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ANTI DIABETIC AND ANTI HYPER-LIPIDEMIC POTENTIAL OF BASIL: A COMMONLY USED HERB IN INDIA

Susmita Roy¹ and Dr Pratiti Ghosh²

Abstract

Diabetes mellitus (DM) is a chronic metabolic disease affecting the health of a large population worldwide. In 2010 an estimated 285 million people had diabetes and within the next 20 years this value is expected to almost double. Although a number of synthetic medicines are available and used for the treatment of DM, they have some side effects. So drugs from natural origin are preferred over these medicines due to their lesser side effects. In the present study the antidiabetic and anti-hyperlipidemic effects of aqueous extract of fresh leaves of basil in different doses were investigated in normal and streptozotocin induced diabetic rats. The effect of these doses on fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), HDL-C content were investigated in streptozotocin induced rat and found significant effect ($p < 0.05$). Maximum reduction of FBG level was found with high dose. It has been observed from the experimental results that reduction in TG, LDL% are highly significant ($0.001 < p < 0.000$). Total cholesterol level was also found to decreased significantly ($p < 0.05$) whereas HDL-C level increases. A significant decrease in creatinine, urea and BUN were also observed. The results indicate the favourable effect of basil in the treatment of diabetes mellitus.

Key words: Streptozotocin, *Ocimum sanctum*, FBG, TG, TC

Introduction

Diabetes Mellitus (DM) is a common and chronic medical condition characterized by hyperglycemia, glycosuria, hyperlipidemia, polyuria, polyphagia, polydipsia, negative nitrogen balance and sometime ketonemia, Insulin and glucagon are two hormones produced by the pancreas which controls the blood glucose level. Elevated level of sugar in blood increases insulin secretion and blood sugar level is normalized. Diabetes is a condition with impairment of insulin secretion or defective insulin response. DM has been found to be associated with many complications including production of reactive oxygen species. There are many expensive drugs available in the market, as well as insulin that are used for treatment of diabetes but some of them cause side effects like disturbances in the functioning of vital organs (Singh et al, 2011). 'So, World Health Organization advocated exploring herbal remedy and naturally occurring medicinal plant for the treatment of diabetes in a better way with minimum side effects' (Takamoto, 2011). On the other hand, medicinal plants have been used from ancient time in the herbal medicine and some of them have therapeutic potential and experimentally documented.

"Holy Basil" or *Ocimum sanctum* (*O. sanctum*) is one of the roughly sixty species of genus *Ocimum*. It is little known in the western world but widely cultivated in India. Although it is originated in India, the main center of diversity is considered to be Africa (Simon et al, 1999). It comes from the botanical family 'Lamiaceae' and commonly known as tulsi in India. Mainly three different types of tulsi leaves are

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commonly cultivated in India, rama tulsi or the green leaf variety, the most common one, the second type dark green-to-purple leaves or krishna tulsi, a third type is vana tulsi a forest variety that often grows wild. In the recent years there is an exponential growth in the field of herbal medicine and the drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Moreover increased scientific interest in plant phytochemicals has brought numerous herbs and spices including basil to the forefront of nutritional research. Since ancient times, tulsi or basil is known for its medicinal properties (Modak et al, 2007, Grover, 2002) and its therapeutic use is experimentally evidenced now a day (Molnar, 2008), (Hussain et al, 2001 Aggarwal, 1996 Chattopadhyay et al, 1993), (Grover, 2002), (Sarkar, 1994), (Rai, 1997).

Basil is virtually calorie-free and rich in antioxidant, vitamins, phenolic and dietary fibre. Introducing basil into the diet by using them as flavouring agent or for garnishing or in aqueous extract form provides us an added health boost. But before using it as a therapeutic agent proper identification of phytochemicals present with mechanism involved behind the activity, investigation of combination dosages of natural plant product and determination of the long-term side effects of natural herbal product formulations individually and in combination, human clinical evaluation in large population are necessary (Molner, 2008).

Objective

In the present study the main objective is to assess the efficacy of *O. sanctum* in different single doses in the treatment of diabetes mellitus.

Table 1: Group no and - Dose of treatment (Modak et al, 2007)

Treated groups	Dose of tulsi
T ₁ (low dose mg /kg body weight)	50
T ₂ (medium dose mg /kg body weight)	100
T ₃ (high dose mg /kg body weight)	200
T ₄ Streptozotocin control	-

Methodology

Selection of experimental animal and Induction of diabetes:-

Healthy adult Wistar albino rats of 90-120 gram weight were used for the study. They were fed with a standard diet and water. As rats are mammals, their system reacts to these chemicals in a similar way as it reacts in the human system (Halim et al, 2006). In the present study twenty five rats were made diabetic by intra peritoneal injection of freshly prepared streptozotocin (STZ), 40 mg/kg body weight. Five rats were taken as non-diabetic healthy controls. Then after 7 days 2nd dose was given. The diabetic rats were divided into following four groups given in table 1 (Modak et al, 2007), each having five rats. Five diabetic rats, which expired during the experiment, were excluded from the study.

Treatment of diabetes:-

The dosing schedule used was once per day. The aqueous solution was fed through oral gavage for 21 days. (Sethi et al, 2012). Time of dosage was at 10:00 a.m. to 10:30 a.m. Oral gavage was performed using a ball ended feeding needle. The rat was restrained in a straight line to facilitate introduction of the gavage needle. The needle was introduced in the space between the left incisors and molars, and gently it was directed caudally toward the right ramous of the mandible. Once the desired position was attained, the dose was injected and the syringe was withdrawn. The animal was monitored after the procedure to ensure that there are no adverse effects. The blood glucose level and body weight was measured once in every week.

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Reagent:-

Streptozotocin (STZ) was obtained from Spechtrochem Pvt. Ltd. Mumbai, India. *Ocimum sanctum* collected belongs to family Lamiaceae and green variety leaves (Rama Tulsi). The leaves were fresh. An aqueous extract was produced by grinding the leaves and extracted in distilled water. The rats were fed with the extract by means of feeding tube at a dose above mentioned. All the kit for biochemical estimation were obtained from Span Diagnostic.

Collection of blood sample

Blood was collected at the beginning, after 7 days, 14 days, retro-orbitally from the inner canthus of the eye using capillaries (Mucaps) in EDTA vial and at the end of the experiment after 21 days they were sacrificed by cervical dislocation under light ether anesthesia and blood was collected.

Estimation of biochemical parameters

Glucose estimation: Glucose was estimated at the beginning, after 7 days, 14 days and end of the experiment by using Glucometer (Contour TS Blood Glucose meter) and Contour TS Blood Glucose strip.

Hemoglobin estimation: Hemoglobin was estimated by cyanmethemoglobin method using kit (Drabkin reagent) from Span diagnostics.

Lipid Profile: Total cholesterol (TC), high-density lipoprotein cholesterol, (HDL-C) and triglycerides (TG) were estimated by enzymatic methods employing kits from Span diagnostics. LDL cholesterol was estimated by using Friedewald WT (1972) formula as follows (Halim et al, 2006):

LDL in mg % = total cholesterol - (HDL-C \times 1/5 triglycerides).

Urea, creatinine and BUN: Urea and creatinine were estimated by enzymatic methods employing kits from Span diagnostics. BUN was calculated by the standard formula as follows:

BUN concentration mg/dl = Urea concentration mg/dl \times 0.467 (Murray R.L.1984).

Statistical Analysis: The results are analysed using mean \pm SD for all experiments and represented graphically. The significance of difference between data pairs was evaluated by analysis of variance (ANOVA).

RESULTS

Table 2: Blood glucose level after treatment with *O. sanctum* in streptozotocin induced diabetic rat

Plasma glucose mg/dl, mean \pm SD

Group	0 days	07 days	14 days	21 days	F	p value
Diabetic untreated (T ₀)	220 \pm 24	243 \pm 14	243 \pm 14.1	244.67 \pm 44	4.44	0.170
Tulsi low dose (T ₁)	381 \pm 7.9	281.2 \pm 7.22	395.3 \pm 12.2	373 \pm 11	1.043	0.365
Tulsi Medium dose (T ₂)	244.75 \pm 94	217 \pm 61.5	188	150 \pm 20.07	4.485	0.102
Tulsi high dose (T ₃)	254.5 \pm 7.7	256 \pm 10.6	157 \pm 4.24	133.67 \pm 9.07	368.0***	0.000

***=1% level of significance, **= 5% level of significance and *=10% level of significance

The effects of oral administration of aqueous extract of Tulsi leaves in diabetic rat are shown in Table1. Whereas in diabetic untreated rat (STZ control T4) plasma glucose continued to rise from 220 ± 24 mg/dl to 243 ± 14.1 mg/dl after 14 days, and 244.67 ± 44 mg/dl after 21 days, in diabetic rats having treatment with Tulsi low dose (T1), plasma glucose fell initially from 381 ± 7.9 mg/dl, to 281.2 ± 7.22 mg/dl after 7 days. But after that there was an increase in the blood sugar level, 395.3 ± 12.2 mg/dl and 373 ± 11 mg/dl after 14

days and 21 days respectively. When treated with Tulsi medium dose (T2) plasma glucose fell from 244.75 ± 94 mg/dl at the beginning of the experiment to 217 ± 61.5 mg/dl, 188 mg/dl and 150 ± 20.07 mg/dl after 7, 14 and 21 days respectively. With high dose treatment (T3) the plasma glucose fell significantly (368^{***}) from 254.5 ± 7.7 mg/dl at day 01 to 256 ± 10.6 mg/dl, 157 ± 4.24 mg/dl and 133.67 ± 9.07 mg/dl for day 07, day 14 and day 21 respectively.

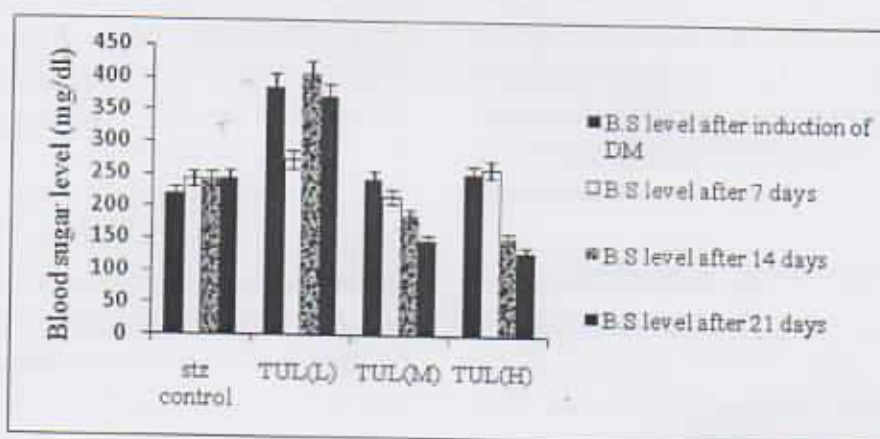


Fig 1: Effect of aqueous extract of leaf of *Ocimum sanctum* (Tulsi) on plasma glucose level in Control, and streptozotocin - induced treated diabetic rats.

TUL= Tulsi, STZ CTRL = streptozotocin control, BS= Blood sugar

Table 3: Haemoglobin level before and after treatment with *O. sanctum* in streptozotocin induced diabetic rat

Haemoglobin level mg/dl. mean \pm SD				
Group	0 days (before experiment)	21 days (after experiment)	F	p value
Diabetic untreated (T ₄)	9.52 ± 0.210	9.83 ± 1.51	0.126	0.740
Tulsi low dose (T ₁)	8.82 ± 0.326	12.6 ± 0.75	63.258 ^{***}	0.001
Tulsi Medium dose (T ₂)	9.36 ± 0.513	13.65 ± 0.212	190.512 ^{***}	0.000
Tulsi high dose (T ₃)	9.54 ± 0.314	17.13 ± 1.26	121.2 ^{***}	0.000

***=1% level of significance, **= 5% level of significance and *=10% level of significance

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Table 2 shows the effect of aqueous extract of *Ocimum sanctum* (Tulsi) in different dose, on the value of haemoglobin measured initially before experiment and at the end of experiment on 21 days in control, diabetic untreated and diabetic treated rats. From the result it has been observed that haemoglobin level increases significantly ($p < 0.05$) after treatment with the herb. Maximum increase was observed with high

dose of the herb from 9.54 ± 0.314 mg/dl to 17.13 ± 1.26 mg/dl (121.2^{***}) followed by medium 9.36 ± 0.513 mg/dl to 13.65 ± 0.212 mg/dl (190.512^{***}) and low dose 8.82 ± 0.326 mg/dl to 12.6 ± 0.75 mg/dl (63.258^{***}) respectively. The haemoglobin level of the diabetic untreated rat was 9.52 ± 0.210 mg/dl at the beginning of the study and at the end of the experiment it was 9.83 ± 1.51 mg/dl.

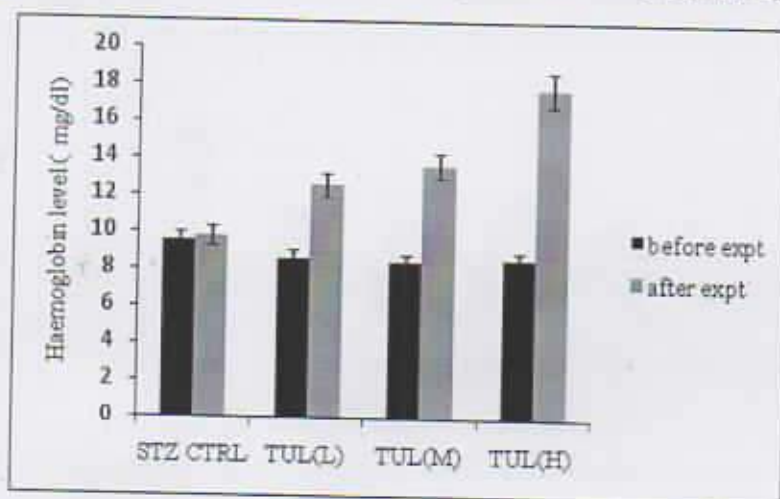


Fig 2: Effect of aqueous extract of leaf of *Ocimum sanctum* (Tulsi) on haemoglobin level in Control, and streptozotocin - induced treated diabetic rats.

