0.001	NS	P<0.001	NS	P<0.001	P<0.001

Therefore, a post hoc analysis of working heart rate between different phases of tasks was also made (Table 4). From this analysis it has been noted that in paddy carrying phase there was significant difference ( $p<0.001$ ) in working heart rate when compared to that of carrying water, carrying wet paddy up to oven (chulla), boiling and storing phases. But in storing phase there was no significant difference of working heart rate with water carrying phase and wet paddy carrying phase.

**Fig 2: Workload and cardiovascular stress index of women workers engaged in different**



From the Table 5 cardiovascular stress indexes were calculated from the resting and working heart rates and it has been revealed that workers exposed to different degree of cardiovascular stresses. The highest CSI was found in the storing phase. The t- test was performed to find the level of significance in the difference of the mean CSI values between storing task and other tasks. The results showed that there was no significant difference in CSI between storing task and carrying wet paddy to oven. The CSI in all other activities was significantly different from that of storing task. Differences of cardiovascular stress index at different phases of tasks indicated that all tasks are not equally stressful to the worker. In the storing phase, the workers required to cover a long distance, which might increase the cardiovascular stress. The same results also noted in

cases of carrying of water and carrying of wet paddy. The cardiovascular stress may be related to the amount of work done by the workers.

**Table 5: Cardiovascular stress index (CSI) at different working tasks**

(Mean  $\pm$  SD).

Sl.No	Post Harvesting Tasks	CSI
1.	Carrying of paddy for wetting	15.39 $\pm$ 10.33**
2.	Carrying of water	21.60 $\pm$ 8.43*
3.	Filtering	14.23 $\pm$ 9.32**
4.	Carrying of wet paddy up to oven (chulla)	25.93 $\pm$ 7.42
5.	Boiling	9.86 $\pm$ 4.04**
6.	Drying	13.88 $\pm$ 4.40**
7.	Storing	26.11 $\pm$ 3.16

\*p<0.05 \*\*p<0.001 w.r.t. storing task

The relative change of CSI and the amount of work done have been compared in different post harvesting tasks (Fig 2). It has been noted that the task in which the work done was low and the CSI was also low and higher value of CSI was observed in those tasks where work done was comparatively lower. Further it has also been noted that the magnitude of CSI tends to be increased where the tasks is performed with postural stress.

### CONCLUSION

The present study indicates that the women workers have different degree of physiological stress in different post harvesting tasks. In order to reduce the cardiovascular stresses of the worker, proper designing and lay out arrangement of different parts of the workstation like, wetting place, chulla, drying place, source of water etc should be done. The distance between these should be reduced to decrease their workload.

Use of different hand tools may decrease the work done and cardiovascular stress. For example, use of paddy puller during drying phase may reduce the physiological stress of the workers. In working period bend posture demands relatively higher energy output than in sitting posture. So, it may be suggested that when the work can be done in sitting posture in the jobs like bend posture should be avoided.

**Acknowledgement:** The author are grateful to DST (Science and Society Division), New Delhi for financial assistance.

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# CUT OFF POINT FOR MATERNAL WEIGHT OF TERM LOW BIRTH WEIGHT NEONATES AMONG BENGALLEES OF KOLKATA, INDIA: ASSESSED BY TWO METHODS

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## ABSTRACT

This study was conducted in a government hospital in Kolkata on 139 mother-baby pairs. Data on age, history of last menstrual period and medical disorder were collected. Mother and newborn measurements were recorded within 24 hours of delivery. Results revealed that mean  $\pm$  SD maternal weight, birth weight and length were  $47.5 \pm 7.0$  kg and  $2601 \pm 377$  g and  $47.8 \pm 1.9$  cm, respectively. The difference in mean weight between mothers who delivered LBW and normal birth weight (NBW) babies were statistically significant ( $t = 8.20$ ,  $p < 0.001$ ). Overall, the prevalence of LBW was 39.6 %. Higher incidence of LBW and lower mean birth weight and length was observed in first quartile or low weight mothers. The rates of LBW decreased and mean birth weight and length increased significantly with increasing maternal weight.

Logistic regression analyses using maternal weight as an independent variable correctly classified 70.9 % and 82.1 % of LBW and NBW cases, respectively. Overall, 77.7% of all cases were correctly classified. Results of Linear regression analysis revealed that maternal post delivery weight had significant impact ( $B = 0.02667$ ,  $t = 7.075$ ) on birth weight. Moreover, maternal weight accounted for 43.3% variation in birth weight after controlling for gestational age.

The receiver operating characteristics (ROC) curve showed that maternal weight of  $< 40.5$  kg was the best cut-off for detecting term-LBW with 80 % sensitivity and 85 % negative predictive power. After validation studies among a larger sample, antenatal caregivers of health institutions and community health workers in the field can use this cut-off value for screening pregnant women at early second trimester of pregnancy, in this ethnic group.

**Key words :** Bengalees; Pregnancy; Maternal weight, Low birth weight, ROC.

## INTRODUCTION

Newborns having a birth weight of less than 2500g are defined as low birth weight

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(LBW) neonates. This is the universally accepted cut-off point provided by World Health Organization (WHO, 1984). LBW is a consequence of intrauterine growth retardation (IUGR) or preterm birth (born before 37 weeks) or in combination of both. LBW babies demonstrate significant growth retardation, as reflected by low body weights, heights and head circumference in comparison to normal weight peers. There is evidence of delayed skeletal growth and maturation in these children (Villar et al. 1990; WHO 1992). Growth retarded adult women (stunted and underweight) are likely to give birth to LBW babies thereby perpetuating a vicious cycle through generations (Ashworth and Feachem 1985).

The prevalence of LBW is high in South Asia including India. In India the rate of LBW is 30% (UNICEF 2004). In urban slums, deprived populations have consistently recorded the highest prevalence of LBW. The weight of an infant at birth is an important indicator of maternal health and nutrition during pregnancy. In developing countries with a higher incidence of LBW, IUGR is a major component of LBW compared to prematurity. IUGR component of LBW is related to nutritional parameter of the mother, such as weight before and during pregnancy (WHO 1995).

It is well established that undernourished women are more prone to have LBW babies (Kramer 1987; Bisai 2004) and pregnancy complications (Baird 1947). Women among less privileged communities in India are malnourished (Barros et al. 1987; Samuel and Rao 1992). A Multi-Center Study (WHO 1995) has provided cut-off values of maternal anthropometry as a risk for poor infant outcome; i.e. pre-pregnancy weight less than 45.0 kg. The cut off for IUGR birth is maternal pre-pregnancy weight of less than 40.0 kg. Similarly the incidence of pre-term deliveries is higher in mothers who are above 50.0 kg of pre-pregnancy weight. At a cut-off point of 48.0 kg for maternal weight, the association with full-term LBW becomes stronger. Based on an earlier study from India (WHO 1995), it has been suggested that maternal pre-pregnancy weight below 41.0 kg or less than first quartile was associated with a higher incidence of LBW.

It must be noted here that most of the government health institutions in India provide antenatal care on or after 12 weeks of gestation onwards. Moreover, in the Indian scenario, only mothers' weights are measured during antenatal checkup. Mothers' heights are not recorded on a routine basis in health institutions. Therefore, an efficient cutoff point is essential for screening pregnant women in different weight for gestational age. Antenatal caregiver of health institutions can use the cut-off value as a screening tool for the identification of the target group.

The aim of the present study is to provide efficient cut off point for maternal pregnancy weight as a screening tool to detect LBW baby which applicable for both hospital and community settings.

## MATERIALS AND METHODS

The cross-sectional study was conducted in a government general hospital in South

Kolkata. It is formerly known as Calcutta, the provincial capital of West Bengal. This hospital serves the needs of lower-to-lower middle class socio-economic people. A total of 176 mother-baby pairs were consecutively examined over a period of four months since May 2004. Those pair lying on the bed after delivery in the obstetric ward. Of these, 139 (79.0 %) term babies who met the recruitment criteria were included in the study, 37 (21.0 %) were preterm baby have been excluded from the present analysis. During the time of inclusion of subject, three criteria were used to check that (a) women did not suffer from any severe medical disorder even before pregnancy; (b) baby did not suffer from any congenital malformation or sickness during the time of examination and (c) singleton live born baby by normal delivery. Data were collected by one to one interview of mothers for confirmation age (completed years), history of last menstrual period (LMP), and medical history, respectively. Gestational age was assessed by Ballard's (1977) physical and neurological maturity scoring method within 24 hours of birth and then matched with gestational age as calculated from the history of LMP. The accounted (average value of both methods) gestational age in completed weeks was used for classification of maturity such as preterm, term and post term, respectively. A scatter plot (figure not shown) revealed very good agreement between the two methods (Ballard's and gestational age by LMP).

Mother-baby pair was examined and measurements were taken within 24 hours of delivery following stabilization. Maternal weight was made and recorded by Salter bathroom scale with minimum clothing to the nearest 100g. Measurements of birth weight of newborns, to the nearest 1g, were made without clothing by triple beam balance (Industrial Trading Co.). Both scales were calibrated daily using standard weight and checks to ensure zero error before weighing each subject. Length of baby was measured using infantometer to the nearest 0.1 cm.

Maternal weight was further divided into following four maternal weight group (MWG) based on quartile cutoff values of maternal weight. The corresponding cut-off values for MWG I, MWG II, MWG III and MWG IV were ( $\leq 42.9$  kg), (43.0 - 47.3 kg), (47.4– 51.6 kg) and ( $> 51.6$  kg), respectively. Normal birth weight (NBW) and LBW newborns were classified on the basis of birth weight greater than equal to 2.5 kg or less than 2.5 kg, respectively. Babies' length at birth less than 47 cm was considered as short (Jelliffe 1978).

Ethical approval and prior permission was obtained from Society for Applied Studies Ethics Committee for the study protocol, before commencement of study. Informed written consent was also obtained from those mothers willing to participate in the study.

Data entry and statistical analysis were done using the EPI-INFO (Dean et al 1995) and MEDCALC (2006) Software. One-way analysis of variance - Scheffe's procedure (Mascie-Taylor 1994a; 1994b) was used to study difference between groups for continuous variables. Chi-square test was used to study the significance of difference

between proportions of categorical outcome and t-test was used to test the compare mean between two groups. Pearson correlation coefficients were used to examine the relation between maternal weight and newborn birth weight. Both linear and logistic regression analyses were done to determine the impact of independent variables on birth weight.

Sensitivity, Specificity, positive predictive value and negative predictive value was calculated by standard statistical method for screening the cut-off point. Receiver operating characteristics (ROC) curve (Metz 1978) was utilized to assess the efficiency of maternal weight in detecting LBW. MEDCALC software was used to test the significance for the areas under the ROC curves (AUC). Cut-off value was also calculated using linear regression equation. Significant level was considered p value less than 0.05.

## RESULTS

The mean (standard deviation) for the total and quartile cutoff values of maternal age, body weight, gestational age and newborn birth weights and lengths were presented in *Table 1*. The mean (SD) age of mother was 22.3 (3.5) years. The mean gestational age was 38.7 (1.5) weeks (average of two methods). No significant difference was found between mean gestational age calculated from the history of LMP and assessment by Ballard's method ( $t = 0.714$ ,  $p = 0.475$ ). Mean maternal weight was 47.5 (6.6) kg; 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> percentile maternal weights were 37.4 kg, 47.3kg and 51.6 kg. The mean birth weight was 2601 (377g); 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> percentile of newborn birth weight were 2338 g, 2597 g and 2878 g, respectively. Similarly, mean length of baby was 47.8 (1.9 cm); 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> percentile of newborn length were 46.5 cm, 48.0 cm and 49.1 cm, respectively. Overall the prevalence of LBW and short birth length were 39.6% and 33.8 %, respectively.

**Table 1: Mean and standard error of mean for the total and percentile cutoff values of maternal age, weight, gestational age and newborn birth weight.**

Variables	Mean (n = 139)	Quartile cutoff		
		25	50	75
Maternal age (year)	22.3 (3.5)	20.0	22.0	24.0
Gestational age (week)	38.7 (1.5)	38.0	39.0	40.0
Maternal weight (kg)	47.5 (6.6)	42.9	47.30	51.60
Birth weight (g)	2601 (377)	2338	2597	2878
Length of baby(cm)	47.8 (1.9)	46.5	48.0	49.1

*Standard deviations are presented in parentheses.*

*Table 2* presents the results of the analysis of variances of newborn birth weight and length by maternal weight group. There existed significant MWG difference in mean birth weight ( $F_{(df1=3,df2=135)} = 26.58, p < 0.001$ ) and length of baby ( $F_{(df1=3,df2=135)} = 22.37, p < 0.001$ ). The lowest mean birth weight and birth length was observed in MWG I while the higher was observed in MWG III, the difference being 490g and 3cm. There was a significant difference in the rate of LBW ( $\chi^2 = 57.22, p = 0.001$ ) between the four MWG. The highest rate (86.5 %) of LBW was observed in the first group. The prevalence of LBW was significantly lower in MWG II (37.1%), MWG III (29.4%) and MWG IV (3.0 %). Moreover, data showed that 37.4 % women had weight of less than 45.0 kg. Among them, 75.0 % women delivered LBW babies.