TUL= Tulsi, STZ CTRL = streptozotocin control

Table 4: Effect of *O. sanctum* on lipid profile level in diabetic control, and streptozotocin - induced treated diabetic rats

Group	T ₀	T ₁	T ₂	T ₃
HDL-C (mg/dl)	19.87±1.25	33.18±7.42	43.98±1.00	47.41±8.63
F		60.43***	401.67***	100.35***
P value		0.001	0.000	0.001
TG (mg/dl)	251.8±10.31	83.7±6.24	89.53±7.37	82.76±2.81
F		277.03***	245.42***	391.14***
P value		0.000	0.000	0.000
Total Cholesterol (mg/dl)	78.3±3.33	51.87±2.39	51.87±3.79	48.7±5.18
F		9.741**	8.906**	25.493***
P value		0.03*	.041	0.007
LDL% (mg/dl)	109.39±9.82	25.25±9.88	33.89±2.23	13.44±9.28
F		100.88***	169.423***	131.810***
P value		0.000	0.000	0.000

***=10% level of significance, ** = 5% level of significance and * = 10% level of significance

Table 3 shows the effect of aqueous extract of *O. sanctum* on lipid profile in different doses, measured at the end of experiment in control, diabetic untreated and treated rats. Study result revealed that HDL-C level was 19.07 ± 1.29 mg/dl for untreated rat whereas after treatment with *O. sanctum* HDL-C level was 53.16 ± 7.42 mg/dl, (60.43^{***}), 45.68 ± 1.66 mg/dl (401.67^{***}) and 47.41 ± 4.63 mg/dl (100.55^{***}) for low,

medium and high dose respectively. A significant reduction in the total cholesterol was observed with *O. sanctum* with all the three doses (9.541^{**} , 8.906^{**} and 25.498^{***} for T₁, T₂ and T₃ respectively). Moreover a significant decrease was observed in TG ($p=0.000$), and LDL% ($p=0.000$) with all the three doses.

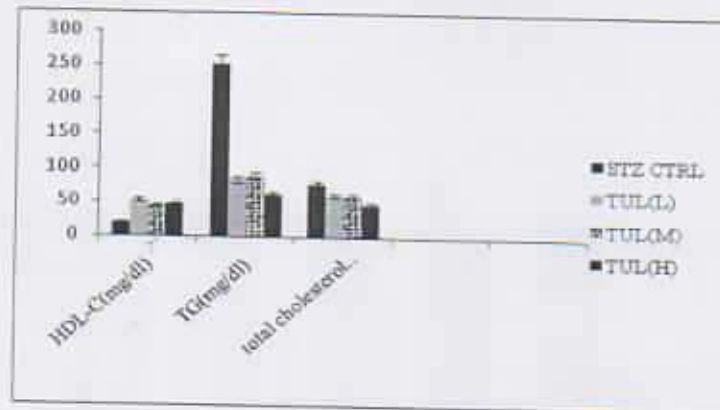


Fig 3: Effect of aqueous extract of leaf of *Ocimum sanctum* (Tulsi) on lipid profile in Control, and streptozotocin-induced treated diabetic rats.

HDL= High density lipoprotein, TG = Triglyceride, TUL= Tulsi, STZ CTRL= streptozotocin control

Table 5: Effect of *O. sanctum* on urea, creatinine and BUN level in diabetic control, and streptozotocin-induced treated diabetic rats

Group	T ₀	T ₁	T ₂	T ₃
Creatinine (mg/dl)	1.28 ± 0.13	0.71 ± 0.011	0.454 ± 0.12	0.43 ± 0.06
F		36.643^{***}	61.958^{***}	104.249^{***}
P value		0.002	0.001	0.001
Urea (mg/dl)	66.4 ± 4.1	57.6 ± 3.22	43.9 ± 2.10	40 ± 1.67
F		8.516^{**}	71.248^{***}	106.352^{***}
P value		0.043	0.001	0.000
BUN (mg/dl)	31 ± 1.31	26.8 ± 1.5	20.5 ± 0.98	18.68 ± 0.78
F		8.516^{**}	71.248^{***}	106.352^{***}
P value		0.043	0.001	0.000

***=1% level of significance, **= 5% level of significance and *=10% level of significance

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The effect of aqueous extract of *O. sanctum* in different dose, on the value of urea, creatinine and BUN measured at the end of experiment in control, diabetic untreated and treated rats are shown in table 4. A significant reduction in creatinine, urea and BUN was observed in the study. At the end of the experiment creatinine level was 1.28 ± 0.13 mg/dl for diabetic untreated rat whereas after treatment with

O. sanctum it was 0.71 ± 0.011 mg/dl, (56.643^{***}), 0.454 ± 0.12 mg/dl (63.958^{***}) and 0.43 ± 0.06 mg/dl (104.249^{***}) for low, medium and high dose respectively. Urea level was found to be 66.4 ± 4.1 mg/dl for diabetic untreated rat and decreases upto 57.6 ± 3.22 mg/dl, (8.516^{**}), 43.9 ± 2.10 mg/dl (71.248^{***}) and 40 ± 1.67 mg/dl (106.352^{***}) for T₁, T₂ and T₃ respectively.

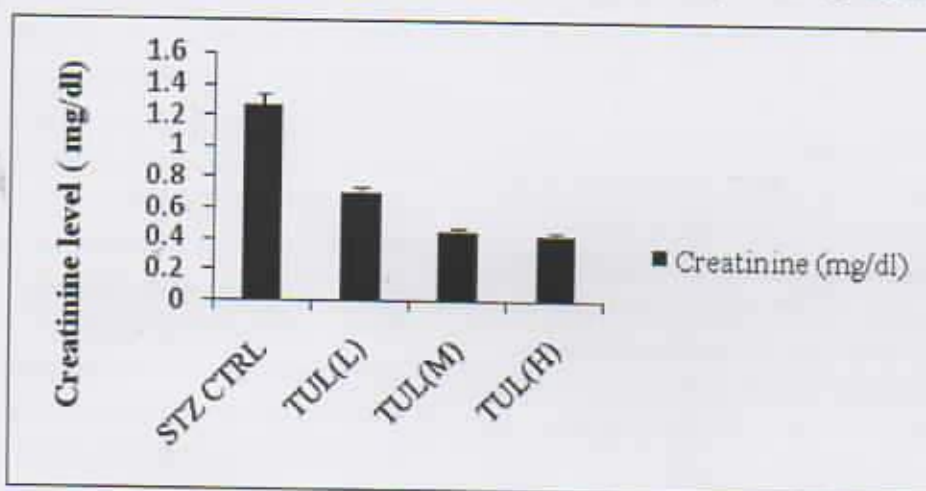


Fig 4a: Effect of aqueous extract of leaf of *Ocimum sanctum* (Tulsi) on creatinine in Control, and streptozotocin-induced treated diabetic rats.

TUL= Tulsi, STZ CTRL = streptozotocin control

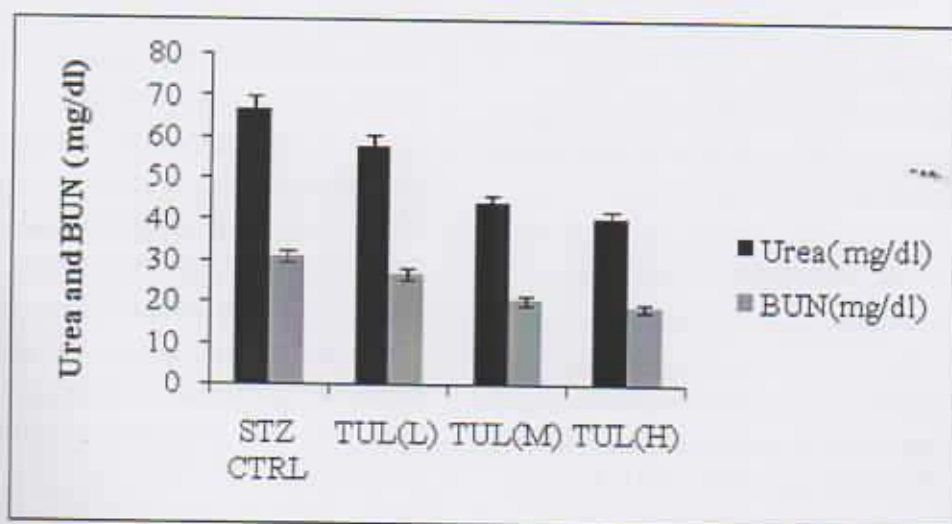


Fig 4b: Effect of aqueous extract of leaf of *Ocimum sanctum* (Tulsi) on urea and BUN in Control, and streptozotocin-induced treated diabetic rats.

TUL= Tulsi, STZ CTRL = streptozotocin control

Conclusion

In the present investigation the effect of *O. Sanctum* on diabetes was studied in adult Wister albino rats. Experimental result showed the anti-diabetic and anti-hyperlipidemic potential of *O. Sanctum* on streptozotocin induced diabetic rats. Treatment of the diabetic animals with *O. sanctum* for 21 days showed reversal of plasma glucose level. A significant change in lipid profile level was observed in the study ($p=0.000$) Result showed that the HDL-C level increases significantly after treatment with *O. sanctum* which is an important part of the study because diabetes is often associated with cardiovascular diseases and elevation in HDL-C level indicates lowering the chance of cardiovascular diseases. Reduction in TG, total cholesterol and LDL% also accounts for its anti-hyperlipidemic activity. Moreover Hb level was found to increase significantly with *O. sanctum* treatment in all three different doses ($p=0.000$) Parameters like blood urea, creatinine and BUN level that are closely associated with diabetic complication and often found high in diabetics were also found to decrease significantly ($p=0.000$) with herbal supplementation of *O. sanctum*.

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DEVELOPMENT OF NATURAL TEA-BASED RTD (READY-TO-DRINK) BEVERAGE FROM DARJEELING BLACK TEA FANNINGS

Madhurima Chatterjee¹, Shourya Basu² and Jayati Pal (Chattopadhyay)³

Abstract

Tea is one of the most popular consumable beverages in world after water. Owing to its increasing demand, tea is considered to be a potential platform in world beverage market. Besides, consumption in hot format, RTD tea-based beverage (cold format) is also gaining popularity in the beverage market exploring its inherent health benefit added with convenience. A particular grade of Darjeeling tea i.e. Darjeeling fannings was identified as raw material for development of tea-based RTD. Mint leaves were identified as another ingredient for its natural goodness and specific flavor profile, compatible with tea aroma. Preliminary screening was done from available varieties of fannings on the basis of percentage extractives and perceived aroma of the extracted liquor. Next, different formulations of RTD beverage were tried out using decreamed liquor prepared from selected Darjeeling fannings, mint extract and sugar syrup. Two formulations were

selected based on chemical and sensory analysis. Formulated beverages were pasteurized, filled in PET bottles and were analyzed. Percent acidity and brix of unprocessed beverage were found to be in the range of 0.4-1.9 g/100ml. and 20-22 degree. Total polyphenol (1980-2700 ppm) and total flavonoid (184-185 ppm) content were found to be much higher compared to common raw and/or processed food products. Flavonoid content did not show much change after heat-processing, whereas, polyphenol content was found to increase for both the formulations in processed beverage. Overall acceptability score of 6.85-7.5 (hedonic scale 0 – 9) by definite panelists also revealed acceptability of both the formulations.

Key words: Fannings; decreamed; RTD; polyphenol; flavonoids

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Introduction

Tea is one of the most popular beverages after water having a global presence with its penetration in wide range of market segment. Owing to its increasing demand, tea is considered to be a potential platform in world beverage market. There are many different types of tea; some teas, like Darjeeling and Chinese greens, have a slightly bitter, and astringent flavour, while others have vastly different profiles that include sweet, nutty, floral, or grassy notes. The most common varieties of tea include: White tea, Green tea,

Oolong tea, Black tea, Pu-erh Tea. The principal component of tea is flavonoids, which includes catechin, flavonols, the aflavins, the arubigins and proanthocyanidins. It also contains other components such as alkaloids, phenolic acids, vitamins, volatile acids. The major catechins present in fresh tea leaves and green tea are : Epicatechin (EC), Epigallocatechin (EGC), Epigallocatechin gallate (EGCG), Epicatechin gallate (EGC). Almost all characteristics of manufactured tea, such as colour, taste, aroma, are attributed either indirectly or

directly to modification of catechins. Tea extract has been demonstrated to exhibit anticarcinogenic activity and is found to be effective against inflammatory responses. It also has cardioprotective and neuroprotective benefits (Williamson and Johnson, 2003). The global market for hot beverages (coffee and tea) is forecasted to reach US\$69.77 billion in value and 10.57 million tons in volume terms by the year 2015 (GIA, 2011). According to a report by Tea Association of USA, consumers spent 52 billion dollar on cold beverages and iced tea represents 9% of total consumer dollar spent on cold beverage (Technomic, 2005). A cold-water infusible tea leaf product has already been described, which brews in cold water to produce iced-tea beverage (Balentine et al., 2002). Tsai et al. (1983) discloses treating black tea with tannase, together with other cell-wall digesting enzymes, to generate cold-water soluble instant tea powders. Literature study shows that Darjeeling tea whole leaf has been explored for preparing cold brew, but there is no such literature on cold brew infusion preparing from other grade of Darjeeling leaves. Some low-cost Darjeeling tea grades can be explored as raw material. This work, therefore, aims at development of natural tea-based beverage from Darjeeling tea-fannings, which will have targeted functional benefit in convenient RTD format. Enhancement of flyover in the final beverage is also aimed with compatible herbs without no external addition of flavorings agent.

2. Methodology:

2.1. Materials:

Darjeeling tea (fannings) and fresh spearmint leaves were sourced from local market. Other raw materials used are sugar, salt and distilled water. All chemicals, oxalic Acid ($\geq 99.5\%$), Sodium hydroxide ($\geq 97\%$), Gallic Acid ($\geq 99.5\%$), Methanol (Assay $\geq 99\%$), Folin Ciocalteu (Phosphomolybdate + Phosphotungstate), sodium Carbonate ($\geq 99\%$), Quercetin ($\geq 99\%$), Aluminium Chloride ($\geq 98\%$), sodium acetate ($\geq 99\%$), phenolphthalein were of GR grade.

2.2. Methods:

2.2.1.: Selection of extraction methods from tea leaves:

10gm of tea sample (Darjeeling tea fannings) was measured in each of the four sets of butter papers. 100ml distilled water was measured in each of the four 250ml conical flasks. Once each conical flask reached the particular temperatures, i.e., 50°C , 60°C , 70°C and 80°C , the tea leaves were added to the water in the conical flasks and the conical flasks were sealed with cotton plugs. The conical flasks were kept in the water bath for 10 minutes. The hot infusion of tea was filtered in four separate conical flasks and were labeled accordingly. Colour, appearance, taste, brix and aroma were noted down and total dissolved solids (by taking 10ml of extract) was calculated. All four infusions were evaluated on the basis of sensory and total solids and 60°C 10 min. was identified as suitable extraction method for preparation of base for the beverage.

2.2.2.: Preparation of De-creamed Liquor

Tea fannings were extracted in hot water (60°C for 10 min.) and brix was checked by hand refractometer. It was cooled to room temperature and was concentrated to 160 brix using rotary vacuum evaporator. The concentrated slurry was then chilled to 40°C for overnight to ensure effective separation of "tea cream". It was then centrifuged to obtain tea-cream as residue and de-creamed liquor (DCL) as supernatant. The de-creamed liquor was the base of the beverage.

2.2.3. Preparation of Mint Extract :

Freshly sourced spearmint (Pudina) leaves were macerated with the help of mortar and pestle. 10 g. of the macerated leaves were added in a conical flask containing 200ml distilled water. The conical flask was kept in a mechanical shaker for about 4 hours and the suspension was then filtered and centrifuged to obtain an aqueous extract of mint.

2.2.4. Preparation of Sugar Solution:

45g of sugar was slowly added to about 60g of hot distilled water (55-60°C) in a container and was heated over a water bath to obtain a sugar solution of 45° brix.

2.2.5. Preparation of different formulations:

Four different formulations were tried out on 100g-scale incorporating decaffeinated liquor, mint extract and sugar syrup in definite proportion. All these formulations were evaluated on the basis of total dissolved solids (0 brix) and the sensory attributes. Appearance, aroma, taste, flavour, mouthfeel and overall acceptability were scored on monadic basis against a hedonic scale (1-9). Final two formulations were selected with high overall acceptability score and comparable dissolved solids for ready-to-drink beverage.

2.2.6. Heat-processing of the beverage:

Formulated beverage was pasteurized maintaining a definite temperature-time condition (90°C and 10 min.). The beverage was then hot-filled into glass bottles and cooled to ambient temperature. Processed beverages were also analysed by sensory and chemical evaluation.

2.2.7. Analytical Methods:

2.2.7.1. Measurement of total dissolved solids:

Total dissolved solids of tea infusion was measured by gravimetric method. Definite amount of sample was weighed in a porcelain dish and it was evaporated to dryness. Dry mass was weighed and total dissolved solids was reported as

$$TDS = [(W_2 - W_1)/W] \times 100$$

where,

W = Weight of solution taken

W₁ = Weight of empty porcelain dish

W₂ = Weight of (porcelain dish + Residue of solution after drying)

2.2.7.2. Measurement of Acidity

Titration acidity was measured by titrating 10 ml. of

beverage sample against 0.1(N) NaOH using phenolphthalein indicator.

2.2.7.3. Estimation of Polyphenol content (in terms of Gallic Acid)

The total phenolic content in ethanolic extracts of mint leaves was estimated by Folin-Ciocalteu reagent, as described by Singleton and Rossi (1965), as described by Thakur et al., 2014. Mother stock of Gallic Acid (standard polyphenol) of concentration 500 µg/ml was prepared in methanol-water (1:1), in a total volume of 100ml. 0.5ml, 1ml, 2ml, 3ml, 5ml of gallic acid solution and a blank was taken in different volumetric flasks of 50ml volume. Raw and processed beverage were diluted in a definite proportion. To each of these volumetric flasks, 1ml of Folin ciocalteu reagent was added. After 5 minutes, 3ml of 20% sodium carbonate was added in all the volumetric flasks and mixed. The solutions were allowed to incubate for 30 minutes in the dark so that the coloured complex does not get disturbed. The absorbance of the samples was measured by spectrophotometer at a wavelength of 760nm.

2.2.7.4. Estimation of Total Flavonoid Content (in terms of quercetin):

Mother stock of quercetin (standard flavonoid) concentration 10mg/100ml was prepared in methanol, in a total volume of 250ml. 0.1ml, 0.3ml, 0.5ml, 0.7ml, 1ml of quercetin and a blank (1ml methanol added instead of quercetin) was taken in different test tubes. 0.5ml methanol, 50 µL of 10% AlCl₃, 50 µL 1(M) CH₃COONa and 1.4ml distilled water was added to the test tubes. Volume was made upto 3ml. The above samples of different concentrations were allowed to incubate at room temperature for 30min. The absorbance of the samples was measured by spectrophotometer at 415nm. Raw and processed beverage were diluted in a definite proportion. 1ml of the above dilutions were taken and colour was developed following the similar procedure as the standard.

3. Findings & Results :

3.1. Extraction:

Extraction of tea fannings at different time-temperature conditions resulted in infusions of different characteristics. Total dissolved solids in the infusion, extracted between 60-80°C for 10 minutes was found to be in the range of 35.02-47.2 % (w/v). At 500°C, there was much decrease in solid % in the infusion (15.78%). Results of sensory evaluation for tea infusion was shown in Table 1. Considering the sensory attributes, it was observed that extraction at higher temperatures (800 and 700°C) resulted in turbid suspension. It might be due to substantial formation of tea-cream, as tea-cream is a complex formed by the interaction between caffeine and polyphenols (Phytochemical functional foods). More amount of extracted polyphenols might result in much cream formation leading to turbid suspension. Perception of tea aroma was also found to be reduced, as temperature decreased from 800°C to 500°C. Among the four conditions, it was observed that extraction at 600°C/10 min. resulted in less turbid tea infusion with perceptible tea-aroma and substantial tea-solids.

Table 1: Sensory evaluation of tea infusion extracted at different temperatures:

Temperature	Appearance	Colour	Aroma
80°C	Turbid	Hazy brown	Strong tea aroma
700°C	Turbid	Hazy brown	Tea aroma
600°C	Less turbid	Dark brown	Tea aroma
500°C	Clear	Brown	Least perception of aroma

3.2.: Preparation of decreamed liquor:

Total dissolved solids of decreamed liquor was found to be 15.04%(w/v).

3.3: Preparation of mint extract:

Total dissolved solids of mint extract was found to be 2.22%(w/v).

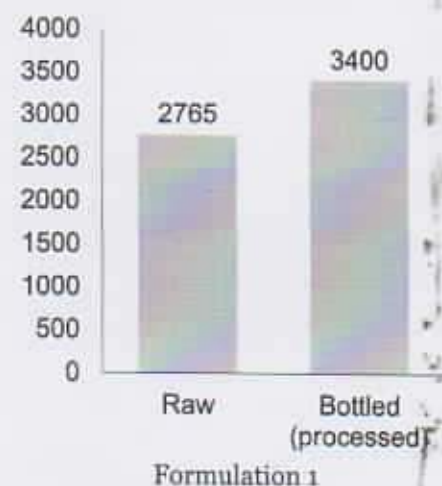
3.4: Preparation of different formulations:

Two definite proportions of tea-solids and mint-solids were identified to develop acceptable formulations. The proportions of decreamed liquor and mint extract were found to be 5:2(w/w) and 5:1(w/w). Soluble tea solids upto 3.00% (w/w) was found to be acceptable in final formulation. Tea solids level at 3.75% (w/w) was found to be strong and astringent. Mint extract delivering more than 0.16g mint solids was found to impart significant bitterness in final beverage.

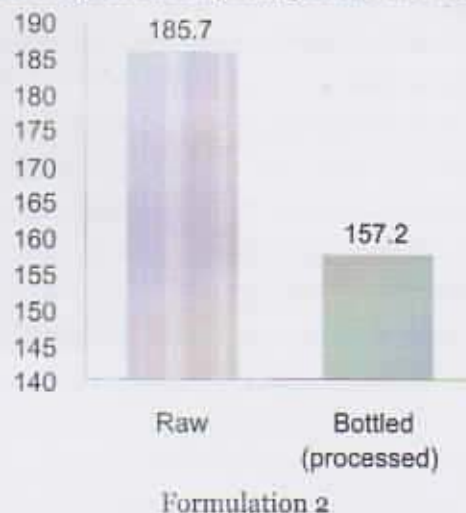
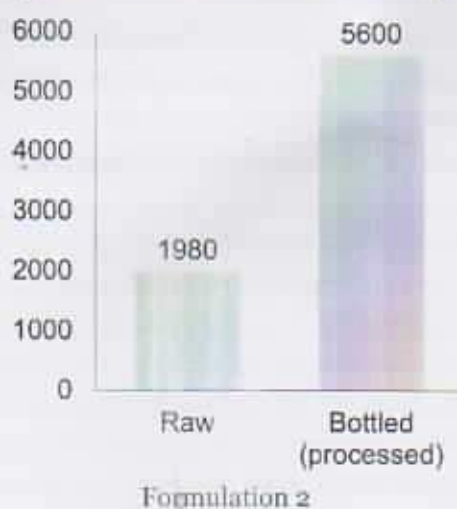
3.5. Analysis of raw and processed beverage:

Brix value for both raw and processed beverage were found to be 20-220. Titrable acidity of raw beverage was 0.31-0.39 (g/100 ml). There was not much change in titrable acidity in processed beverage as compared to raw one. In final processed beverage, it was 0.425. Total polyphenols in the raw beverage (in terms of GA) was found to be in the range of 1900-2700 ppm and total flavonoid content (in terms of Quercetin) was much less (184-185 ppm). Effect of heat treatment on total polyphenol content for both the selected formulations was given in figure 2.

Fig. 2: Effect of heat-treatment on polyphenol content:

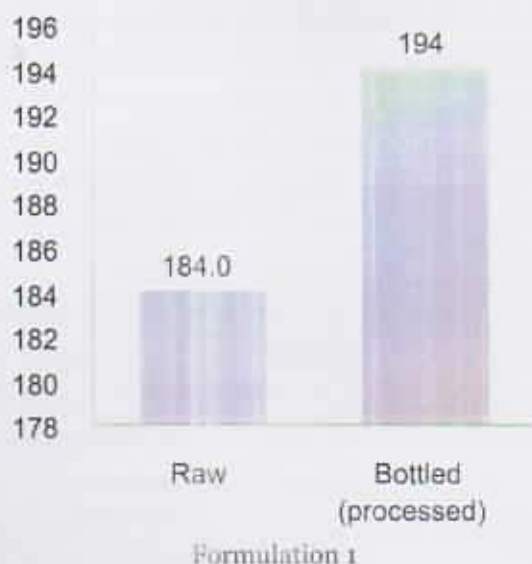


Development Of Natural Tea-Based RTD (Ready-To-Drink) Beverage From Darjeeling Black Tea Fannings



For both the formulations, the polyphenol content increased after heat-processing the raw beverage. This observation is unlike the findings observed for mint extract by Thakur et al.,2014 .The increase in polyphenol content (in terms of gallic acid) could be attributed to cleaving of galloyl groups of major esterified catechins during thermal process (Theppakorn, T,2016) .This phenomena also happens during thea flavin formation by fermentation of fresh and withered tea leaves.

Effect of heat treatment has also been studied on total flavonoid content of the beverage and was shown in fig.3.



Changes in flavonoid content was found to be correlated with the nature and composition of flavonoid profile (Andlauer et al.).Total flavonoid content in both the raw and processed beverages were found to be much higher than the catechin content (lower than 30 ppm) in most of the bottled or canned green tea beverages(Chen et al.,2001). Increase in flavonoid content after heat treatment might be correlated with endogenous biotransformation of precursor or intermediates into flavonoids, as observed in boiling of *Cosmos caudatus* (Kunth) leaves in water by Anam et al(2011).

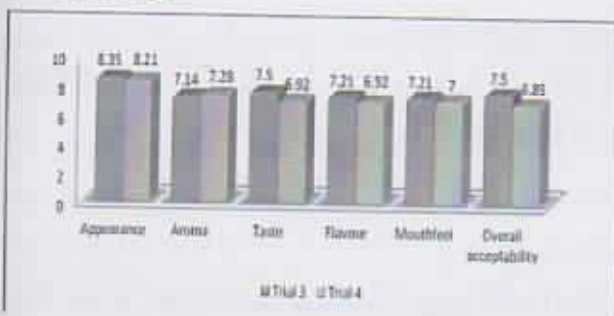
3.6. Sensory analysis of raw and processed beverage:

Fig. 4: Sensory scores of formulation 1 and 2 of raw beverage



Overall acceptability score was found to be 5.98-6.4 for raw beverage.