**Table 2: Mean birth weight and percentage of LBW by maternal weight.**

Maternal weight group	Sample size (n)	Mean birth weight (g)*	Length of baby (cm)**	LBW** (%)
MWG-I	37	2286 (317)	46.3 (1.6)	86.5
MWG-II	35	2600 (313)	48.0 (1.6)	37.1
MWG-III	34	2626 (318)	48.0 (1.5)	29.4
MWG-IV	33	2931 (253)	49.3 (1.6)	3.0

Standard deviation is presented in parentheses.

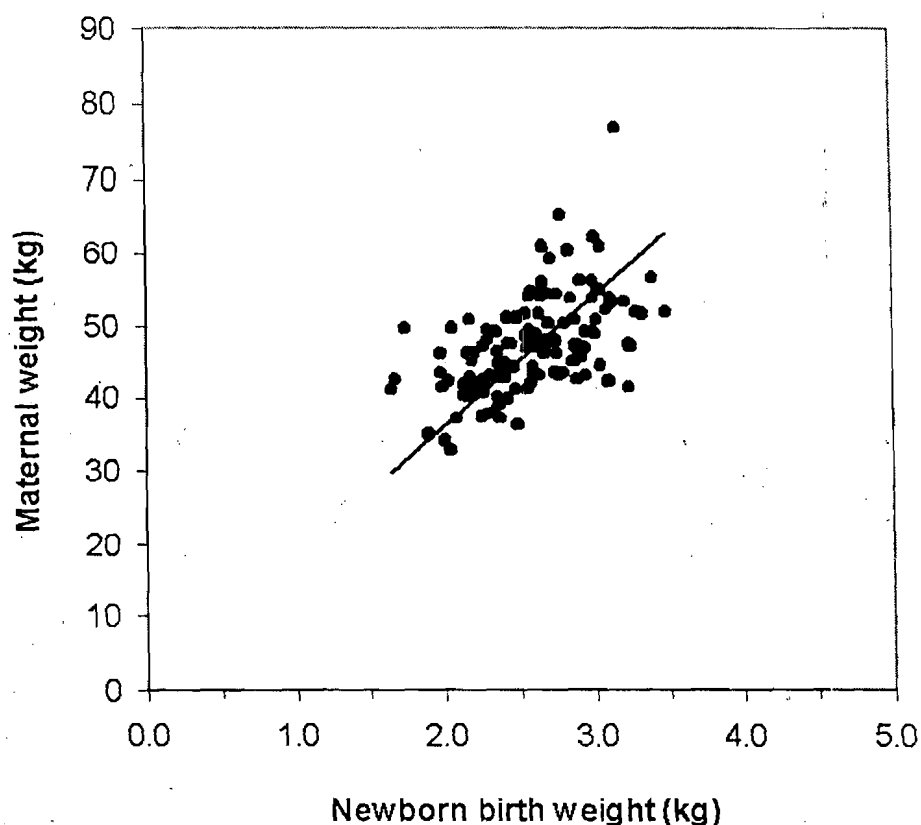
\*  $F(df1=3,df2=135) = 26.58, p < 0.001$ ,

\*\*  $F(df1=3,df2=135) = 22.37, p < 0.001$ ,

\*\*\*Chi-square ( $df=3$ ) = 57.22,  $p < 0.001$ .

The relationship between maternal weight and newborn birth weight is shown in *Figure 1*. It was observed that maternal weight was significantly positively correlated with newborn birth weight ( $r = 0.55, p < 0.001$ ) and birth length ( $r = 0.51, p < 0.001$ ). Regression equation for maternal weight predicted 32 g and 0.2 cm increase in birth weight and birth length for each kg increase in maternal weight. After controlling for gestational age, maternal post delivery weight had remain significant impact on birth weight ( $B = 0.02667, t = 7.075$ ) and birth length ( $B = 0.147, t = 6.916$ ). Thus, maternal weight accounted for 43.3 % and 25.3 % of variation in birth weight and birth length, respectively.





**Figure 1: Relationship between maternal weight and newborn birth weight.**

Means and 95 % confidence interval (CI) of maternal age, gestational age and body weight by birth weight category (LBW Vs NBW) were presented in **Table 3**. The mean ages of the two groups of mothers (LBW: 21.6; NBW: 22.7) were similar ( $t = 1.728$ ,  $p = 0.086$ ). In contrast, difference in mean maternal weight ( $t = 8.20$ ,  $p < 0.001$ ) and gestational age ( $t = 4.658$ ,  $p < 0.001$ ) between two groups of mothers was statistically significant. As expected, mothers having LBW newborns were, on average, 7.68 kg lighter than the mothers who had NBW babies.

**Table 3: Maternal characteristics by newborn birth weight category.**

Maternal Characteristics	LBW (n=55)	NBW (n=84)	t-value
Age (year)	21.6 (20.7-22.6)	22.7 (22.0-23.4)	1.728
Gestational age (week)	38.0 (37.6-38.4)	39.1 (38.8-37.4)	4.658*
Weight (kg)	42.9 (41.7-44.1)	50.6 (49.3-51.8)	8.200*

Confidence Interval (95 %) of mean is presented in parentheses.

\*  $p < 0.001$ .

Logistic regression analysis with gestational age and maternal weight as independent variables showed that these were good predictors of LBW (**Table 4**). Using gestational age as an independently variable, 72.7% of overall cases were correctly classified. Of these, 52.7% and 85.7% of LBW and NBW cases, respectively, were correctly classified. Using maternal weight as an independent variable, 70.9% of LBW and 82.1% of NBW were correctly classified. Overall, 77.7% of all cases were correctly classified. Using these two independent variables together, 80.6% of overall cases were correctly classified. Of these, 74.6% of LBW and 84.5% of NBW were correctly classified.

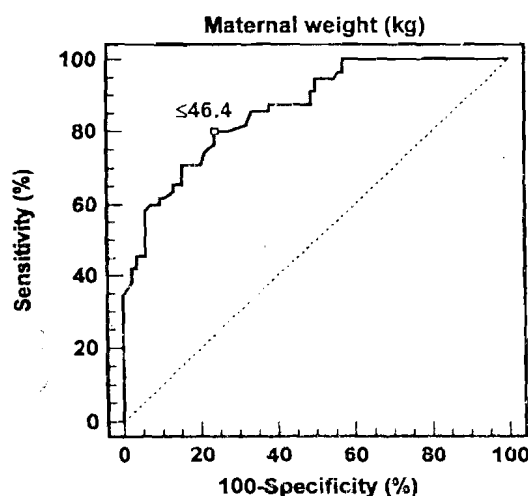
**Table 4: Logistic regression of gestational age and maternal weight with birth weight category.**

Model	Independent Variables	% Classified correctly			B	SeB	Wald
		LBW	NBW	Overall			
1	GA	52.7	85.7	72.7	0.6111	0.1493	16.746*
2	MW	70.9	82.1	77.7	0.3428	0.0601	32.575*
3	GA + MA	74.6	84.5	80.6	0.6271 <sup>a</sup>	0.1894	10.967*
					0.3586 <sup>b</sup>	0.0662	29.334*

GA = Gestational age, MW = Maternal weight.

<sup>a</sup> for GA, <sup>b</sup> for MW, \*  $p < 0.001$

Firstly, a series of sensitivity and specificity for LBW by maternal weight were performed. Cut-off value was obtained at the point marked in the ROC curve (**Figure 2**).



**Figure 2. Receiver operating characteristics curve of low birth weight**

The figure indicates ROC curve (sensitivity Vs 100-specificity) for LBW turned at the point of maternal weight = 46.4 kg. The area under curve (AUC) was 0.863 (CI: 0.794-0.915). Regression equation [Maternal weight (y) = 9.5832 x (birth weight) + 22.58] for birth weight of 2500 g had a corresponding value of maternal weight equal to 46.54 kg. Data showed maternal weight of < 46.5 kg had maximum negative predictive power (85.3 %) for delivering LBW babies compared to maternal weight < 46.0 kg (80.7 %) and < 47.0 kg (84.9 %) (**Table 5**). The sensitivity of maternal weight < 46.5 kg was 80.0 %, similar to maternal weight < 47.0 kg (80.0 %) and higher than maternal weight of < 46.0kg (70.9 %).

**Table 5: Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) for LBW by maternal weight.**

Maternal weight (kg)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
<46.0	70.9	79.8	69.6	80.7
<46.5	80.0	76.2	68.7	85.3
<47.0	80.0	72.6	66.7	84.9

#### DISCUSSION

The prevalence of LBW in the present study was 39.6 %. Previous studies (Bisai 2004; Sen et al 2005; Bisai et al. 2006; Bisai et al. 2007) have reported rates of LBW of 41.0%, 36.0%, 36.6% and 34.0%, respectively. However, using weight criterion of less than equal to 2500 g, Pakrasi et al (1985) had reported a prevalence of LBW of 46.0% among women of Kolkata. The average rate of LBW in India, as reported by UNICEF, is 30% (2004). Overall the rate of LBW in South Asia including India is very high as compared to developed countries. This might be due to several factors including low maternal weight, poor maternal nutrition and other associated factors (WHO, 1982, 1994, 1995). The mean birth weights of these studies varied between 2575 g to 2667 g. Over the period of last 30 years the mean birth weight among women of Kolkata has remained fairly consistent, which is lower than mean birth weight observed from developed countries (WHO 1995). This implies that no considerable progress has been made with regard to increasing birth weight of newborns in Kolkata.

The present investigation revealed that 33.8% of all newborns were short (< 47 cm). Similar results had been earlier reported by Nyaruhucha et al. (2006) from a study conducted in Tanzania. The mean birth length of the present study (47.8 cm ± 1.9) was also similar to that reported in the Tanzanian study (47.0 cm ± 1.8).

The present study also showed mothers who delivered NBW newborns were significantly (7.68 kg) heavier than those mothers who delivered LBW newborns. Similar

findings of 2.9 kg and 3.74 kg higher mean maternal body weight have been earlier reported by Bisai (2004) and Bisai et al. (2007), respectively. Thus, these studies (Bisai 2004; Bisai et al. 2007, and the present study) indicated that the mean weight of mothers delivering LBW newborns varied between 42.9 kg to 45.5 kg.

The present study revealed that a high percentage of mothers were undernourished (as measured by anthropometry) as compared to developed countries. This can be inferred from the fact that in this study more than 37.0 % mothers had a weight of less than 45.0 kg, whereas in the US only 5 % mothers had prepregnancy weight of less than 45.5 kg (The Collaborative Perinatal Study of the National Institute of Neurological Disease and Stroke 1972). Earlier studies among the Bengalee population in the same hospital (Bisai 2004; Bisai et al 2007) had reported that 41 % and 45 % women had post delivery weights of less than 45.0 kg. Among them, 54.2 % and 42.0 % mothers delivered LBW babies. Similarly, in the present study more than 75.0 % these mothers (< 45.0 kg) gave birth to LBW newborns.

The present investigation showed that the weight of mother was significantly correlated with birth weight ( $r = 0.55$ ,  $p < 0.001$ ) and birth length ( $r = 0.51$ ,  $p < 0.001$ ) of newborns (irrespective of sex). Earlier studies by Bisai (2004), Mohanty et al (2006) and Bisai et al (2007) had also reported a significant ( $p < 0.001$ ) correlations between birth weight and maternal weight (Bisai:  $r = 0.25$ , Mohanty et al:  $r = 0.38$ ; Bisai et al:  $r = 0.31$ ).

In the present study, the regression equation for maternal weight predicted 32g increase in birth weight for each unit increase in maternal weight. Similarly, 14g (Bisai 2004) and 17g (Bisai et al 2007) increments in newborn birth weight have been noted for 1 kg higher maternal weight in earlier studies.

A few studies have reported that maternal weights during pregnancy have been shown to be good predictors of birth weight (WHO 1995; Karim and Mascie-Taylor 1997), as well as perinatal survival (Kramer 1987). Most of the studies were able to find out the relationship between birth weight and nutritional status of mothers as measured by anthropometry (WHO 1995; Kramer 1987; Bisai 2004; Taylor and Howie 1989; Mascie-Taylor 1993). The present study attempted to examine to what degree post delivery maternal weight is useful and efficient in predicting low weight at birth of term (gestation age > 36 weeks) neonates of pregnancy.

Based on ROC curve, this study provided a cut-off point for maternal weight of = 46.4 kg (AUC = 0.863, SE = 0.03, 95% CI: 0.794-0.915,  $p < 0.001$ ) for LBW. A very similar cut-off of 46.5 kg (maternal weight) was obtained by regression equation. Therefore, maternal weight < 46.5 kg had risk ratio of 4.69 with 80.0 % sensitivity and 76.2 % specificity for LBW. The positive and negative predictive values of this cut of point were 68.7% and 85.3 %, respectively. Similarly, a recent study provided a cut-off for maternal early second trimester weight of < 46.0 kg for LBW, with risk ratio of 1.7, 66% sensitivity and 53% specificity (Bisai et al. 2007).

Another study from the same hospital analyzed post delivery data of 176 mothers (Bisai 2004) and the data showed that maternal weight of less than 46.5 kg was the best cut off for detecting LBW, with 62.5% sensitivity and 59.6 % specificity. An earlier study from Varanasi, India (Mohanty et al. 2006) had reported that maternal weight < 45 kg at first trimester was the predictor of term LBW with sensitivity and specificity of 62.0% and 67.2%, respectively. Another study from Bangladesh (Karim and Mascie-Taylor 1997) had found maternal weight < 50 kg (at term) to be the best predictor of LBW with sensitivity and specificity of 69% and 68%, respectively.

In urban India, there are very little provisions of primary health centers to provide antenatal care during pregnancy. Moreover, most of the government health institutions provide antenatal care on or after 12 weeks of gestation onwards. In rural India, 39 % women do not receive any antenatal care during pregnancy (NFHS-2 2000) although in urban Kolkata, 98% mothers received at least one antenatal check-up from a doctor (NFHS-2 2001). Therefore, an efficient cutoff point is essential for screening pregnant women based on weight for gestational age. Those at high risk can then be referred to tertiary health centres for better management.

In conclusion, it can be seen that a weight of less than 46.5 kg was the most efficient cut-off point for detecting Term-LBW among Bengalee women of Kolkata. This cut-off point can be utilized as a screening tool to detect high-risk mother at early second trimester of pregnancy. It is well recognized that maternal weight within 48 hours of delivery can be taken as proxy for weight at 14 weeks of gestation (Moller and Lindmark 1997), as significant weight gain occurs in second and third trimester of gestation. Thus, antenatal care givers of health institution and community health workers can easily use this cut off value for monitoring pregnant women. The women can be given appropriate advice for better birth outcome based on this cut-off point. It is recommended that further studies be undertaken on larger samples among this ethnic group to validate this cut-off point. Such validation studies would ultimately lead to the acceptance of this cut-off point in this ethnic group.

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# INITIAL GROWTH AND SURVIVAL OF MAIZE (*ZEA MAYS* L.) CV. BR 154 (SARACURA) EXPOSED TO HYPOXIA UNDER DIFFERENT FLOODING CONDITIONS

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## ABSTRACT

This work was carried out to evaluate the initial growth and survival of cultivating maize (*Zea mays* L.) BR-154 (Saracura) under hypoxia conditions. Maize seeds were put to germinate in different saline solutions (CaCl<sub>2</sub>, MgCl<sub>2</sub>, 6H<sub>2</sub>O, KCl and mannitol) and in distilled water used as control. Four days after germination, the roots were measured and the plantlets were transferred to vermiculite pots. The survival of the seedlings was evaluated on the third and sixth days after the transplant. To verify the effect of different osmotic pressures in the initial growth of the plants, another group of seed were put to germinate in mannitol solution under different osmotic pressures (0, - 0.12, -0.25, -0.37 and -0.50 MPa). The results indicate that the saline solutions promoted a significant reduction ( $p < 0.05$ ) of the initial growth during the germination and an increase in the survival of the plants after flooding compared to the control. The reduction in growth of the plantlets treated with CaCl<sub>2</sub> was drastic, resulting in higher survival rate compared to the other treatments. Increase in osmotic pressure during germination and flooding, significantly reduced ( $p < 0.05$ ) the seedling growth by causing the mobilization of the carbonic backbone from the seed towards other organs of the plant and water content. These effects could be associated with the reduction of necrosis in the plantlets after flooding, typical symptom of the hypoxia process.

**Keywords** : Brazil, calcium, flooding, hypoxia, magnesium, maize, mannitol, potassium

## INTRODUCTION

Flooding caused by heavy rains and/or bad draining of the soil in agricultural areas could hold back the full agricultural use of some cultivated areas. Maize like many other crops, are flooding-intolerant as flooding provokes a lack of oxygen in the ground (hypoxia) (Vitorino,

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1999). The BR 154 (Saracura) maize cultivar developed by Embrapa (National Research Center of Maize and Sorghum-Brazil) is tolerant to temporary overflow (flooding). This cultivar was developed from a large genetic base, after nine cycles of massive selection under excess of humid conditions in the ground, having the capacity to support temporary flooding or to be used in areas with intermittent overflow (Parentonni *et al.*, 1997).

The physiological consequences of hypoxia process in majority of the plants involve a reduction in the accumulation of dry substances and productivity, and if it continues for extensive periods may lead to the death of the plants (Parentonni *et al.*, 1997). It was observed that the American maize cultivar B73Ht, cultivated in the state of Illinois USA, can survive under hypoxia for a period of three days (Lemke-Keys & Sachs 1989). In Brazil, BR-154 cultivar (Saracura) is one of the most tolerant to flooding. During flooding conditions, the weight of spike was reported to be increased by 25%; compared to 14% under normal water conditions in the ground (Parentonni *et al.*, 1997). The Federal University of Lavras (UFLA) initiated in a project in 1998 along with the the State University of Ohio (USA), The University of Illionois (USA) and the National Center of Research of Maize and Sorghum (Embrapa, Brazil), to characterize the specific factors of tolerance to flooding of BR 154 to identify and charaterize the genes tolerant to the overflow or flooding. These genes could then be transferred to other maize cultivars with better agronomic features, becoming more tolerant to flooding, making it possible to use them in agricultural areas with intermittent flooding, making it possible to use them in agricultural areas with intermittent flooding.

The objective of this work was to evaluate the physiological effects of different saline solutions in the initial growth and the survival of maize seedlings (BR 154) when exposed to hypoxia and under different osmotic pressures.

### MATERIAL AND METHODS

All the experiments were conducted using maize (*Zea mays* L.) seed of the Brazilian cultivar BR-154 (Saracura). Five different treatments were used including solutions of calcium chloride ( $\text{CaCl}_2$ , Sigma), magnesium chloride ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , Sigma) potassium chloride (KCl, Sigma) and mannitol (Sigma) and distilled water (used as control). For the hypoxia assays, all the saline solutions were used at the same concentration (0.0689 M, -0.25 MPa) as suggested by Vitorino (1999). For germination tests, 25 seeds were placed in germitest paper (Fisher) moistened with the respective solutions. After four days in the growing chamber (28°C), the same seed were transferred to PVC tubes (Fisher) and submerged in the same solutions used in the germination, after adding TRIS (Sigma) (5 mM) and ampicillin (Sigma) (100 mg L<sup>-1</sup>). The pH was adjusted with HCl at 8.0 as suggested by Sab & Sachs (1996). Oxygen content inside each of the tubes was decreased using nitrogen solution (Fisher) (1 L min<sup>-1</sup>) per three minutes. The

tubes were then sealed and left in dark ( $26 \pm 2^\circ\text{C}$ ) for four days. After flooding, the seedlings were transferred to vermiculite trays, and were kept in a growing chamber ( $26 \pm 2^\circ\text{C}$ , 12 hours of light). The initial growth was evaluated measuring the length of the roots after the germination and flooding treatments. The seedling survival was evaluated based on the development of green leaves after the third and the sixth days of transplant.

The effects of different osmotic pressures over the initial growth of the plants were carried out using the following mannitol solutions :  $9.24 \text{ gL}^{-1}$  ( $-0.12 \text{ MPa}$ );  $18.49 \text{ gL}^{-1}$  ( $-0.25 \text{ MPa}$ );  $27.73 \text{ gL}^{-1}$  ( $-0.36 \text{ MPa}$ );  $36.98 \text{ gL}^{-1}$  ( $-0.50 \text{ MPa}$ ) and distilled water (as control). The methods for the germination and the induction of hypoxia process were same as described above. After flooding, fresh and dry matter of all seedlings were measured. Psychological damages in the seedlings caused by hypoxia were evaluated by checking the presence or absence of mesocotyl necrotic lesions, which is a typical symptom of lack of oxygen in maize seedlings (Vitorino 1999). All the assays were carried out in an entirely casualized delineation, with five treatments and three repetitions. The statistical analyses were done using the Scott-Knott test at 5% probability level.

## RESULTS AND DISCUSSION

Seed germination during the four day treatment in different saline solutions resulted in a significant ( $p < 0.05$ ) reduction in the initial growth of the roots compared to the control (distilled water). Mannitol and KCL treatments decreased the growth of the roots by 18% whereas root reduction using  $\text{CaCl}_2$  and  $\text{MgCl}_2$  solutions was observed to be around 55%. The root growth reduction is probably related to the decrease of the seed osmotic potential and also due to reduction in the water availability, leading to a lower utilization of seed metabolic reserves. No root growth was observed after flooding, for all treatments (Fig.1). Hypoxic stress caused the strong root growth inhibition.

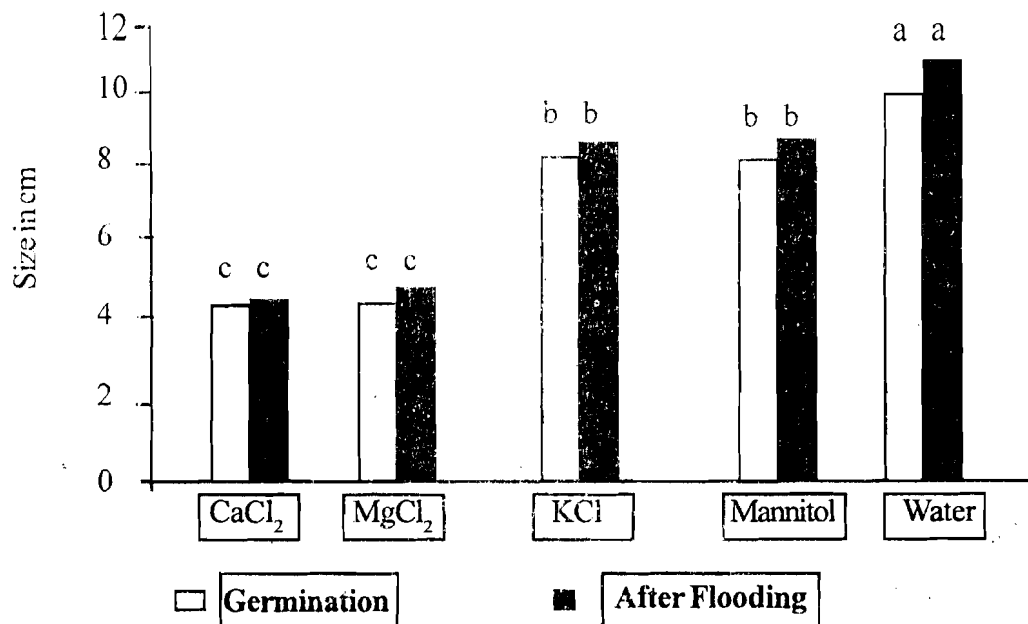
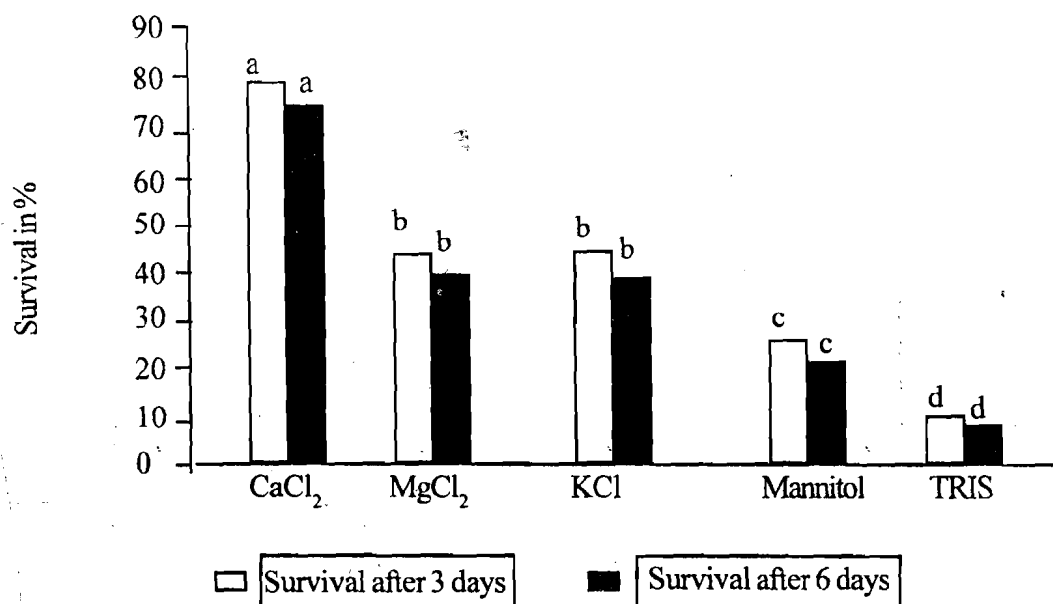