Fig.5: Sensory scores of formulation 1 and 2 of raw beverage



Overall acceptability score has been increased by 0.4-1.6 in processed beverage. It might be due to balanced and rounded note of tea and mint aroma developed after processing.

4. Conclusion:

Darjeeling tea fannings has been identified as a potential raw material for preparation of natural tea-based RTD. Mint extract can be successfully incorporated as a compatible herb to enhance flavour in the final beverage. A heat-processed tea-based beverage has been successfully developed with two unique combinations of tea-solids and mint-solids with average overall acceptability score of 7.0. Maximum limit of acceptable tea-solids and mint solids in final beverage have been identified. Heat-processing has increased total polyphenol and total flavonoid content, which needs to be further monitored through a detailed storage study.

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IS QUALITY OF LIFE OF MOTHER A CENTRAL CAUSE FOR CHILD UNDER-NUTRITION: A STUDY IN NORTH 24 PARGANAS

Dr. Debaprasad Sarkar

Abstract

Background: Prevalence of under-nutrition among under-five children is very high in many developing countries in the World. While there has been significant improvement in demographic trends, control of infectious diseases and growth of infrastructure, health indicators in India, though those are still far from optimal. The analysis of the situation of children in India would be incomplete without paying attention to the child health and nutrition. As a step towards reducing the level of under-nutrition, there is need to identify the important determinants of under-nutrition in the specific context.

Objective: The main aim is to establish the link between low Health Utility Scores of mother and under-nutritional status of under five children.

Data: A primary survey has been conducted on mother having under 5 children in six villages from three administrative blocks (Barrackpore-II, Hasanad-II & Swarupagar) on random basis in the district North 24 parganas, West Bengal. The total sample size of 900 individuals, the head of each households are chooses purely on random basis.

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Introduction

It has long been recognized that the well-being of a population is not solely captured by the levels and growth of consumption and income. Social indicators such as life expectancy, infant and child mortality and educational outcome serve as complementary in economic development. Long term health-human

Methodology: The first part of the paper deals with the evaluation and computation of child under-nutrition status and quality of life scores of mother. In course of evaluation of child under-nutrition status, Z-score height for age is calculated using primary anthropometric information on child (in Anthro 3.1 software). The quality of life scores of mother are derived through Health Utility Index HUI3 scoring system on the basis of 8 different attributes of subjective health. The second part of this paper deals with the impact of mother's Health Utility Index (HUI) on child under-nutrition status which is utmost important from policy perspective.

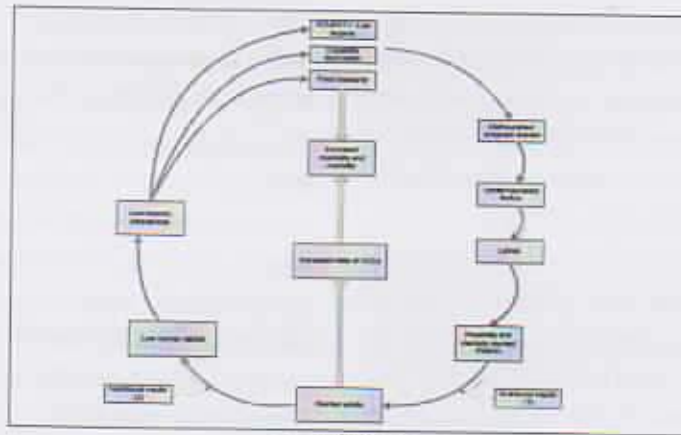
Findings: The findings of this paper suggest that the mother's HUI, mother's education and nutrition and child birth history are most important determinants for child under-nutrition atleast in North 24 parganas.

JEL Classification: D63, I18, J13, O15...

Keywords: Child under-nutrition, z-scores, Quality of Life, Health Utility Index (HUI), socio-economic sub-group.

capital is severely affected if the individual does suffer from malnourishment which may cause an intergenerational vicious cycle, a worse health capital stock may be passed from adults to their children (Strauss and Thomas 1998; Alderman, Hoddinott & Kinsey 2006; World Bank 2006; Pathak & Singh 2011) as shown in Fig.1.

Figure –I The vicious cycle of poverty and malnutrition.



Malnutrition is caused due to insufficient, excessive or imbalance consumption of dietary energy and nutrients (Babatunde et al., 2007). It manifests in different forms, such as under nutrition, over nutrition and micronutrients malnutrition (Smith and Haddad, 1999). Children that are malnourished tend to have increased risk of morbidity and mortality and often suffer delayed mental development, poor school performances and reduced intellectual achievement (Strauss 1990, World Bank 2006). While there has been significant improvement in demographic trends, control of infectious diseases and growth of infrastructure, health indicators in India are still far from optimal. India shares about 36 and 17 per cent of the world's poverty and population respectively but it contributes to one-fifth of the world's share of diseases (World Bank 2008). According to the National Family Health Survey (NFHS)-III-2005-06, child malnutrition in India is disproportionately high and the results are striking: 46 per cent of children under three are underweight, compared with 28 per cent in Sub-Saharan Africa and 8 per cent in China – another country with an enormous rural poor population; in addition to the 46 per cent who are underweight, 39 per cent are stunted, 20 per cent are severely malnourished and 80 per cent are anemic (Mendelson 2010). Numerous studies have already been conducted pertaining to the determinants of malnourishment

and its consequences in the developing countries especially in India. Radhakrishna, & Ravi (2004) have used NFHS-II data in the logit model and have observed that the risk of malnourishment decreases with standard of living of the households but descriptive statistics suggest that it persists even among the top quintiles. Sarkar (2014), Kanjilal et al.(2010) have used NFHS-III data and employed multi-level OLS for finding out the relationship between socio-economic status (SES) and nutritional status of children. Broadly speaking, these findings are more or less consistent with the findings of Sarkar (2014), Das and Sahoo (2011) who have used NFHS-III data and employed logistic regression models which revealed that education of the mother, poverty, social group membership, birth order, nutritional status of mother etc are important predictors of child under-nutrition. Apart from some household level factors like income or wealth, mother's health is a most important factor because it can cover family disease profile, nutritional inferences, genetic fact flow along with the upbringing of ritual, cultural and mental health of child. However, little effort had been devoted to examining the bi-directional relation between mother's quality of life and nutritional status of children where as mother's quality of life is the most crucial for child health and future life. Moreover, this analysis has a in-depth relevance for different socioeconomic subgroups of the population. As a step

towards reducing the level of under-nutrition, there is need to identify the important determinants of under-nutrition in the specific context, particularly in case when India is lagging behind far to combat with the lower quality of life of mother.

Objective and Research Questions of the study

Malnutrition is a chronic epidemic in India since long past which is partly due to inadequate food and nutrient supply along with low quality of life of mother, lack of primary knowledge of child care. Even after a long journey, a little focus has been given on the health and nutritional status, the most important cause of child health. Thus, the main aim is to establish the link between low Health Utility Scores of mother and under-nutritional status of under 5 children. In this respect the main research questions that are addressed in this paper are:

- 1) How can the under-nutritional status of under 5 children be assessed and analyzed?
- 2) How can the subjective quality of life or HUI of mother having under 5 children be assessed and analyzed?
- 3) How can the subjective quality of life or HUI of mother having under 5 children be related to under-nutrition status of under 5 children?

Analytical framework

Studies of determinants of children's nutritional status follow the household production framework of Becker (1965) and Strauss and Thomas (1995). Starting with a simple household utility maximizing model, we assume that a household has preferences that can be characterized by the utility function, U which depends on consumption of a vector of commodities, C , leisure, L , and the quality of children represented by the lower level of malnutrition status, CM :

$$U = u(C, L, CM) \dots\dots\dots 1$$

Where CM is measured using standardized anthropometric measures of height for age z-score (*haz*). The assumption in such a model is that good nutrition, as represented by the vector of low

malnutrition status of children is desirable in its own right, and it is likewise assumed that households make consumption decisions on the basis of reasons other than nutrition (Pitt and Rozenzweig, 1995).

Household maximizes its utility from the quantity and quality of the children and also the consumption of other commodities subject to a budget constraint which, in turn, determines the optimal values of consumption and also quantity and quality of children. Representative household maximizes a quasi-concave utility (assuming household's preferences are inter-temporally separable) as a function of average consumption c of commodities by household members and child malnutrition index CM subject to current period budget constraints, including wealth constraints (which depends on household income or wealth and also prices of consumption and child health goods) and a time specific nutrition production function (depends on birth characteristic, the duration of breast feeding, mother's health conditions, etc.). Along with determining the optimum value of average consumption (C), this constrained optimization exercise determines the i -th child's malnutrition level CM_i (in implicit form) as decreasing function of all correlates, as follows:

$$CM_i = cm_i(X_i, X_p, X_h, \epsilon) \dots\dots\dots (1)$$

where X_i the individual child characteristics (e.g., gender, age, birth character) of the child, X_p is the set of maternal health characteristics (e.g., maternal health or Health Utility Index (HUI), maternal education, duration of breastfeeding etc) and X_h the household and community level environment (e.g., wealth, rural, medical and health care practices and facilities available etc.) into which the child is born. Assuming, that all the right hand side variables are exogenous, Eqn. (1) can be considered as a reduced form equation which forms the basis of much discussion of the socioeconomic literature on child malnutrition (e.g., see Heller and Drake, 1979; Thomas and Strauss, 1992). Care must be taken

However to interpret the estimated coefficients as well as increase in all the explanatory variables, the level of malnutrition is reducing.

The specified nutritional production function allows us to estimate the following equation:

$H_{it} = f(\text{child characteristics, maternal health variables, household-community variables, } \epsilon_{it})$

Where i denote the i -th group (defined by year, region or gender), ϵ_{it} is random error terms assumed to be uncorrelated with the covariates included in the reduced form nutritional outcome models. Individual child variables include age, gender and birth history of the child. Maternal health variables include mother's HUI, education (school years completed), age at first baby birth, underweight mother, and duration of breastfeeding. Higher mother's HUI is the source key of the Health and nutritional status of child and are positively related. Underweight of the mother captures both the nutritional and some extent genetic effects. Maternal education is expected to improve nutrition through altering the household preference function and also through better child care practices. Mother's age at first birth is taken to capture the effects resulting from family background. Household level characteristics can be divided into parental characteristics related to household structure, like, income level or asset position, rural residence number of dependents and headship and the community level characteristics of the household. The structure of the household is relevant to test whether presence of older siblings may improve a child's nutritional status, and also whether composition of the household affects the nutritional status of a child (Sahn and Stifel, 2003). In the present study, community variables like access to health care as well as environmental factors such as water and sanitation (Strauss and Thomas 1995) are taken to analyze.

Data, Variable definitions and Methodology:

Data:

A primary survey has been conducted on mother having under 5 children in five villages from three

administrative blocks on random basis in the district North 24 parganas, West Bengal. Selection of north 24 parganas is purely purposive on the basis of the idea that north 24 parganas is the representative of state average as well as national average in almost all health indicators. A two stage random sampling method is used to select Six villages from three Administrative blocks (Barrackpore-II, Hasanad -II & Swarupagar) to assess the heterogeneity within the homogeneity. The total sample size of 900 individuals, the households are chooses purely on random basis. Around 34% of child are under-nourished (negative z-score).

Variable definitions:

Child Nutritional measures (Dependent Variable)

The health status H can be captured by different variables, which are typically either self-reported, subjective measures, or objective measures such as height, weight, or body mass index (Falkner and Tanner, 1986). Anthropometric indices are constructed by comparing relevant measures with those of comparable individuals (in regard to age and sex) in the reference populations (O'Donnell et.al.; 2008). We focus here on three commonly used measures of nutritional status, height for age, weight for age (Trapp and Menken, 2005) and height for weight. Which are termed as z-score (standard deviation score) and defined as the difference between the value for an individual (X) and the median value (μ) of the reference population for the same sex and age (or weight), divided by the standard deviation (σ) of the reference population $z = (x - \mu) / \sigma$. For example, consider an 18-month-old girl who heights 82.2 cm. On the basis of the reference standard height-for-age for girls, it can be established that the median weight for healthy girls of this age is 85.5 cm and that the standard deviation in the reference population is 1.0 (say). On this basis, the following calculation can be made:

$$Z\text{score} \left(\frac{H}{A} \right) = \frac{82.1 - 85.1}{1} = -3.4$$

This provides the basis of estimating prevalence of malnutrition in the populations or subpopulations. Among the three child malnutrition measures, weight for age and weight for height confound the effect of short- where as height for age has the long- term effect on health and nutritional problems; as most regresses in cross sectional studies are stock rather than flow variables it is generally not practical to study this variable with such data (Alderman, 2000).

Independent Variables:

In the present analysis Subjective health related quality of life mother is the key explanatory factor along with other explanatory factor are age in month of the child, birth order, birth interval gender, duration of breastfeeding, mother age at first baby birth, underweight mother, family income, family composition, safe drinking water and sanitation facility, and caste.