Fig. 1: Size of roots, after germination and followed by flooding. Columns followed by the same letters do not differ significantly (Scott-Knott 5%)

The MgCl<sub>2</sub> and CaCl<sub>2</sub> solutions, did not differ significantly ( $p > 0.05$ ) between themselves and caused the highest reduction in the initial growth of the roots (Fig. 1). This indicates an additional effect of magnesium and calcium bivalent cations, that exceed the effect caused by the increase of the osmotic pressure, reducing root growth, since all the saline solutions used in the experiment were adjusted to same osmotic concentration ( $-0.25$  MPa). Calcium is physiologically related with cellular growth, forming covalent links with negative forms of polygalacturonic acids from pectin and cellulose fibers, avoiding cellular expansion (Buchanan, 2000). These data are in common with the ones obtained by Vitorino (1999), where seed germination with CaCl<sub>2</sub> solution showed a significant reduction in the initial growth of the plants and a higher tolerance to hypoxic stress. The survival of the seedlings after flooding with different saline solutions was verified in the third and sixth days after the seedlings were transferred to vermiculite trays. Seed treated with CaCl<sub>2</sub> solution showed the highest number of developed seedlings (80%) followed by MgCl<sub>2</sub> and KCl solutions (45%); mannitol (20%) and control (5%) (Fig.2)



**Fig. 2 :** Survival of the seedlings after 4 days of flooding, analyzed on the third or sixth days after transplanting to vermiculite. Columns followed by the same letters do not differ significantly (Scott-Knott 5%)

The comparison between the results observed during the initial growth of the seedlings (germination process) and during the survival of the plants, after flooding, showed an opposite relationship. Saline solutions which caused the highest root inhibition leading to a smaller development of the seedlings were the same ones that caused the highest number of seedlings survival after flooding. However, plants exposed to KCl solution produced an equal effect on initial growth as plants treated with mannitol (Fig. 1), but with a greater rate of survival after flooding (Fig. 2). In fact, the survival of plants flooded with KCl was equal to MgCl<sub>2</sub> (Mg<sup>2+</sup> is a divalent cation). It seems that the monovalent K<sup>+</sup> contributed to the increase in the tolerance to hypoxic stress.

Plants germinated and flooded in CaCl<sub>2</sub> solution exhibited lower initial growth and higher survival rate after flooding, compared to the other treatments (Fig. 1 & 2). Subbaiah *et al.* (1994) related the presence of calcium with the expression of genes resistant to flooding, where this cation would have important signaling functions. Furthermore, survival of the plants in the presence of calcium shows that calcium plays an important role in the maintenance of the integrity of the cell wall structure, with a delay effect of cellular lysis (Conway, 1995). Post-harvest experiments further illustrated the positive effect of this cation in the preservation of the cell wall integrity (Conway, 1995). Calcium promotes covalent links between carboxylic groups of polygalacturonic acid from pectins (Jarvis, 1984), turning the cell-wall less vulnerable to degradation enzymes (Burns & Pressey 1987). Dantas (1999) evaluated enzyme activities associated with

the cell-wall metabolism as polygalacturonase, xiloglucano endo-transglucosilase and cellulase, and verified and increase in the *in vitro* activity of these enzymes without, however, promoting lysis of *in vivo* cells. This fact was attributed to the increased concentration of calcium in apoplast conferring a structural activity of the element, also increasing the production of calcium pectates, promoting a higher stability of matrix pectic. This in turn leads to the reduction in the activity of the enzymes involved in the degradation process of the cell wall.

The germination tests with mannitol solution under different osmotic pressures showed that with the increase of osmotic pressure, the mobilization of carbonic skeletons (dry matter) from the seed towards the plants diminished (Fig. 3). The water content analysis from the seed and seedlings (during germination and flooding tests) with mannitol solution was also observed to decrease and was inversely proportional to the increase of the osmotic pressure (Fig. 4).

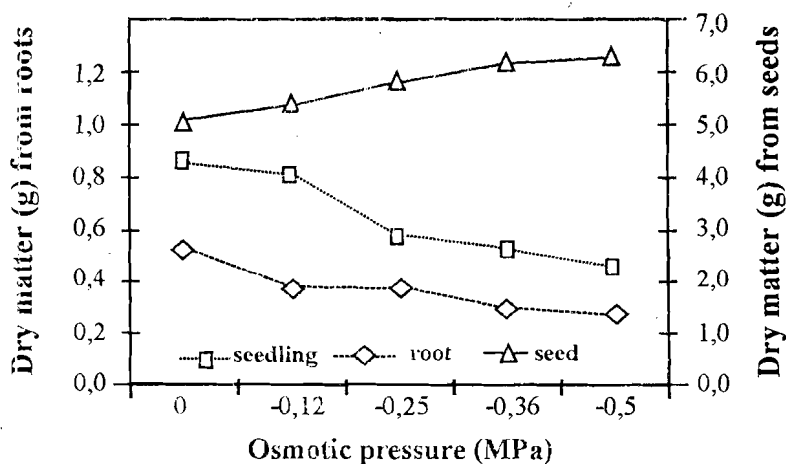


Fig. 3 : Dry matter (g) from roots, seedlings and seed after four days germination.

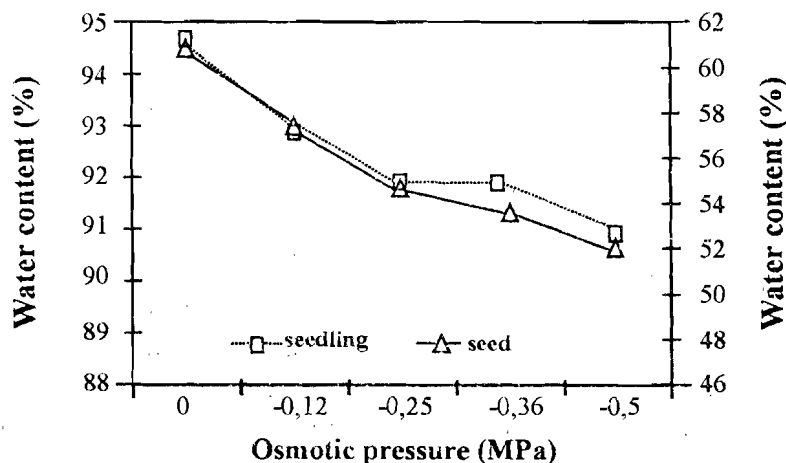


Fig. 4. : Water content in seed and seedlings after four days of flooding in mannitol solution under different osmotic pressure.

There seems to be a direct relationship between the carbonic skeletons from the seed to the plants and the osmotic adjustment. The low water content in the cells causes the reduction of the transport of carbonic skeletons and the lack of energy for the rest of the plant, along with the lack of pressure necessary for the cellular wall elongation (Jarvis, 1984). Flooding during four days with mannitol solution under different osmotic pressures resulted in a lower number of plants with necrotic injuries in the coleoptiles up to  $-0.36$  MPa pressure (Fig. 5). This inhibition, caused by the decrease of the osmotic potential, may have contributed with a smaller degradation of the cellular wall during hypoxic stress, and in consequence, the reduction of the number of plants with characteristic symptoms flooding process.

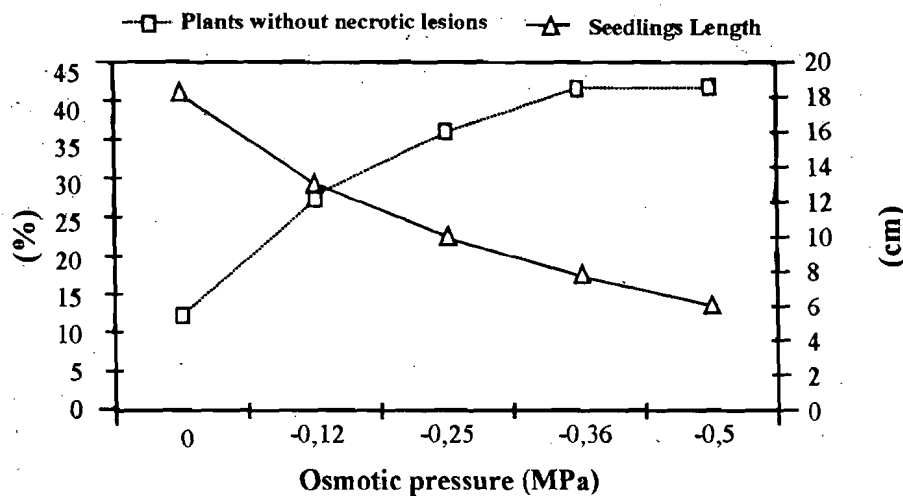


Fig. 5 : Percentage of seedlings without symptoms, after four days of flooding with mannitol solution under different osmotic pressures.

### CONCLUSIONS

Calcium possesses specific physiological function therefore, it presented significant difference in the increase of seedling survival in relation to mannitol. The gradual increase of the osmotic pressure in the flooding solutions, increased the survival of the seedlings under hypoxic stress and inversely interfered with the mobilization of nutrients from the seed towards the rest of the plant, reducing the seedling growth and the number of plants with coleoptiles necrosis, characteristic symptom of the oxygen deficiency.

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# AN ASSESSMENT OF TANNASE AND GALLIC ACID PRODUCTION BY *ASPERGILLUS ACULEATUS* DBF9 FROM DIFFERENT PLANT TANNIN

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## ABSTRACT

Different tannin rich plant materials were tested for their tannase and gallic acid producing abilities. It was found that amount of tannin is comparatively higher in *Shorea robusta*, *Bruguiera* sp., *Terminalia belerica*, *Acacia auriculiformis*, *Cassia fistula*, *Cassia siamea*, *Anacardium occidentale* and *Acacia arabica* as compared to that of *Sonneratia* sp., *Delonix regia*, *Alstonia scholaris*, *Azadirachta indica*. A large amount of tannase and gallic acid production was noticed in the raw tannin obtained from *Terminalia belerica*, *Cassia siamea*, *Acacia arabica* and *Bruguiera* sp. by *Aspergillus aculeatus* DBF9. Among them, maximum production of tannase and gallic acid was found in the raw tannin obtained from *Cassia siamea*.

**Key Words :** Gallic acid, Plant Tannin, Tannase, *Aspergillus aculeatus*

## INTRODUCTION

Gallic acid (3, 4, 5-tri hydroxy benzoic acid), phenolic units of gallotannin has several applications in chemical and pharmaceutical industries. Gallic acid has huge demand in India though it is an imported item. It is used for the production of propyl gallate, which is mainly used as an antioxidant in fats and oils as well as in beverages (Weetal, 1985; Gathon *et al.*, 1989), for the preparation of trimethoprim, a broad spectrum antibiotic (Hadi *et al.*, 1994) and as a photosensitive resin in semiconductor production (Yamada *et al.*, 1989). Besides, gallic acid is also used in the manufacture of pyrogallol, inks, photographic developer, in testing free mineral acids, dihydroxy acetone and alkaloids (Budavari, 1989).

Conventionally gallic acid is produced through acid hydrolysis, though microbial fermentation of tannic acid is preferred today. Pourrat *et al.* (1985) mentioned that fermentation of tara pod powder in a bioreactor is suitable for gallic acid production. They recovered 30%

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gallic acid through fermentation of tara pod powder. Pourrat *et al.* (1987) reported gallic acid production from tannin of *Rhus coriaria*. Deschamps *et al.* (1980) studied chestnut tannin degradation by *Corynebacterium sp.* in a 2L jar-fermenter. They have studied the pattern of tannase production and analysed the hydrolytic product by thin layer chromatography (TLC). Production of gallic acid by *Aspergillus sp.* from gallo-tannin was studied by Vermeire and Vandamme (1990). Misro *et al.* (1997) studied the production of gallic acid using the immobilized cells of *Rhizopus oryzae*. They found that 78.5% tannin conversion was possible after 4 days of incubation. Mukherjee and Banerjee (2004) reported gallic acid production from tannins of myrabolan and teri pod powder through modified solid state fermentation by two fungal strains *Rhizopus oryzae* and *Aspergillus foetidus*. Therefore it has been found that production of gallic acid through fermentation is possible provided that plenty of raw material (tannin) and desired organism for bioconversion are in hand. In West Bengal plenty of tannin containing plant materials are available in different forest areas. Gallic acid production is possible using those tannin rich plant materials through microbial fermentation. Tannase producing ability of *Aspergillus aculeatus* DBF9 has reported earlier (Banerjee *et al.*, 2001). In the present investigation tannase and gallic acid production by *Aspergillus aculeatus* DBF9 has been carried out through liquid submerged fermentation of locally available raw tannin containing plant materials.

## MATERIALS AND METHODS

### **Microorganism:**

Previously isolated potent tannase and gallic acid producing fungal strain *Aspergillus aculeatus* DBF9 (Banerjee *et al.*, 2001), has been used in the present study.

### **Inoculum preparation:**

*Aspergillus aculeatus* DBF9 was transferred to tannic acid agar media and incubated for 3 days at 30 °C. Spores formed in plate were suspended in 0.02% Triton x-100 solution. 2% inoculum containing  $10^5$  spores/ml was used for fermentation.

### **Culture condition:**

Gallic acid producing abilities of different tannin rich plant materials were carried out in 250ml Erlenmeyer flask containing 50ml media. The culture medium consisted of raw tannin, 20.0 g/l;  $K_2HPO_4$ , 0.5 g/l;  $KH_2PO_4$ , 0.5 g/l;  $MgSO_4 \cdot 7H_2O$ , 1.0 g/l; and  $(NH_4)_2HPO_4$ , 3.0 g/l. Fermentation was carried out at 30 °C for 48 hrs. Cells were removed by filtration and centrifugation after fermentation. Supernatant obtained after centrifugation was assayed for tannase activity

### **Assay of tannase:**

Tannase activity was estimated by measuring the residual tannic acid after enzymatic reaction according to the method of Mondal *et al.* (2001). Enzyme solution (0.05 ml) was incubated with 0.3 ml of 1.0% (w/v) tannic acid, in 0.2 M acetate buffer (pH 5.0) at 40°C for 10

min and then the reaction was stopped by addition of 2.0 ml bovine serum albumin (BSA) (1mg/ml), which precipitated the remaining tannic acid. A control reaction was done side by side with heat denatured enzyme. The tubes were then centrifuged (5,000 'g, 10 min) and the precipitate was dissolved in 2.0 ml of SDS-triethanolamine (1 % w/v SDS in 5 % v/v triethanolamine) solution and the absorbency was measured at 550 nm after addition of 1.0 ml of FeCl<sub>3</sub> (0.13 M) (Systronics spectrophotometer 105).

One unit of tannase activity is defined as the amount of enzyme required to hydrolyze 1.0 μ mol of ester linkage of tannic acid in 1 min under specified condition.

#### **Determination of tannin content of plant material :**

Initially 10.0 g of plant parts (bark and fruit) was taken in 50 ml of distilled water and was boiled for 30 min. The water extract of different plant materials was collected and dried by vacuum evaporator. Tannin content of different plant specimens (bark and fruit) were measured by Folin-Denis method (Schanderi, 1970). 0.2 ml of plant extract was mixed with 8.3 ml of distilled water, 0.5 ml of Folin-Denis reagent (10.0g sodium tungstate and 2.0g phosphomolybdic acid in 75.0 ml H<sub>2</sub>O and 5.0 ml phosphoric acid mixture) and 1.0 ml of 15 % Na<sub>2</sub>CO<sub>3</sub> was added. After 30 min the optical density of the mixture was measured at 700 nm. The concentration of tannin was determined using tannic acid as standard. Depending on their tannin richness (≥ 4.0% dry weight), eight plant materials were selected for gallic acid production by fermentation with *Aspergillus aculeatus* DBF9. Results shown here are the average of three experiments.

#### **Estimation of gallic acid :**

Gallic acid in the culture broth was estimated by the method of Bajpai and Patil (1996). Culture supernatant was diluted to 100 fold in 0.2M acetate buffer, pH 5.0. The absorbence was recorded at two selective wavelengths of 254.6 and 293.8nm. The concentration of gallic acid was measured using specific extinction coefficient, by the following equation; Concentration of gallic acid (mg/ml) = 21.77 (A<sub>254.6</sub>) - 17.17 (A<sub>293.8</sub>).

#### **Growth measurement :**

Growth of the organism was estimated after drying the biomass at 60°C for 24h.

## RESULTS

#### **Determination of tannin content of some plant materials:**

Tannin content of some locally available plant materials were determined and presented in Table 1. Mainly barks and fruits were considered for tannin estimation. It was found that higher amount of tannin was present in fruit as compared to bark. Among the selected plant species, the fruits of eight plants contained higher amount of tannin. Maximum amount of tannin (8.7%) was found in the fruits of *Shorea robusta*. Whereas plants like *Bruguiera sp.*, *Terminalia belerica*, *Acacia auriculiformis*, *Cassia fistula*, *Cassia siamea*, *Anacardium occidentale* and *Acacia arabica* posses 4.2 to 6.4 % tannin in their fruits and 0.62 to 1.18 % tannin in their barks. Very negligible

amount of tannin was recorded from the barks of *Ziziphus jujuba*, *Ficus benghalensis*, *Eucalyptus sp.*, *Alstonia scholaris*, *Tectona grandis* etc.

**Table 1:** Tannin content of some commonly available plant species

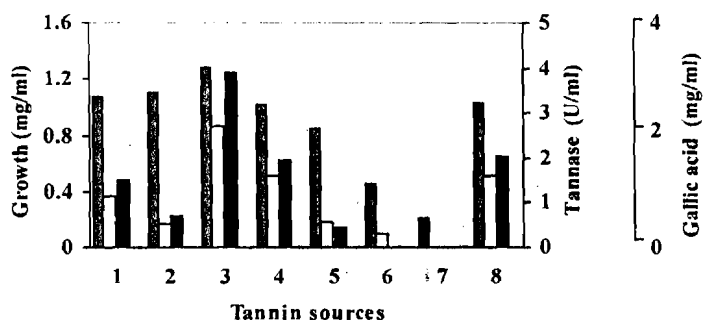
Plant materials	Plant part used	Tannin content (% dry weight)
<i>Mimosa pudica</i>	fruit	1.76
	bark	0.29
<i>Shorea robusta</i>	fruit	8.7
	bark	1.27
<i>Bruguiera sp.</i>	fruit	6.4
	bark	1.18
<i>Sonneratia sp.</i>	fruit	2.87
	bark	1.12
<i>Rhizophora sp.</i>	fruit	3.77
	bark	0.97
<i>Terminalia belerica</i>	fruit	4.56
	bark	0.82
<i>Acacia auriculiformis</i>	fruit	5.4
	bark	0.62
<i>Tectona grandis</i>	fruit	0.97
	bark	0.32
<i>Delonix regia</i>	fruit	2.13
	bark	0.62
<i>Ziziphus jujuba</i>	fruit	0.17
	bark	0.12
<i>Cassia fistula</i>	fruit	6.34
	bark	0.97
<i>Cassia siamea</i>	fruit	5.18
	bark	0.88
<i>Alstonia scholaris</i>	fruit	3.54
	bark	0.34
<i>Casaurina equisetifolia</i>	fruit	2.11
	bark	0.68
<i>Anacardium occidentale</i>	fruit	4.22
	bark	0.79

Plant materials	Plant part used	Tannin content (% dry weight)
<i>Acacia arabica</i>	fruit	5.79
	bark	0.89
<i>Azadirachta indica</i>	fruit	1.12
	bark	0.47
<i>Ficus benghalensis</i>	fruit	0.93
	bark	0.31
<i>Eucalyptus sp.</i>	fruit	1.18
	bark	0.32
<i>Polyalthia longifolia</i>	fruit	1.74
	bark	0.45

#### ***Tannase and Gallic acid production through fermentation of raw tannin :***

Production of tannase and gallic acid through fermentation of raw tannin from selected eight plants was studied and is presented in Fig 1. It was found that tannase and gallic acid production was maximum within the medium containing raw tannin obtained from the fruits of *Cassia siamea*. Good amount of gallic acid was also produced in the raw tannin obtained from fruits of the *Terminalia bellerica*, *Anacardium occidentale* and *Bruguiera sp.* No gallic acid production was recorded in the media containing *Shorea robusta* and *Acacia auriculiformis* tannin.

#### **DISCUSSION**



**Fig 1:** Effect of raw tannin on growth (▨), tannase (□) and gallic acid (■) production by *Aspergillus aculeatus* DBF9. Raw tannin used: 1. *Terminalia bellerica*; 2. *Cassia fistula*; 3. *Cassia siamea*; 4. *Anacardium occidentale*; 5. *Acacia arabica*; 6. *Shorea robusta*; 7. *Acacia auriculiformis*; 8. *Bruguiera sp.* Fermentation was carried out in 250ml Erlenmeyer flask containing 50ml media.

Selection of a substrate for enzyme and subsequent product formation by fermentation depends on several factors like cost, availability and suitability of the substrate for obtaining the desired product of fermentation and thus requires screening of several agroindustrial residues (Pandey *et al.*, 1999). In the present experiment, cost effective substrate for biotransformation were selected on the basis of their tannin content. It was found that amount of tannin is comparatively higher in *Shorea robusta*, *Bruguiera sp.*, *Terminalia sp.*, *Acacia auriculiformis*, *Cassia fistula*, *Cassia siamea*, *Anacardium occidentale* and *Acacia arabica* as compared to that of *Sonneratia sp.*, *Delonix regia*, *Alstonia scholaris*, *Azadirachta indica*. Maximum amount of tannin (8.7%) was observed in the fruits of *Shorea robusta*. Earlier 12% tannin was reported in the seeds of *Shorea robusta* (Makkar, 1988). Variation in the tannin content from plant to plant and in their different parts is not clear. Presence of higher amount of tannin in fruit may be for the protection of future generation from microbial attack. Differences in tannin content of plant parts may be due to variations in their metabolism. Gallic acid production was noticed in the raw tannin obtained from *Terminalia belerica*, *Cassia siamea*, *Acacia arabica* and *Bruguiera sp.* Among them, maximum gallic acid production was found from the fruits of *Cassia siamea*. This differential gallic acid production may be due to the variation of amount of hydrolysable tannin content among the plants. Mukherjee and Banerjee (2004) used tannin rich plant material myrabolan and teri pod powder as a source of raw material for tannase and gallic acid production by *Rhizopus oryzae* and *Aspergillus foetidus*. No tannase and gallic acid production was recorded with raw tannin obtained from *Acacia auriculiformis*. This plant may possess higher amount of condensed tannin and gallotannin may be absent in the extract of the plant. This is for the first time; tannase and gallic acid production has been carried out using raw tannin of *Cassia siamea* through fermentation by *A. aculeatus* DBF9. Use of such low cost raw material ensured the possibility for exploitation of this organism in large-scale production of gallic acid.