Subjective health related quality of life measure:

According to World Health Organization (WHO) 1948, Health is defined as "A state of complete physical, mental, and social well-being not merely the absence of disease". This definition specifies the positive aspect of health state keeping negative quality of health. If all aspect of health can be taken together to assess the health state of an individual then it can represent the Quality of Life (QOL). As per the definition by WHO (1994) "an individual's perception of his/her position in life in the context of the culture and value systems in which he/she lives, and in relation to his/her goals, expectations, standards and concerns. It is a broad-ranging concept, incorporating in a complex way the person's physical health, psychological state, level of independence, social relationships, and their relationship to salient features of their environment." If it is possible to assign any value life in any duration as modified by all those important aspect of QOL including subjective assessment of the impact of impairments, functional states, perceptions and social opportunities that are

influenced by disease, injury, treatment, or policy then it can be named as Health-Related Quality of Life (HRQL). Thus, HRQOL, Health Status, Functional Status are terms that are often used interchangeably but they measure different things. The HRQL, a multi-dimensional concept can broadly categorised as i) Mortality based measure ii) Subjective or morbidity measure (based on patient's perception), ii) Self-reported in most conditions and iii) multi-dimensional. The Health-Related Quality of Life (HRQL) is a method of measurement under Subjective or morbidity measure of health. Again, HRQL is of either specific or generic. The Specific HRQL instrument evaluates a series of health related dimensions specific to disease. On the other hand, the Generic instruments can be used with any population to cover perceptions on overall health and also questions on social, emotional and physical functioning, pain and self-care. The HUI3 provides a generic measure of health status (HQRL) and a preference-based scoring system. The HUI3 classification system has constructed to have content validity in regards to the specification of attributes and levels (Feeny et al.1995a). The Health Utilities Index Mark 3 (HUI3) is an indirect preference-based measure of overall HRQL that has been used in hundreds of clinical studies covering a wide variety of health problems. The HUI3 includes a comprehensive generic health status classification (i.e. profile) system and a utility scoring function (Feeny et.al., 2002; Furlong et.al., 2001). The classification system is comprised of 8 attributes: vision, hearing, speech, ambulation, dexterity, emotion, cognition, and pain. Each attribute has 5 or 6 levels of functioning, thereby defining 972,000 possible unique health states (Horsman et.al., 2003). The overall HUI3 scoring system provide utility (preference) scores on a generic scale ranging from -0.36 to 1.00, where worst possible health = -0.36, dead = 0.00, and perfect health = 1.00 (Horsman et.al., 2003). In the present study one of the most popular Generic measure of HRQL, HUI3 is used to assess overall health utility score of mother

having under 5 child on the basis of health quality related 8 attributes like vision, walking, distress etc. on 1 to 6 relative scale.

The Health Utility Score is derived as

$$u^* = 1.371(b_1 * b_2 * b_3 * b_4 * b_5 * b_6 * b_7 * b_8) - 0.371 \dots (2)$$

Where u^* the utility of a health state on the utility scale is where dead a utility of '0', perfect healthy has a utility of '1'. Again b_i are the individual specific item score for different attributes (Vision, hearing etc.),

$$u^* = 1 - u^* \& u^* = \frac{\bar{u}}{\bar{u} \sum}, \text{ where } \bar{u} = \left\{ \frac{1}{C} \right\} \left[\prod_{j=1}^8 (1 + C * C_j * \bar{u}_j) \right]$$

Again calculation of 1st and 2nd constant of Multi-Attribute Utility Function is derived from

$$1 + C = \prod_{j=1}^8 (1 + C * C_j) \text{ where } \prod_{j=1}^8 \text{ is the product of all } (1 + C * C_j) \text{ from } C_1 \text{ to } C_8$$

Other Related Maternal Health Variables

Along with the HUI of mother some other characteristics of the mother included in the analysis are a dummy variable for whether the mother was younger age at time of first birth of the child. Adolescent mothers typically have higher risks of poor pregnancy outcomes (for the medical literature on this issue see Conde-Agudelo et al., 2004; Gilbert et al., 2004; Fraser et al., 1995). While duration of breast feeding in months and underweight may reflect biological factors it also reflects socioeconomic considerations including the need for financial contribution to household. Underweight mother dummy may also reflect biological factors and socioeconomic considerations. According to the household decisions literature, educational status of mother could influence those resources that the mother may receive for herself as well as for her child, possibly leading to adverse nutrition consequences (Smith et al., 2003) and does have a significant impact while male does not (Behrman and Wolfe, 1984; Thomas, Strauss and Henriques, 1991; Murthi et al., 1995). Education in completed years of the mother is included in this analysis as an indicator of information access related to mother and child health care. Decision making power of mother in spending,

family wealth or income and drinking or smoking habits are also very important in predicting child nutritional status (Gilbert et al., 2004).

Methodology:

After specifying variables affecting child under-nutrition status and their measurement procedure a socioeconomic description is analyzed to know the viability of the objectives of the preset analysis. The pair-wise correlation coefficients are then derived to present the significant linkages between child under-nutrition and mother's Health Utility Scores and all other related socioeconomic factors. After finding a sufficient evidence of coexistence of bi-variate linkages a multi-co linearity test for explanatory variables are done to prepare the regression specification.

Empirical specification for Ordered Logit Model:

A more general under-nutrition classification (proposed by WHO, 2006) that distinguishes between mild ($0 > z\text{-score} > -2$), moderate ($-2 \geq z\text{-score} > -3$), severe ($z\text{-score} \leq -3$) is used to define ordered response category as $CM_i = 1$ if severe under-nutrition, $= 2$ if moderate under-nutrition and $= 3$ if under-nutrition. This provides the basis of estimating prevalence of under-nutrition in the populations or subpopulations with under-nutrition category. Given the ordered nature of the nutritional status index CM_i , classified from the child specific value of different z-scores, the ordered probabilistic model specified in (2) for estimation could be more appropriate with the ordered category of dependent variable (Amemiya (1981); Cameron & Trivedi (1986); Greene (1993); Gerdtham and Johannesson (1997), Wooldridge (2002, 2009)). We assume a linear dependence between the latent variable CM_i^* and X_i , β and the random disturbance term v_i and X does not contain a constant, m and k are mother's and others factors respectively.

$$CM_i = \sum_m \beta_{mi} x_{mi} + \sum_k \beta_{ki} x_{ki} + v_i, v_i = N(0, \sigma^2) \dots (2)$$

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A positive estimate indicates that an increase in the respective variable shifts weight from category 1 into category 3, which means that the probability of category 3 increases and alternatively the probability of category 1 decreases. On contrary the negative estimate implies that the probability of decrease in category 3 basis on the respective references. If the regression contains a constant term, the full set of coefficients is not identified. The absolute value of the estimated parameter has no such importance like OLS, but if it is negative in sign implies that the respective representative (= 1) category of independent variable has lower fixed impact compare

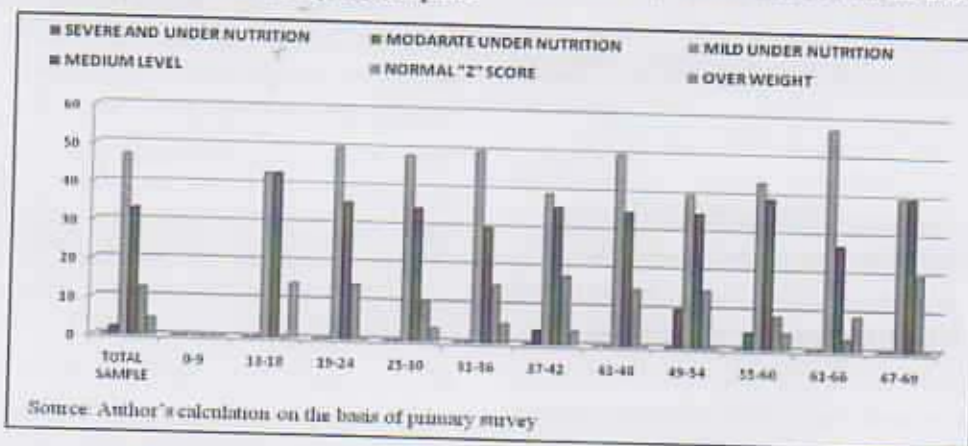
to other category (=0), taking other things remain unchanged. The estimated coefficients (β_i) shifts the Z-score by that amount, this may change the prediction of the category of dependent variable, or it may not.

Results:

Descriptive Results

The sample statistics and description of stunted children are shown below for overall sample and for some of the selected household, maternal and child variables.

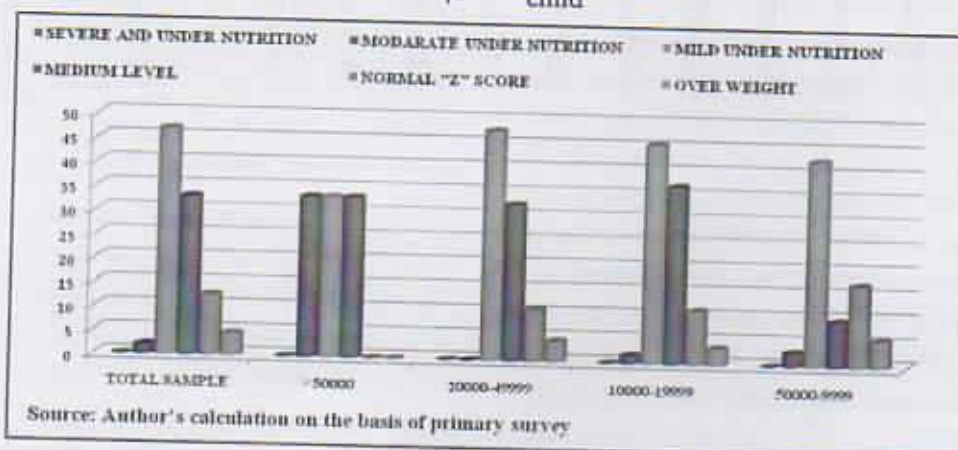
Figure:2 Age and under-nourishment of child



Almost half of children under five years of age (48 percent) are stunted in India. But, very few of the under five children (14%) are stunted for the present sample. The proportions of children who are mild under-nourished are around 50%, increases rapidly with the child's age through age 23 months to 48

months and decreases thereafter. It is notable that at age 18-23 months, when many children are being weaned from breast milk, are fallen into under-nourished state (Fig-2).

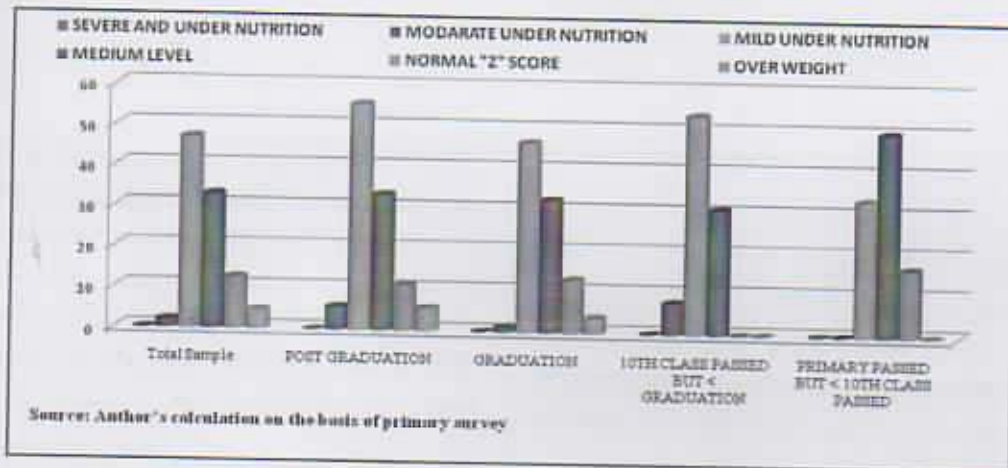
Figure:3 Family income and under-nourishment of child



With increase in the family income of the household under-nourishment level of child should reduce but for the present sample under-nourishment is predominant irrespective of family income. One astonishing fact is observed here that sample with highest family income (>50,000) contains huge

moderate under-nourishment (34%) child. This might be the result of modern rapidity of nuclear family and associated factorials.

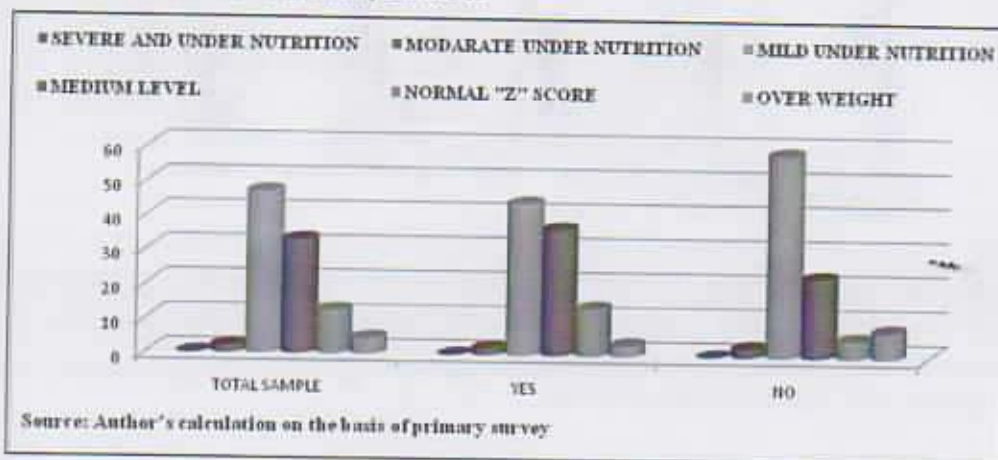
Figure:4 Mother's education and under nourishment of child



As per literature Under-nutrition of child has a strong negative relationship with the mother's education, but for the present analysis percentage of undernourished children has no such link, rather it

has somewhat in-deterministic relation with mother's education.

Figure:5 Food habit and under-nourishment of child

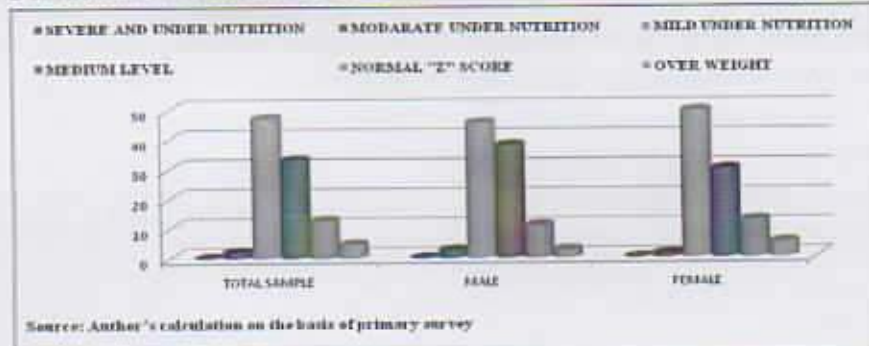


Even during the first six months of life, when most babies are breastfed, 20-30 percent of children are undernourished. It is notable that at age 18-23 months, when many children are being weaned from breast milk, 30 percent of children are severely stunted. After six months, children are generally starts to take food supplements. In finding the

judgments over the quarry of child having timely and balanced nutritious food as per requirement according to age female child are more vulnerable with respect to link with nutritional status, compare to male counterpart.