#### ACKNOWLEDGEMENT

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# EFFECT OF ENVIRONMENTAL COLD STRESS ON METABOLIC CHANGES IN HIBERNATING TOAD, *DUTTAPHRYNUS MELANOSTICTUS* (Schneider, 1799)

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## ABSTRACT

The present investigation reports the effect of cold stress on different blood- plasma biochemical - parameters in the Indian toad (*Duttaphrynus melanostictus*). Plasma protein, plasma glucose and blood urea are analysed in summer and in winter. It clearly indicates that the total plasma protein is decreased significantly in hibernating toads. But plasma glucose and blood urea level are significantly increased in hibernating toads. SDS-PAGE analysis also indicates absence of polypeptide bands corresponding to the molecular weight 80-85kDa and 24-26kDa respectively. So, it indicates that environmental cold stress is an important modulator of their hibernation.

**Key words :** SDS-PAGE, Plasma glucose, Plasma protein, Blood urea

## INTRODUCTION

Decrease in environmental temperature in winter acts as cold stress on hibernating toads resulting in slowing down of heart rate, breathing and metabolism so that these animals could survive with reserve food. Acclimatization to low temperature causes changes on their system to maintain catalytic potential or to alter the relative activities of different metabolic pathways at low temperature (Hochachka and Somero 1984). Available reports indicate that environmental cold stress in hibernating toads causes significant changes in plasma-protein and glucose (Churchill and Storey 1993, Edwards et al. 2004, Costanzo and Lee 2005,) blood urea (Pasanen 1977, Jorgenensen 1997, Costanzo and Lee 2005.) and in SDS-PAGE pattern (Das et al 2004, Bulbul and Kutrup 2007).

In the present investigation, effect of cold stress on the Indian toad, *Duttaphrynus melanostictus* natural condition has been envisaged. This toad is available in plenty during summer but is not found during winter in West-Bengal. An attempt has been made to study the changes in some blood-parameters such as plasma protein, plasma glucose, blood-urea and SDS-PAGE analysis of this amphibian.

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## MATERIALS AND METHODS

Five adult pond toads, each weighing 50-55 gms were collected from Midnapur in May, 2007 as non-hibernating toads, when air temperature was 34.4°C and from the mud hole in their hibernating state in December 2006, when air remained around 16.2°C. Blood samples were collected by cardiac puncture using 21 gauge needle and 5 ml syringe. These were transferred to EDTA coated vacutainer tubes for determination of protein, urea, SDS-PAGE and in Sodium fluoride coated vacutainer tubes for determination of glucose. Plasma was separated by centrifugation at 3000 r.p.m. for 10 minutes and the supernatant was taken for bio-chemical analysis.

### BIO-CHEMICAL ANALYSIS

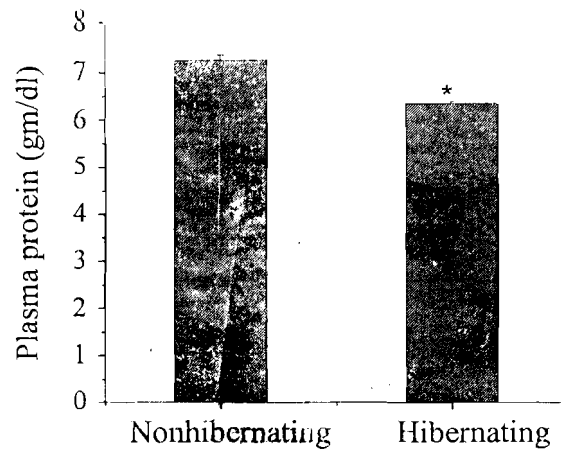
Plasma protein was estimated photometrically using the standard curve against the known protein as BSA, following the Lowry method (Lowry et al. 1951). Plasma glucose was estimated photometrically by Glucose- Oxidase protocol using the standard kit (Merck-Diagnostica-PDLFT0879). Blood urea was estimated photometrically by the DAM technique using the standard kit (Merck- Diagnostica-PDLFT0082). Sodium dodecylsulfate- polyacrylamide gel electrophoresis

(SDS-PAGE) was performed following the technique of Laemmli (1970), using 1.5 mm x 10 cm polyacrylamide slab gels consisting of 10% resolving and a 6% stacking gels containing 1% SDS. Plasma containing 360ng of protein for each of the specimen were loaded in the gel. A sample of 10 µl of protein marker (Genei-PMWM-M1065) was loaded in the gel in this experiment. The gel was run at a constant amperage of 20mA until the tracking dye was within 1-2 mm from the bottom of the resolving gel. After electrophoresis the gel was stained with 0.25% Coomassie Brilliant Blue R250 (CBB) in a solution of methanol, water and glacial acetic acid (40:50:10) and destained in the same solution without CBB.

Statistical analysis was done using Microcal Software, Inc. Version:6.0.

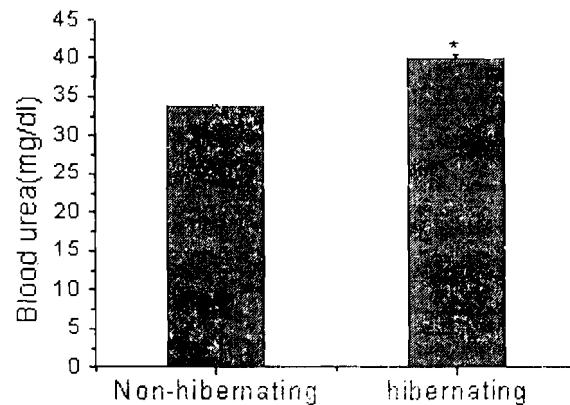
### RESULTS AND DISCUSSION

Plasma protein significantly decreased in hibernating toads as compared to that of non-hibernating ones (Fig. 1). Available reports indicate that cold exposure in winter inhibits the protein synthesis in the blood and causes changes in enzyme activities of *Rana pipiens* in hibernating season (Churchill and Storey, 1993). Environmental cold stress increases protein catabolism. Available reports also indicate that serum of *Duttaphrynus stomaticus*, which is found in the plains of West Bengal, represents a clear distinct band above the albumin band which is found missing in *Duttaphrynus himalayanus*, which is found in temperate zone, reflecting the adaptation to cold climatic conditions (Das et al 2004). Blood urea level significantly increased as compared to



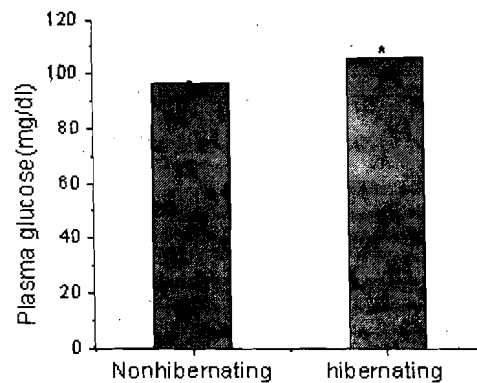
**Figure 1:** Comparison of plasma protein (expressed in gm/dl) in non-hibernating and hibernating toads. At the 0.05 level the two means are significantly different (\*  $P < 0.05$ ).

those of non hibernating toads (Fig.2). Available reports indicate that urea, the end product of nitrogen metabolism is the predominant organic osmolyte which accumulates during dehydration in hibernating (Jorgensen 1997). Elevated urea level is effective cryoprotective agents (Costanzo and Lee 2005). Here, decreased hydration is accompanied by a marked reduction in the resting state of oxygen consumption which is inversely correlated with urea concentration. So, present findings are unique in making a strong link between elevated urea and hypometabolism at the organismal level in hibernation. Perhaps, cold stress triggers changes in gene expression of urea sensitive enzymes.



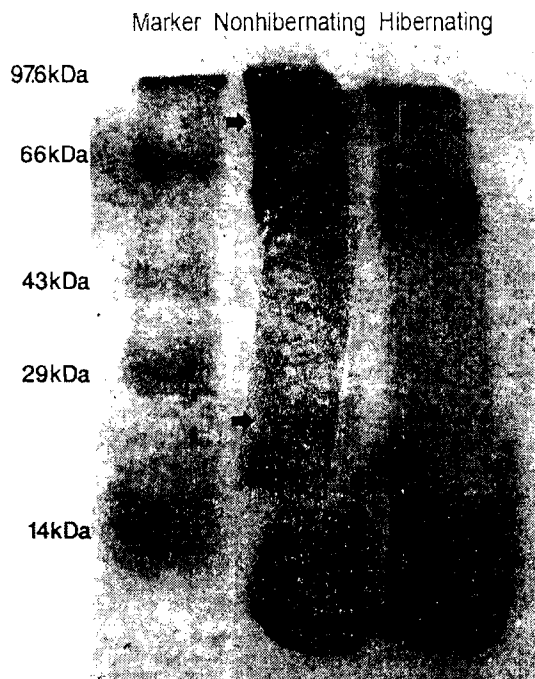
**Figure 2:** Comparison of blood urea (expressed in mg/dl) in non-hibernating and hibernating toads. At the 0.05 level the two means are significantly different (\*  $P < 0.05$ ).

Plasma glucose level of hibernating toads significantly increased as compared to non-hibernating ones (Fig.3). Increased glucose level, as found in the present work has also been reported in case of *Rana sylvatica* (Churchill and Storey 1993, Edward et al. 2004, Costanzo and Lee 2005). Available reports indicate that elevated glucose level is an effective cryoprotective agents in hibernation (Costanzo and Lee 2005). Here, elevated glucose level is associated with the enhanced rate of glycogenolysis and neo-glucogenesis. The elevated glucose level in blood is not only derived from lactic acid but also from amino-acids. This in all probability might explain the decreased amount of plasma protein in hibernating toads during hibernation, in the absence of food intake. During this period, glucose can not be stored as glycogen and fat. Glycogen released by pancreas stimulates the liver to convert stored glycogen into glucose and raises plasma glucose level during hibernation.



**Figure 3:** Comparison of blood urea (expressed in mg/dl) in non-hibernating and hibernating toads. At the 0.05 level the two means are significantly different (\* $P < 0.05$ ).

Distinguishable changes in the band pattern between non-hibernating and hibernating blood samples have been presented in Fig.4. Absence of protein expressions corresponding to the molecular weight ranging between 80-85kDa and 24-26kDa in hibernating toads, suggests that these proteins might be playing an important modulatory function during hibernation. Further in depth investigations are how ever needed to identify this protein and detailed enzyme-assay, relating to the physiological changes needs to be ascertained.



**Figure 4:** Photograph of SDS-PAGE .Marker range from 14kDa to 97kDa. Absence of protein expressions corresponding to molecular weight ranging between 50-55kDa and 24-26kDa in hibernating toads..

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# ANTHROPOMETRIC APPRAISAL OF NUTRITIONAL STATUS OF ADULT FEMALE ORAONS AND SARAKS OF RANCHI DISTRICT JHARKHAND INDIA - A COMPARATIVE STUDY

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## ABSTRACT

This cross-sectional study was undertaken among two adult (aged > 18 years) female samples, representing the Oraons, a tribe and the Saraks, an endogamous caste of Ranchi district In Jharkhand, to compare their anthropometric profile and the prevalence of chronic energy deficiency (CED), based on body mass index (BMI). Anthropometric measurements including height, weight, BMI and mid upper arm circumference (MUAC) were measured using standard techniques and equations. Internationally recommended BMI cut-off points were used to evaluate and to compare the nutritional status of the two adult sections. Significant differences between the means of some anthropometric characteristics of the two populations were observed. The degree of CED (BMI < 18.5) was found to be high in both populations (Oraons = 62.50 %; Saraks = 46.36%). According to the World Health Organization standard, the prevalence of CED was high and the state was serious in both populations. In conclusion, this study provided evidence that the nutritional states of the adult Oraons and the Saraks, were not satisfactory.

*Key Words* : Oraons, Saraks, Body Mass Index; Chronic Energy Deficiency.

## INTRODUCTION

Anthropometry is of substantial interest to the public health professionals, dieticians, scientists and policy makers. Undernutrition, overweight and obesity of different age and of both the sexes are the matters of deep concerns about their social and health-related implications. The use of anthropometry is a resourceful indicator of nutritional and health status of adults (WHO, 1995; Lee and Nieman, 2007). The body mass index (BMI) is indicative of overall adiposity (Bosc, 1996, 2002). Although nutritional status of the adults can be evaluated in many ways, the BMI is most widely used because its use is inexpensive, non-invasive and suitable for large-scale surveys (Lohman *et al.*, 1998; Ferro-Luzzi *et al.*, 1992; James *et al.* 1994). Therefore, BMI is

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the most established anthropometric indicator used for assessment of adult nutrition status. BMI is generally considered a good indicator of not only the nutritional status but also the socio-economic condition of a population, especially adult populations of developing countries (Ferro-Luzzi *et al.*, 1992; Shetty *et al.*, 1994; Khongsdier, 2002; Adak *et al.*, 2006).

It has been recently suggested (Datta Banik *et al.*, 2005, 2007; Datta Banik, 2007; Datta Banik and Sain 2007; Bose *et al.*, 2005, 2006abcd) that there is urgent need to evaluate the nutritional status of various tribes of India. The tribals reside in the interior rural areas of forest, plateau, hills and mountains of the country. The tribal populations of India are socially and economically at risk (Ghosh and Bharati, 2006).

### ***The Oraons of Jharkhand***

Jharkhand includes a foremost part of the Chhotanagpur plateau which is studded with a range of hills and more than 27% of land under forest cover, which provides a unique habitat to the tribal communities (Mandal *et al.*, 2002). The Scheduled Tribes constitute around 30.25% of the total population of the state of Jharkhand (Census of India, 1981). There are about 30 major tribal communities in the state. Among them the Santals, Oraons, Mundas, Hos, Loharas, Kharwars, Kharias and the Bhumijis are the predominant tribal groups. The Oraons are the second largest tribal community, next to the Santals in the state of Jharkhand with a total population of 9,66,413 out of which 7,49,073 are settled in Ranchi district followed by the districts of Palamau (1,28,191) and Hazaribagh (34,648). They were the originally the inhabitants of the Chhotanagpur region (Hazaribagh district). They are also distributed in Bihar, West Bengal, Tripura, Assam, Maharashtra, some parts of Madhya Pradesh and Orissa. The Oraons speak *Kurukh*, which belongs to the sub-groups of the Dravidian language family. They also know Hindi. The Oraons have several exogamous totemic clans and their clan names are used by them as surnames (viz. Kujur, Tirke, Ekka etc.). Land is their main economic resource. They are settled cultivators. But during lean seasons they depend on forest produce. A number of Oraons work as wage labourers and industrial workers and some of them are employed in government and private organizations. The Oraons have their own religion, folk songs and folk tales. According to the 1981 Census, about 58.43% of them follow some religious traits of the Hindus, 21.05% are Christians and 20.52% belong to other religions (Roy, 1915; Dalton, 1866, 1872; Caldwell, 1875; Gait, 1903; Das and Raha, 1963; Hahn, 1900, 1903 and 1905).

### ***The Saraks of Ranchi District, Jharkhand***

Sarak or Sarawak, a small caste of Chotanagpur who seems to be a Hinduised remnant of early Jain people. Saraks of Manbhum, while retaining the tradition that their ancestors were Jains, appear themselves to have completely adopted Hinduism. They worship the ordinary Hindu Gods and Goddesses; nor have they retains, any of the characteristic 'Tirthankars' or glorified saints of the Jains. Etymologically, Sarak is a derivative of *Sravaka* of which *sra* means

'*sradha*' (respect), *va* means 'vivek' (consciousness) and *ka* means 'kriya' (work). *Sravaka* is Sanskrit, means a listener. (Risley, 1891). In the descriptive ethnological accounts of Hunter (1877), O' Mally (1910) and Coupland (1911) one gets casual references of the community. During the last two decades also local literature depicting the exclusive nature of this community began to appear in regional and national languages, (for instance, Bijoyjee 1985; Chakrabarti, 1981; Chakrabarty, 1984; Bhattacharya, 1986; Singh, 1996). They are pure vegetarian and seldom per take onion, garlic, lentil, carrot, beat-root that are believed to have stimulant effect. Temperamentally they are a very mild, docile and peace loving community. Although Saraks profess and practice Jainism in their way of life they have been greatly influenced by the Hindus and tribals as they also follow side by side the rites and rituals of the latter communities (Bhattacharya, 1997).

The Saraks are skillful agriculturists. The Saraks are a peasant community spread over the eastern region of India in West Bengal, Bihar and Orissa. In West Bengal, they live in the district of Purulia, Burdwan, Bankura and Midnapur in varying strength. In Bihar, they are found in the Santhal Parganas, Ranchi, Bokaro, Singbhum and Dhanbad district. Bhattacharya (1986) mentioned Saraks a small community which was transforming into a Hindu community. They observe the *Gajan* festivals of Lord *Siva* as well as other Hindu festivals (Chakrabarti and Bhattacharya, 2000). There are 7 gotras or exogamous groups, *Adi or Adi Deb*, *Dharma Deb*, *Rishi Deb*, *Sandilya*, *Kashyapa*, *Ananta* and *Bharadvaja*.

Several studies have focused on anthropometric characteristics and nutritional status of adult men and women of different ethnic groups of both tribal and non-tribal populations (cited in Bose and Chakraborty, 2005). In view of this, the objective of the present study was to report nutritional status, based on BMI, of the adult (age 18 years and above) female Oraons and the Saraks in the district of Ranchi in the state of Jharkhand, India. It is the first report dealing with the levels of anthropometric characteristics and nutritional status of the Saraks. This cross-sectional study focuses on anthropometric variations and also evaluates nutritional status of the adult female Oraons and the Saraks in Ranchi district of Jharkhand, India.

## MATERIALS AND METHODS

The present cross-sectional study among the adult (age 18 years and above) Saraks (110 females) and the Oraons (216 females) was conducted during July 2007. The area of study was located in five villages in and around Bundu (police station, block and sub-division), about 45 kms away towards south from the city of Ranchi, the provincial capital of the state of Jharkhand. The study was undertaken in five villages, viz. Bundu, and Manjhituli (notified area under Bundu block), Paramdih, Beradih and Paramdih (under Beradih Panchayat) The area of study was also extended to an another village, Nawadi under the block and police station of Ichagarh which is towards south and 45 kms. away from Bundu. The big Sarak village, Nawadi near Rangamati



region in the bordering areas of the two neighbouring districts of Ranchi and Paschimi (West) Singhbhum is under the Sub-Division of Chandil and Panchayat of Deultanr.

The anthropometric data of the adult (age 18 years and above) female (n = 216) Oraons were collected from five villages, viz, Amanburu, Ulidih, Nahelgara and Nawadi and Manjhituli near Bundu.

The other social and cultural factors like ethnicity, endogamy, clan exogamy, marriage distance and direction etc were also kept in mind in order to restore the purity of data of a particular community, either caste (the Saraks) or a tribe (the Oraons). All available female subjects from the villages were included in the study. Pregnant and lactating women were excluded from the investigation. The response rate was 87%.

#### ***Anthropometric measurements, derived ratios, indices and equations***

Anthropometry is used to evaluate or to determine the prevalence of undernutrition within and between population(s). All Anthropometric measurements of lightly-clothed subjects were taken by the trained investigators using standard anthropometric techniques followed by Weiner and Lourie (1969), Lohman *et al.*, (1988); and Lee and Nieman (2007). Stature or height (cm) is the vertical distance from floor to vertex of the head. The subjects head is held with the Frankfurt plane and bare footed. Mid-upper arm circumference (MUAC) is an indicator of the amount of fat and muscle in the upper arm. In population level, a reasonable correlation exists between MUAC and BMI in adults.

Height and weight were taken to the nearest 0.1 cm and 0.5 kg, using standard Martin's anthropometer and weighing scale (Libra, New Delhi, India), respectively. Technical errors of measurements (TEM) were within acceptable limits. Derived anthropometric Indices and ratios were computed using the following standard equation and classifications were presented following international standards (Lohman *et al.*, 1988; WHO, 1971, 1995).

Abbreviations of the anthropometric measurements, indices and ratios used are (in alphabetical order):

BMI (Kg/m<sup>2</sup>) = Body Mass Index

BW = Body Weight (in kg)

MUAC (cm) = Mid Upper Arm Circumference

ST (cm) = Stature or Height

#### **Body Mass Index (BMI)**

BMI may be appropriate for population-level assessments of chronic undernutrition. The classification of BMI provides a useful framework for the analysis of height and weight data from chronically undernourished adult populations.

$$\text{BMI} = \text{Weight (kg)} / \text{height (m}^2\text{)}$$

The classification of categories of chronic undernutrition of categories BMI-

VALUE	NUTRITIONAL STATUS
< 16.0	Grade III Thinness } Grade II Thinness } UNDERNUTRITION Grade I Thinness }
16.0 - 16.99	
17.0 - 18.49	
18.50 - 24.99	NORMAL
25.0 - 29.99	Grade I Overweight (Overweight)
30.0 - 39.99	Grade II Overweight (Obesity Grade I)
> 40.00	Grade III Overweight (Obesity Grade II)

(Ferro-Luzzi *et al.*, 1992; WHO, 1995)

#### *Mid-Upper Arm Circumference (MUAC)*

The arm contains subcutaneous fat and muscle; Changes in mid-arm circumference (MUAC) tend to parallel changes in muscle mass and hence are particularly useful in the diagnosis of protein energy malnutrition or starvation (Harries *et al.*, 1984; Bray *et al.*, 1978; Collins *et al.*, 2000).

MUAC measurement was made using a flexible, non - stretch tape. The subject stood erect and sideways to the measurer with the head in the Frankfurt plane, arms relaxed and legs apart. The measurement was taken at the midpoint of the upper right arm between the acromion process and the tip of the olecranon. After locating the midpoint the right arm was relaxed so that it was hanging loosely by the side, with the palms facing inwards. The tape was wrapped gently but firmly around the arm at the mid point. Measurement was taken to the nearest 0.1 cm.

#### *Cut-off values of Mid Upper Arm Circumference (MUAC)*

VALUE (cm)	SEX	NUTRITIONAL STATUS
≥ 22.0	Female	Normal
<22.0	Female	Undernutrition

(Pitanga and Lessa, 2005 ; Lee and Nieman, 2007)

Most of the equations were computed following standard (WHO, 1995; Vanitalie *et al.*; 1990; Lohman *et al.*, 1981) formulae. Student t-tests were performed to test for differences in mean Anthropometric characteristics between the two different samples. Pearson correlation coefficients (r) were used to study the interrelationship between age and anthropometric

characteristics. All statistical analyses were done using the SPSS Statistical Package. Statistical significance was set at  $p < 0.05$ .

Ethical approval was obtained from Vidyasagar University Ethics Committee before commencement of the study. Informed consent was also obtained from local community leaders and each participant.

## RESULTS

**Table 1 :** Descriptive Statistics of Anthropometric Characteristics among the adult female oraons (n = 216) and the saraks (n = 110).

Sl. No.	Variables	Population	Range	Mean $\pm$ SE	t	Sig
1.	Age (Years)	Oraons	18-70	33.19 $\pm$ 0.91	6.264	0.0001
		Saraks	19-85	44.36 $\pm$ 1.53		
2.	Height (cm)	Oraons	131.0-174.0	149.56 $\pm$ 0.46	-2.809	0.05
		Saraks	124.7-161.3	147.39 $\pm$ 0.62		
3.	BW (kg)	Oraons	20-71	40.51 $\pm$ 0.47	1.964	0.050
		Saraks	26-70	42.2 $\pm$ 0.78		
4.	BMI (kg/m <sup>2</sup> )	Oraons	8.77-27.21	18.08 $\pm$ 0.18	3.522	0.001
		Saraks	14.12-31.51	19.41 $\pm$ 0.33		
5.	MUAC (cm)	Oraons	14.7-31.8	21.94 $\pm$ 0.17	2.244	0.025
		Saraks	17-29	22.60 $\pm$ 0.24		

The descriptive statistics (mean  $\pm$  standard error) of all anthropometric variables, including derived ratios and indices of the two adult female samples, the Oraons (n=216) the Saraks (n=110) are presented in *table 1*. Results of the student t-test with respect to all parameters are also shown to understand the level of significant differences between the two female samples.