Figure:6 Food habit and under-nourishment of child

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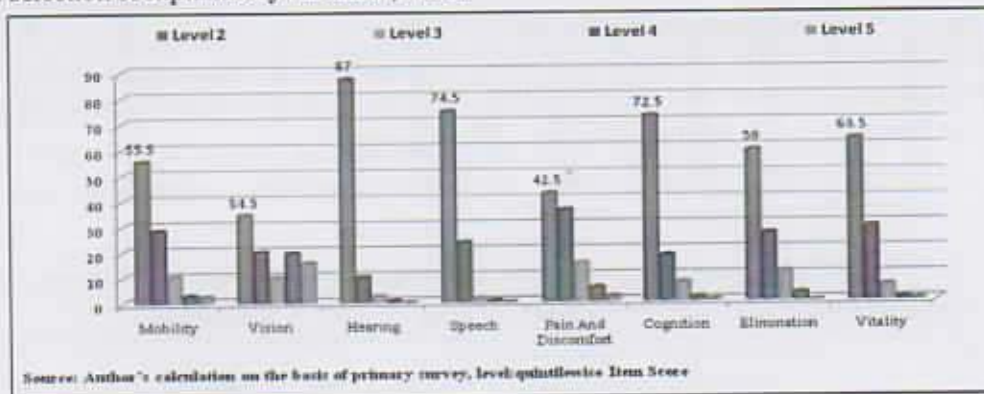


Overall, girls and boys are about equally under-nourished. Under-nutrition is generally lower for first births than for subsequent births and consistently increases with increasing birth order for all measures of nutritional status. Short birth intervals are associated with higher levels of under-nutrition. The analysis of descriptive results is thus a proper guideline for selection of explanatory variables, which

have more explanatory power.

The analysis of the socio-economic distribution (sample statistics and description) of Health Utility Scores of mother having under 6 children are shown below.

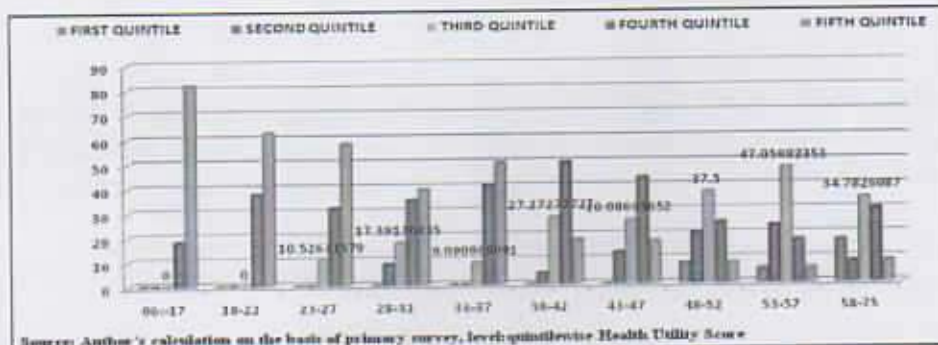
Figure:7 Item or Category wise distribution of mother's Item scores



The item-wise distribution of mother having under 6 children shows a more or less positively skewed pictures except the case of Vision & Pain and discomfort. The positively skewness is normal in a society where most of the people are healthy. Thus, the distribution of quintile-wise Item Score of mother

shows a messy variety for Vision & Pain and discomfort, implies the fact that mother having under 6 children are suffering in seeing & Pain and discomfort of different form.

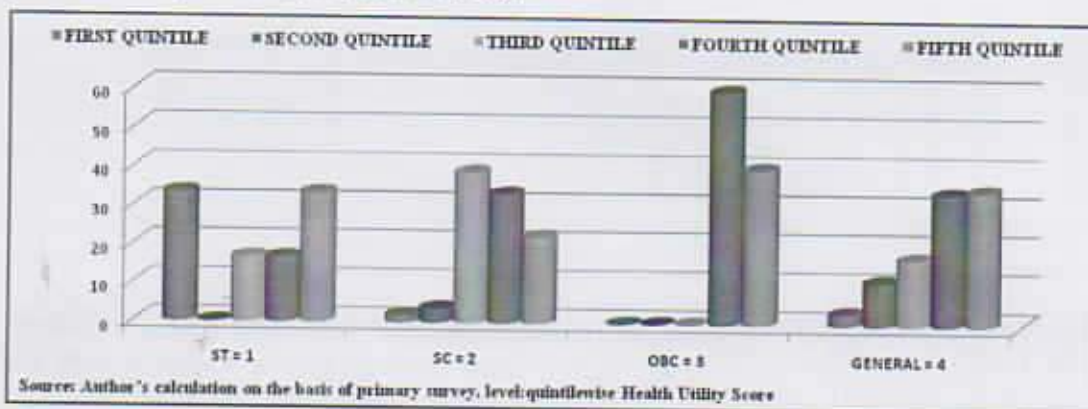
Figure:8 Age (in months) of child and mother's Health Utility Scores



The Health Utility Scores of mother is negatively skewed with respect to age (in month) if age of child is below 3 years, this is might be the case when all concentration of family is entirely on child, hence mother's health is neglected then. Above the age of 3 years by the same logic mother's health are then

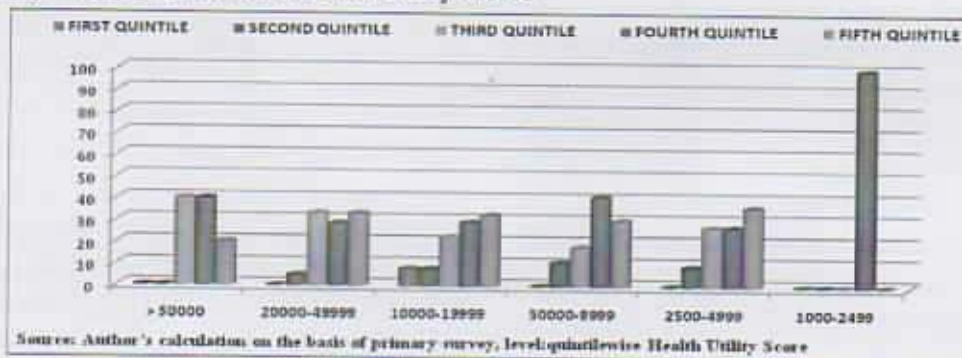
relatively distributed evenly or normally with a less number of mothers engrossed with good Health Utility Score.

Figure:9 Caste group and mother's Health Utility Scores



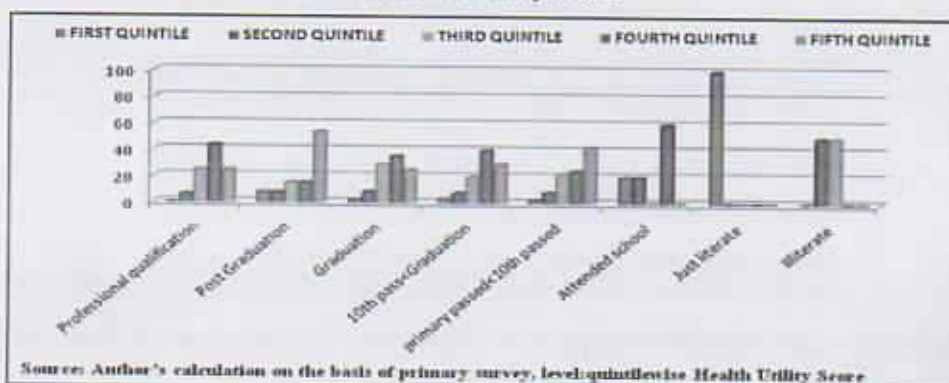
With respect to the societal caste group, mother's HU scores for the scheduled tribe subsection of population are more vulnerable compare to other societal caste group of population.

Figure:10 Family income and mother's Health Utility Scores



In higher family income group mothers health are mostly above the average compare to lower income group but only surprising fact is that mothers in the daily wage group are near to perfectly healthy.

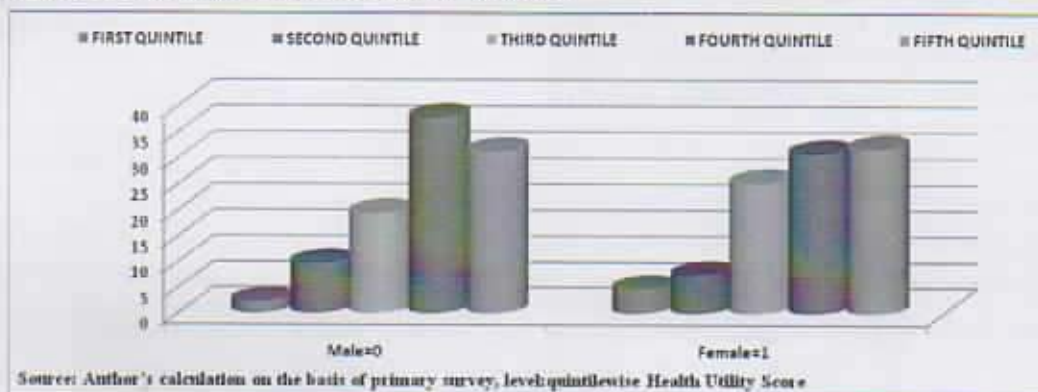
Figure:11 Educational attainment and mother's Health Utility Scores



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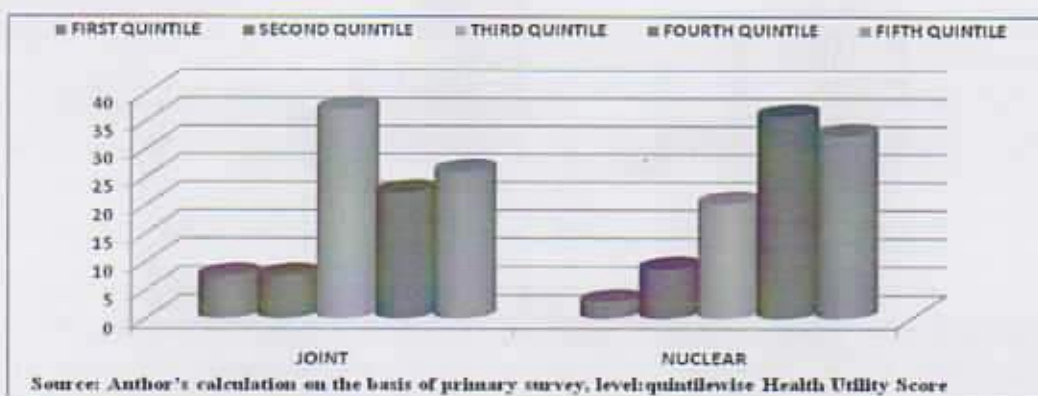
Mothers who completed atleast primary education, have quite justifiable negative skewed distribution, whereas mothers with just literate or illiterate have lower quality of lower HUI.

Figure:12 Gender of child and mother's Health Utility Scores



The distribution of mother's Health Utility Scores follows more or less same type of negatively skewed distribution. Thus, mother's Health Utility Scores is independent of the gender of child or child preferences. Similarly, family structure has no such partial impact on mother's level of Health Utility Scores (Fig.13).

Figure:13 Family structure and mother's Health Utility Scores



Link between Child Under-nutrition and Mother's Health Status:

In explaining the link between child under-nutrition and mother's Health Utility Scores, the correlation coefficient between them are noted in the following table 1 & 2.

Table:1 Correlation coefficient between Mother's HUI Score & Child under-nutrition

Correlation coefficient		
	Under-nutrition	Non under-nutrition
Low HUI Score	0.43*	0.13
High HUI Score	0.26	0.51

Source: Calculated on the information collected from primary survey, ** significant at 5% level.

Table 2 shows the strong significant association between under-nourished child - Low HUI and well nourished child - High HUI. So there is a strong positive association between nutritional status of child and mother's HUI. If relationship is derived in more disaggregate level with respect to quintile wise distribution of mother's HUI, the same tendency of significant association has observed (Table 2). The same tendency of association is observed in almost all socioeconomic subsections of society.

Table 2 Correlation coefficient between Mother's HUI Level & Child under-nutrition Level

Correlation coefficient					
	Level 1	Level 2	Level 3	Level 4	Level 5
under-nutrition	0.60	0.23	0.412	0.35	.27
Non under-nutrition	0.101	0.33	0.31	.48	0.35

Source: Calculated on the information collected from primary survey, '**' significant at 5% level.

To present the specific form of relation from mother's health levels and other related socioeconomic factors towards child under-nutrition status, a multivariate regression is used as per the equation 2.

Determinants of under-nutrition with Ordered Response Dependent Variable

As mentioned earlier, the dependent variable is ordered categorical which equals 1 if the child severely under-nourished, 2 if moderately under-nourished and 3 if mild under-nourished. Since the ordered probabilistic model relies on the maximum likelihood estimation procedure, the resulting parameter estimates not likely represent the probability that a child will be under-nourished. A positive sign on a parameter implies that the variable will lead to

increased under-nutrition, while a negative sign indicate that the variable will reduce under-nutrition. The absolute value of the estimated parameter has no such importance like OLS, but if it is negative in sign implies that the respective representative (= 1) category of independent variable has lower fixed impact compare to other category (=0), taking other things remain unchanged. The estimated coefficient (β_i) shifts the Z-score by that amount, this may change the prediction of the category of dependent variable, or it may not. Instead of reporting coefficients, the odd ratios and the corresponding marginal effects of each explanatory variable of under-nourishment in each of mild, moderate and severe as shown in Table-3. If the odd-ratio (OR) < 1, one can argue that the probability of being under-nourishment increases, the opposite will happen if OR > 1.