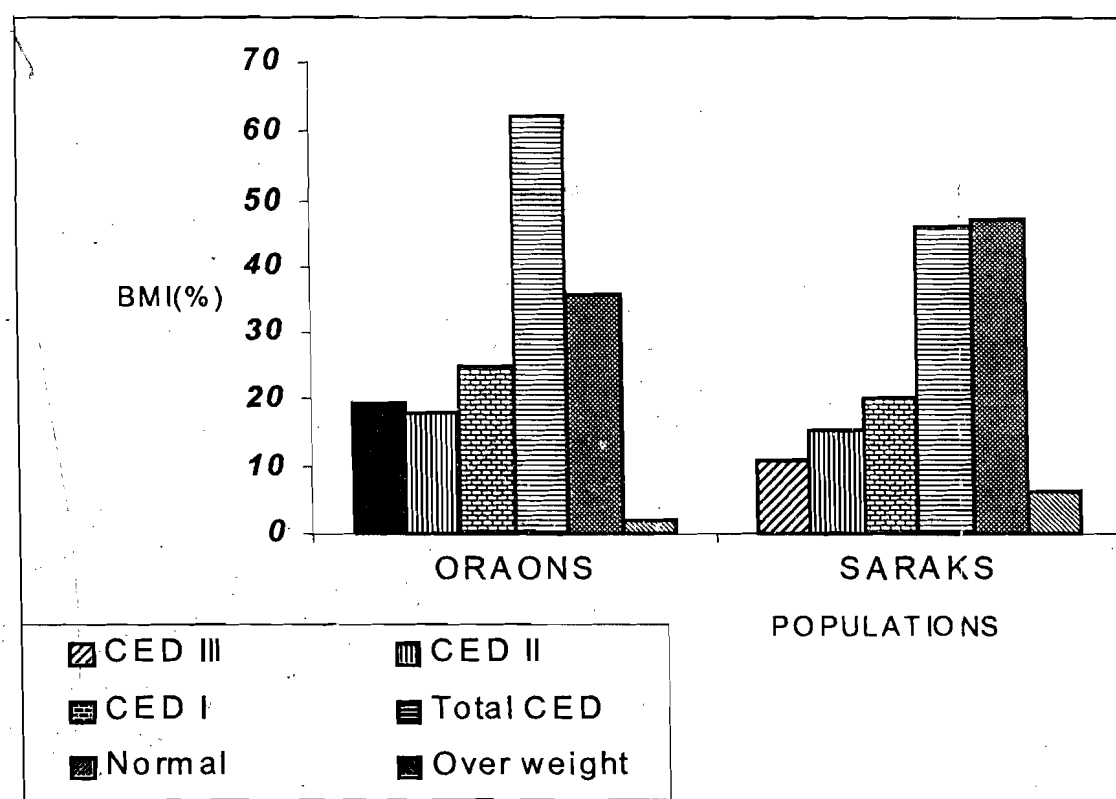
The mean ages (33.19 years  $\pm$  0.91 ranging from 18 - 70 years for the Oraons and 44.36 years  $\pm$  1.53 ranging from 19 - 85 years for the Saraks) of the two samples exhibit that on average, the Oraons represent much younger female population than the Saraks with significant difference ( $p < 0.0001$ ). The Sarak females show higher mean values of all variables with the exception in stature, compared to the Oraon females. Highly significant differences ( $p < 0.001$ ) are observed between the two samples and with respect to the characters like Age (33.19  $\pm$  0.91 for the Oraons and 44.36  $\pm$  1.53 for the Saraks) and BMI (18.08 Kg/m<sup>2</sup>  $\pm$  0.18 for the Oraons and 19.41  $\pm$  0.33 for the Saraks), Moderately significant differences ( $p < 0.05$ ) are observed for the variables like Height (149.56 cm.  $\pm$  0.46 for the Oraons and 147.39 cm.  $\pm$  0.62 for the

Saraks) and Body Weight. However, very less significant differences between the two adult female sample, are exhibited in cases of MUAC ( $21.94 \text{ cm} \pm 0.17$  for the Oraons and  $22.60 \text{ cm} \pm 0.24$  for the Saraks  $p < 0.025$ ).

**Table 2 :** BMI and Nutritional status among the adult female Oraons (n = 216) and the Saraks (n = 110).

Nutritional Status	BMI CUT-OFF VALUE	ORAONS	SARAKS
CED III	< 16.00	42 (19.44%)	12 (10.9%)
CED II	16.00-16.99	39 (18.06%)	17 (15.45%)
CED I	17.00 – 18.49	54 (25.00%)	22 (20.00%)
Total CED	< 18.49	135 (62.50%)	51 (46.36%)
Normal	18.50 - 24.99	77 (35.65%)	52 (47.27%)
Over weight	25.00 – 29.99	4 (1.85%)	7 (6.36%)

The health and nutritional status of the two samples representing the adult sections of the tribal population, the Oraons and an endogamous caste group, the Saraks of the Ranchi District in the State of Jharkhand are measured and assessed by the WHO (1995) recommended cut-off values of Body Mass Index (BMI). The frequency of Chronic Energy Deficiency (CED) of the two samples is also presented in the same table. The prevalence of undernutrition (CED; BMI  $< 18.49 \text{ Kg/m}^2$ ) is much higher among the adult female Oraons (62.50%) compared the adult female Saraks (46.36%). Both the populations however, are suffering from severe under nutrition. 35.65% of the adult female Oraons and 42.27% of the adult female Saraks represent normal (BMI  $18.50 - 24.99 \text{ Kg/m}^2$ ) and healthy sections (*Figure 1*).

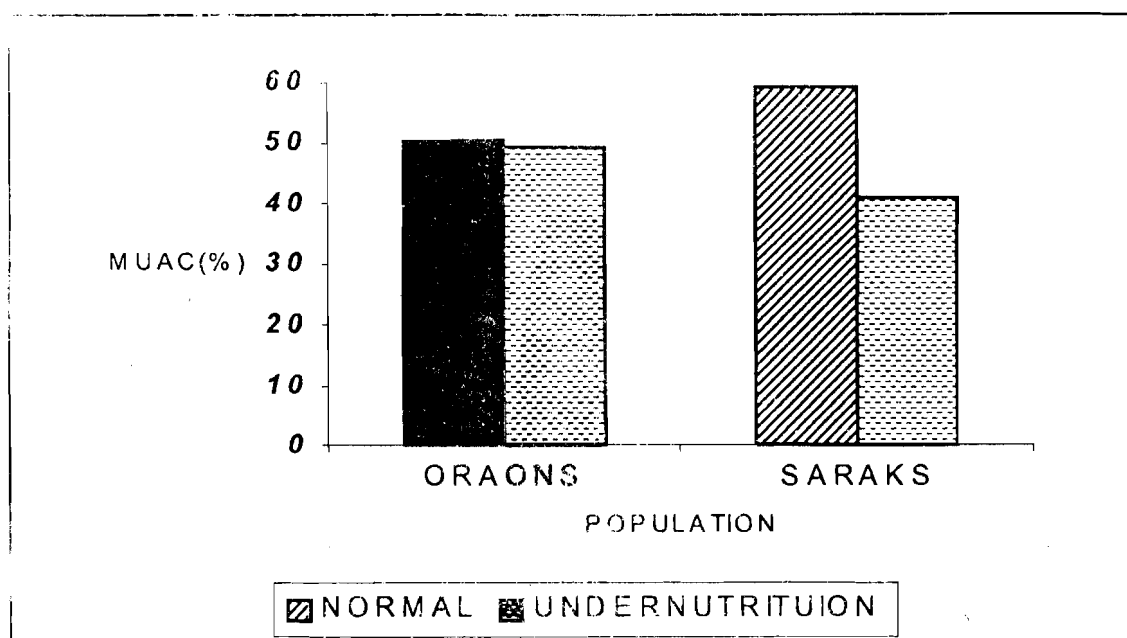


**Figure 1 :** BMI and Nutritional status among the adult female Oraons (n = 216) and the Saraks (n = 110).

**Table 3 :** Nutritional status measured by cut-off points of mid upper-arm circumference (MUAC) among the adult female Oraons (n = 216) and the Saraks (n = 110).

NUTRITIONAL STATUS	MUAC RANGE (cm.)	POPULATION	
		ORAONS	SARAKS
NORMAL	≥22.00	109 (50.46%)	65 (59.09%)
UNDERNUTRITION	<22.00	107 (49.54%)	45 (40.91%)

Nutritional statuses are also measured by the standard Cut – off values of Mid Upper Arm Circumference (MUAC) for the adult females. Higher prevalence of under nutrition (49.54%) is also observed here among the adult Oraons compared to the adult Saraks (40.91%) very high



**Figure 2 :** Nutritional status measured by cut-off points of mid upper-arm circumference (MUAC) among the adult female Oraons (n = 216) and the Saraks (n = 110).

**Table 4 :** Correlation of variables among the adult female Oraons (n = 216) and the Saraks (n = 110).

Sl. No.	Variables	AGE		BMI		MUAC	
		Oraons (n = 216)	Saraks (n = 110)	Oraons (n = 216)	Saraks (n = 110)	Oraons (n = 216)	Saraks (n = 110)
		r	r	r	r	r	r
1.	Age	1.000	1.000	-0.319**	-0.99	-0.082	-0.170
2.	Height	-0.150*	-0.355**	-0.053	-0.087	0.188**	0.310**
3.	BW	-0.355**	-0.268**	0.839**	0.859**	0.607**	0.830**
4.	BMI	-0.319	-0.99	1.000	1.000	0.539**	0.738**
5.	MUAC	-0.082	-0.170	0.593**	0.738**	0.142*	0.305**

\*\*Correlation is significant at the 0.01 level (2 tailed)

\* Correlation is significant at the 0.05 level (2 tailed)

Table 4 represents Pearson correlation coefficients (r) of Age, BMI, MUAC, separately, each with all the other anthropometric characteristics discussed in *table 1*. Significant correlations ( $p < 0.01$ ) are observed in both the adult female samples (Body Weight with Age, BMI and MUAC; MUAC with BMI; Height with MUAC. Significantly Negative correlation of age with

height among the Oraons ( $r = -0.150$ ,  $p < 0.05$ ); among the Saraks ( $r = -0.355$ ,  $p < 0.01$ ) and of age with body weight ( $r = -0.355$  among the Oraons and  $r = -0.268$  among the Saraks,  $p < 0.01$  in both the samples) are observed. No significant correlations are observed in cases of age with BMI, MUAC and BMI with height, in both the adult female samples.

## DISCUSSION

Adults constitute the important proportion of the populations all over the world and especially in developing countries. Less information are available on their anthropometric evaluation of nutritional status and/or socio-economic conditions (Oguntona and Kuku, 2000; McLorg, 2005). Even more insufficient data are available on the nutritional profile of adults of the different tribal populations of India (Bose and Chakraborty, 2005). It is very significant to appraise the nutritional state of adults because of its function in ensuring an overall better quality of life (Dandekar, 1996; Kikafunda and Lukwago, 2005).

Many studies (Burr and Phillips, 1984; Chumlea *et al.*, 1986; Shimokata *et al.*, 1989; Delaure *et al.*, 1994; Chilima and Ismail, 1998; Oguntona and Kuku, 2000) have been carried out on health and nutritional status among elderly individuals utilizing anthropometry from different countries and ethnic groups. However, only a few studies (Bagga, 1997; Ghosh *et al.*, 2001; Bose and Das Chaudhuri, 2003) are available on age changes in anthropometric characteristics among the adult individuals in Indian context. The present study therefore presents unique data on anthropometric and nutritional profile of the adult female Oraons and Saraks, two ethnic groups of Eastern India. These results are in concordance with studies from other parts of the world on different ethnic groups (Pirlich and Lochs, 2001; Suzana *et al.*, 2002). High prevalence rates of undernutrition in both the Oraon and Sarak women are the striking features of this study. Using WHO classification (1995) of nutritional status according to BMI values, 62.50% of adult Oraon women and 46.36% of adult Sarak women in this study were thin. The rate of undernutrition among adult Oraon women was observed to be much higher than those reported from other populations in developing countries (Chilima and Ismail, 1998; Zverev and Chisi, 2004) including India (Bose and Das Chaudhuri, 2003). Kikafunda and Lukwago (2005) reported a higher prevalence of undernutrition (68%), among elderly women from Mpigi District of central Uganda, in comparison with the cases of the adult Oraon women of this study. These results indicate that undernutrition is a severe crisis among the adult Oraon as well as Sarak women.

## CONCLUSION

This study is a kind of preliminary record of data and information of anthropometric characteristics and nutritional status of the adult female Oraons, a tribe and the Saraks, a caste in Ranchi district of Jharkhand. The results show that a remarkable section of population is suffering from undernutrition. Low economic status and living at the below subsistence level, non availability

of proper nutrition and lack of other supports from the state as well as local government and non government agencies are the major causes behind the poor nutritional situation of both the communities. Immediate attention for adequate food and nutrient supplementation is required for both the Oraons and the Saraks. Significant ethnic variations are observed between the adult female Oraons and Saraks in Ranchi district of the state of Jharkhand in eastern India with respect to the anthropometric criteria, viz. body weight, BMI and MUAC. From the above results it is revealed that both the adult females, are suffering from sever undernutrition, as measured by BMI and MUAC. The state of health is even worse for the case of the Oraons, a tribe compared to the Saraks.

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