Table-3 Ordered Logit coefficients, Odd Ratio and Marginal Effects of under-nutrition (z-score(h/a))

Variable	Iteration completed - 5			Marginal Effect (dy/dx) of Categorical Underweight (3= if -2 < Z-score < 0, 2= if -3 < Z-score < -2, 1= if -3 and below)		
	Coefficient	Odd Ratio	z-values	Severe	Moderate	mild
Predicted Probability	-	-	-	0.18	0.27	0.55
Age in month	-0.016***	0.98	-22.35	0.0024***	0.0017***	-0.004***
2 nd Birth Order	0.36***	0.71	14.60	0.051***	0.032***	-0.084***
Higher Birth Order	0.26***	0.63	4.50	0.068***	0.045***	-0.113***
Birth Interval 0-24 months	-0.34***	0.64	-11.93	0.063***	0.045***	-0.109***

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Female child	-0.08***	0.92	-3.97	0.012***	0.009***	-0.021***
Breastfeeding (months)	-0.002***	0.998	-2.81	-0.0003***	0.0002***	-0.0004***
Mother age 1 st birth	0.023***	1.02	7.62	-0.003***	-0.002***	0.006***
Mother's education(v)	0.049***	1.05	15.83	-0.007***	-0.005***	0.012***
Family income	0.32***	0.999	3.73	0.021***	0.019***	-0.020**
Mother's HUI	0.45***	1.57	17.57	-0.065***	-0.046***	0.112***
Family composition	0.17***	1.19	7.93	-0.025***	-0.018***	0.043***
No safe Water facility	-0.054**	1.06	2.12	-0.008*	-0.006*	0.014**
No toilet facility	-0.097***	0.91	-3.54	0.014***	0.010***	-0.024***
SC	-0.47***	0.63	-14.92	0.07***	0.042***	-0.115***
ST	-0.55***	0.58	-16.67	0.089***	0.047***	-0.136***
OBC	-0.38***	0.68	-14.36	0.058***	0.037***	-0.095***
/cut1	3.15					
/cut2	4.50					

Source: Computed by author using unit level records from survey.

Note: *** $p < .01$, ** $p < .05$, * $p < .10$. Pseudo $R^2 = 1 - LU/LR$, where LU is the unrestricted log likelihood values and LR is the restricted log likelihood values (Magee 1990). Iterations completed 4 for all four cases. N is the sample size.

The odd ratios are the exp (logit coefficients), represent the odds of $y=1$, when x_i increases by 1 units if the OR $>1 (<1)$ implies the odds of severity of under-nourishment ($Y=1$) increases (decreases). In the present study, odds of severity of under-nourishment ($Y=1$) increased in case of mother's HUI scores, mother age at first birth, mother's education level, income of households, rural residence, family composition, and no safe water facility. Age of the child is positively related to the probability of under-nutrition for severe and moderate category but not in case of mild category, as shown in Table- 3. Probability of stunting (moderate and severe) is found to be increasing with birth order, birth intervals, birth size and female child. Birth history of child has likely to increase probability of being severely stunted by 5.1% for 2nd birth order, 6.8% for higher birth order, and 6.3% lower birth interval. Mother's education has a significant negative impact on malnourishment of a child. It is observed that educated mothers are better aware about the nutritional requirements of their children and they usually provide improved healthcare as a result of

their general awareness (Webb and Block, 2004). An increase in wealth-scores of households reduces the chances of under-nourishment. Unit increase in mother's HUI score would decrease the chance of reporting severely stunted and moderately stunted by 6.5% and 4.6% points respectively as against only 1.1% increase in the probability of being mild under-nourished (h/a). Our results also reveal that probability of under-nutrition will decrease for non-under weight mothers. This finding is consistent with the previous studies (Smith and Haddad, 1999; Pal 1999). Mother age at first birth is negatively related to the probability of stunting. Duration of breastfeeding (in months) accentuates the probability of being stunted. Probability of being stunted reduces as family size (>6 persons in a household) increases which is contrary to the earlier findings done by Lanjouw and Ravallion, 1995; Pal, 1999. Access to health facility and Safe water facility at the household level reduce the probability of being stunted. Toilet facility at the household level is found to have a strong and significant effect on child under-nourishment. Probability of being under-nourished is influenced by

social class (SC/ST/OBC), this means that chances of under-nourishment increases significantly if the child either belongs to SC or ST or OBC community.

Major Findings and Conclusions:

The present study analyses the determinants of under-nutrition among under-five children in Ordered Probabilistic setup. Descriptive analysis is the main guideline behind the selection of explanatory variables. The major findings of this paper are as follows. The regression analysis in Ordered Probabilistic setup, revealed that child's birth history, mother's education level and nutrition level and mother's HUI score, family composition and toilet facility are the significant determinants of child under-nutrition. This analysis suggests increasing attention being paid to female children, as well as reduced care and attention for older and weaned children. Mother's education and mother's nutritional level are significant in all our regression models pointing to the fact that child nutrition will improve with increased mother's education and nutrition. Family composition, access to health facility and availability of toilet also reduce the probability of under-nutrition. Comparing with previous research in other countries also household size has the significant effects and contrasting on under-nutrition in our sample.

Overall, the mother's HUI, mother's education and nutrition and child birth history are most important determinants for child under-nutrition atleast in North 24 parganas. What is needed therefore is to target pregnant women with specific education and health care programs. Second, the provision of clean water, toilet facility and primary health service should be taken seriously by government. In order to overcome from this health-poverty nexus, government has already been adopted various programmes like Integrated Child Development Services (ICDS), provision of Mid-Day Meal, Health for All, Janani Surakshya Yoyona (Mother Reproductive Security) under National Rural Health

Mission, National Rural Employment Guarantee Act (NREGA) etc. The performance of all these programmes is not satisfactory, so a proper monitoring is needed to the existing programmes.

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ALTERNATING SOURCE OF FOOD: - JACKFRUIT SEED

Soutami Bhattacharyya¹ and Ina Mukherjee²

Abstract

In the present work the nutritional and functional properties proximate composition flour is estimated and utilization of jack seeds have been done. The seeds were lye peeled and milled into flour. The functional property of the flour were analysed by standard AOAC method. The functional property indicates that jack seed flour has 70ml/100gm of water absorbtion capacity, and 129ml/100gm oil absorbtion capacity and 5.46gm/100gm of swelling power. The roasted seed flour was analysed for estimation of protein carbohydrate and moisture content. The roasted jackseed flour contain 37gm/100gm of carbohydrate, 4gm/100gm of protein and 11% moisture. The seed flour is used to prepare biscuits. Sensory evaluation of the biscuits reveals that biscuits made by margarine is more acceptable than the biscuits made by butter with 10% of jackseed flour incorporated with normal wheat flour. The colour, appearance, texture, aroma and overall acceptability were decreased with time.

Key Words: jackfruit seeds, nutritional and functional properties and biscuits.

Introduction

Jackfruit is the longest edible fruit in the world. It has almost 100-500 seeds in one fruit. Most of the people in our country yet unknown about the benefits of Jackfruit seeds. There are 2 main varieties of jackfruits: one is small, fibrous, soft, and mushy, and the carpel are sweet, with a texture like that of a raw oyster the other variety is crisp and crunchy, but not very sweet. The large seeds from this non leguminous plant are also edible, even though they are difficult to digest The jackfruit significantly contributes to the nutrition of the people of our country as a source of vitamins, minerals and calories (Molla et al.,2008). Jackfruit seeds make up around 10 to 15% of the total fruit weight (30-365/fruit) and have high carbohydrate and protein contents

It has different benefits. The seeds give around 135 Kcal/100 Gm., they are the rich source of complex Carbohydrate (32 Gm. /100 Gm.), dietary fiber, vitamins like A, C and certain B vitamins, and minerals like Calcium, Zinc and Phosphorus. As jackfruit is highly seasonal and the seeds have shorter shelf life, hence go waste during seasonal glut. So the seed flower can be an alternative product which can be stored and utilized both for value addition and to blend with other grained flours without affecting the functional and sensory profile of the product. Moreover the incorporation of seed flour to deep fat fried product has found to reduce the fat absorption to a remarkable extent.

So we should not throw away the seeds and use the seed in different ways such as roasted seed flour can be used to prepare biscuits, can be included in curries etc. It can also be roasted or boiled or used in many

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preparation as it contains similar composition as that of grains.

Objective

To prepare biscuits by using jackfruit seed flour and its sensory evaluation.

Methods and Materials:

1. Seed treatment and seed flour preparation:

The jackfruit seeds are collected from local market during season. The seeds are then cleaned with fresh water. After that are sun dried for 6-7 days. The white coat of the seeds are then peeled off easily. Then the seeds are sliced into thin chips and roasted. At last the roasted chips are powdered in a grinding machine and stored in polyethylene pouch.

2. Testing of Seed Flour

Preparation of Experimental sample:

The roasted seed powder was mixed with 10 vol. of 0.035 M potassium phosphate buffer and then homogenized in a homogenizer. The resulting solution was 4 times filtered with filter paper and the filtrate was centrifuged at 10000 rpm. Then the supernatant was mixed with 5% trichloro acetic acid and again centrifuged. The precipitate was collected and treated as the sample to measure the protein content of the roasted seed flour.

Estimation of Protein: The amount of protein was measured by using lowry method. A standard curve is prepared by using BSA solution. Then by using the standard curve the amount of protein present in the sample was measured.

Estimation of Carbohydrate:

At first standard glucose solution is prepared and titrated against benedict solution. Then the sample is hydrolyzed with HCl. After hydrolysis it is the deproteinised by using zinc sulphate. Then the filtrate is used to titrate the benedict solution. The burette reading was taken and calculation was done.

Estimation of moisture content:

Moisture content of the seed flour is measured with the help of moisture analyser by using the following formulae,

$$\frac{\text{Initial weight (Gm.)} - \text{Final weight (Gm.)}}{\text{Sample weight (Gm.)}}$$

Estimation of fat absorption capacity:

The fat absorption capacity is estimated by using the following formula with the help of centrifugation process.

$$\% \text{Fat absorption capacity} = \frac{\text{Weight of sample after centrifugation} - \text{Weight of sample before Centrifugation}}{\text{Weight of original sample taken} \times 100}$$

Estimation of swelling power:

Swelling power of the roasted seed flour was determined according to the method of Schoch (1994) by using the formulae,.....

$$\text{Swelling power (g/g)} = \frac{(W3 - W2) \times 1}{W1}$$

Where,

W1 = Weight of seed flour sample

W2 = Weight of the centrifuge tube with seed flour sample

W3 = Weight of the centrifuge tube with swollen material

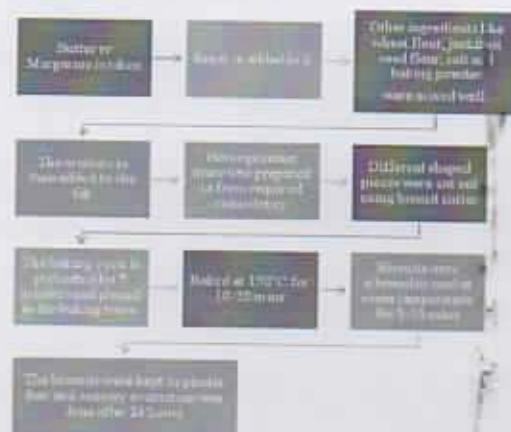
Estimation of te water absorption capacity:

Twenty grams of seed flour was taken.

Required quantity of water added to get dough of moderately stiff consistency.

The amount of water required was noted and expressed in percent.

Preparation of Biscuit:



Alternating Source Of Food: - Jackfruit Seed

Sensory Evaluation of The Biscuit: The product obtained after baking was subjected for sensory evaluation. The formulated biscuits were compared with the control sample that is without jackfruit seed

flour. The score record sheet was prepared based on the nine point hedonic scale.

Result:

Table:1 BIOCHEMICAL PROPERTY

PROPERTY	AMOUNT
CARBOHYDRATE	37gm/100gm
PROTEIN	4gm/100gm
MOISTURE	11%

Values are mean of triplicate.

Result of the experiment to assess the amount of carbohydrate(37gm carbohydrate), protein (4gm protein) and moisture content(11%) of the roasted

Table: 2 FUNCTIONAL PROPERTY

PROPERTY	VALUE
Water absorption capacity(ml/100gm)	70
Fat absorption capacity(ml/100gm)	129
Swelling Power (gm/gm)	5.46

jack seed flour is depicted in the table 1. From the table 2 it can be easily seen that water absorption capacity is 70ml/100gm, fat absorption capacity is 129ml/100gm and swelling poer is 5.46 gm/100gm.

COMPARISON OF TASTE OF BISCUITS (DIFFERENT LEVEL OF JACKSEED FLOUR INCORPORATED) MADE BY BUTTER AND MARGARINE

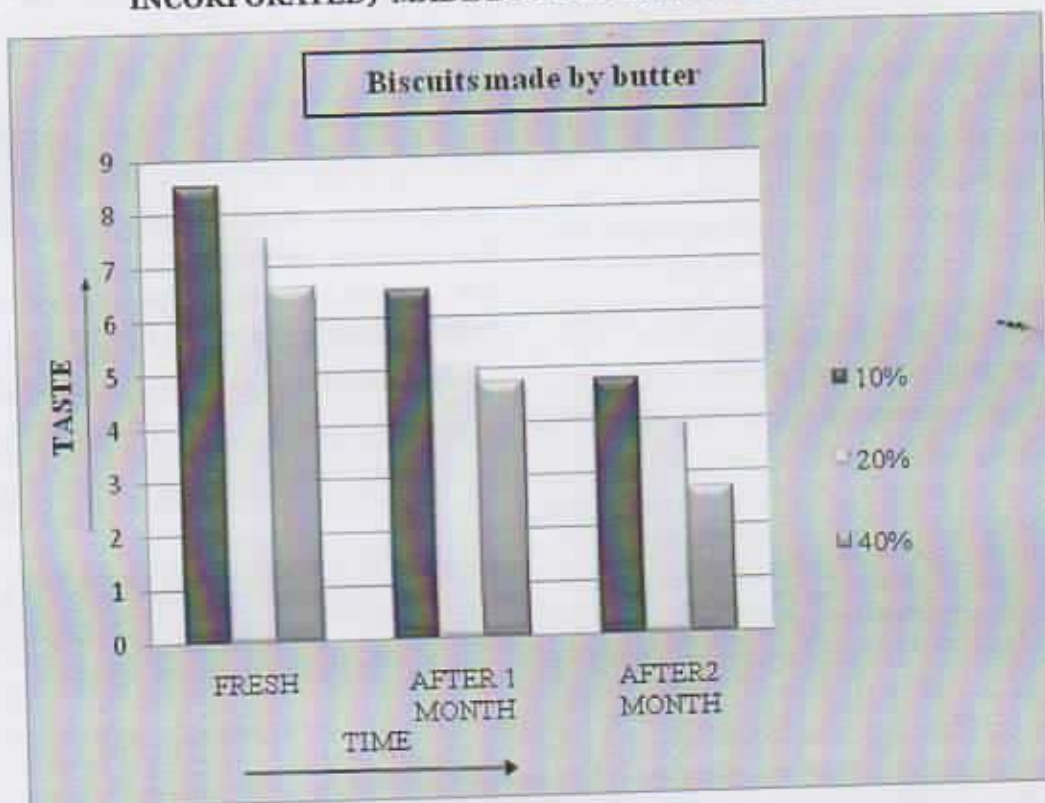


Fig No: 1

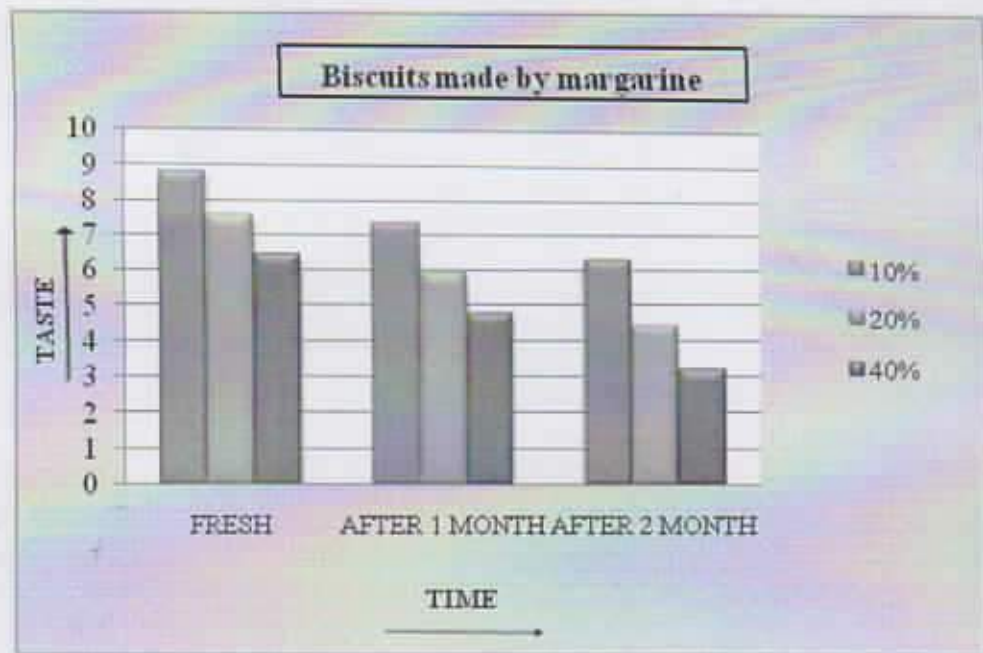


Fig No: 2

The results obtained from the sensory evaluation of the biscuits (shown in figure number 1 and 2) reveals that the taste of the biscuits made by margarine is better than the biscuits made by butter. The taste is also decrease with the time and the increased level of jack seed flour incorporation.

SENSORY EVALUATION OF BISCUITS (MADE BY BUTTER) FOR COLOUR & APPEARENCE, TEXTURE, AROMA AND OVERALL ACCEPTIBILITY

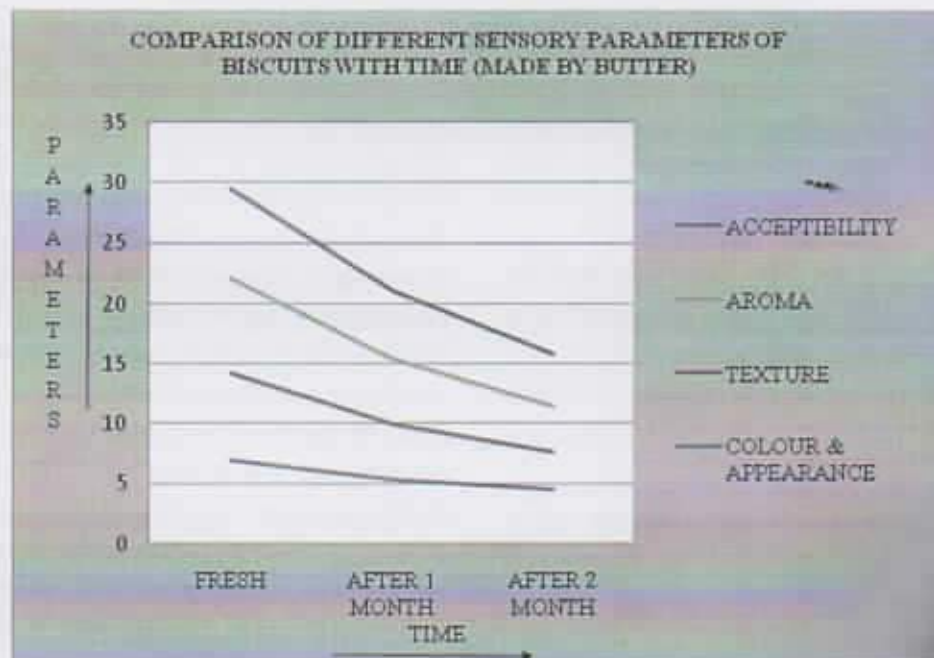


Fig No: 3

Alternating Source Of Food: - Jackfruit Seed

Fig No 3 shows that the aroma, texture, colour and appearance and overall acceptability of the biscuits are decreased with time and with the increase in the amount of jackseed flour incorporation.

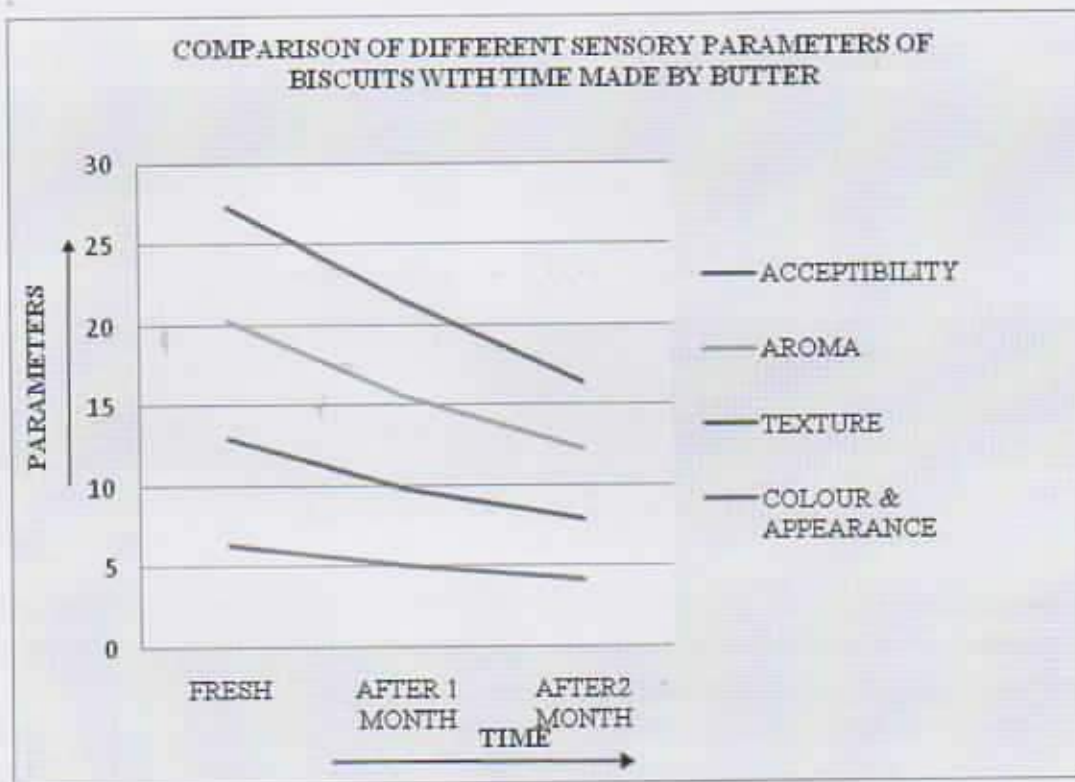


Fig No: 4

The sensory evaluation of the biscuits made by butter reveals that (shown in figure no. 4) aroma, texture, colour and appearance and overall acceptability of the biscuits are decreased with time and with the increase in the amount of jackseed flour incorporation. By comparing fig 3 and fig 4 it can be said that the acceptability of the biscuits made by margarine is better than the biscuits made by butter.

Conclusion:

In the present study the total carbohydrate, protein and moisture content of roasted jackfruit seed flour was estimated. The flour contains about 38gm carbohydrate, 4gm of protein and 11% moisture. It also has 70% water absorption capacity, 129% fat absorption capacity and 5.46gm/100gm of swelling power. So it can be easily utilised to prepare biscuit. In

the present study biscuits were also prepared by using the jackfruit seed flour with refined wheat flour in blended condition and sensory evaluation of the biscuits were also carried out. According to this study the different parameters such as taste, colour and appearance, aroma, texture and total acceptability were more or less same in case of both type of biscuits that is one is made by butter and another was made by margarine. All these parameters have been decreased with the passing of time. Fresh product shows higher scoring than 1 & 2 month old product. So further experiment is required on this study to retain all the properties of the biscuit same in case of 1 & 2 month old product. Preservatives may be used to preserve the seed flour and biscuits and it requires further experiment.

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COMPARATIVE STUDIES ON THE NUTRITIONAL QUALITY OF DIFFERENT VARIETIES OF BANANA BLOSSOMS CULTIVATED IN WEST BENGAL, INDIA

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Abstract

The flower of the banana plant also known as "banana blossom" or "banana heart" has huge nutritional value and healthy benefits. The main objective of this study was to evaluate and compare the nutritional quality of five different varieties of Banana Blossoms namely Martaman, Mohavog, Seed Banana, Giant Governor, and Bagda Kathali cultivated in West Bengal. The samples were collected from local market and analysed. The results shown that significant differences exist ($p < 0.05$) in nutrients, anti-nutrients, antioxidants and minerals content among different varieties of Banana Blossoms. From the study it has been concluded that the nutritional quality of different

varieties of Banana Blossom varies significantly. Among all varieties of banana blossoms, Martaman and Mohavog were found to be more nutritious because of their increased ash, minerals and antioxidant content as compared to others. Crude fibre content was found to be highest in Bagda kanthali variety. Moreover, they are the poor sources of protein but rich sources of fibre, total phenols and minerals except the blossoms of Giant governor variety which is less nutritious as compared to others.

Key Words

Banana Blossoms , Nutrients, Anti-Nutrients, Antioxidant And Minerals.

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Introduction

Banana, a typical climacteric fruit mainly grows in tropical and subtropical regions, produced by several kinds of large herbaceous flowering plants in the genus *Musa* and belongs to the family *Musaceae*. The scientific names of most cultivated bananas are *Musa acuminata*, *Musa balbisiana*, and *Musa* × *paradisiaca* for the hybrid *Musa acuminata* × *M. balbisiana*,

depending on their genomic constitution. The old scientific name *Musa sapientum* is no longer used. In a series of papers published from 1947 onwards, Ernest Cheesman showed that Linnaeus' *Musa sapientum* and *Musa paradisiaca* were actually cultivars and descendants of two wild seed-producing species, *Musa acuminata* and *Musa balbisiana*, both first described by Luigi Aloysius Colla (Constantine

the original on 2008-09-05. Retrieved 2014-09-05). The currently accepted scientific names for most groups of cultivated bananas are *Musa acuminata* Colla and *Musa balbisiana* Colla for the ancestral species, and *Musa × paradisiaca* L. for the hybrid *M. acuminata* × *M. balbisiana* (Royal Botanic Gardens, Kew. Retrieved 2013-01-06).

Almost all the parts of banana tree have wonderful uses and the banana flower is no exception. The flower of the banana plant is also known as banana blossom or banana heart (Yunchalad et al., 1995). Blossom of the banana plant (*Musa acuminata* colla) is often consumed as a vegetable in many Asian countries such as India, Sri Lanka, Malaysia, Indonesia and the Philippines (Walker, 1931).

The banana blossom is a large, dark purple-red blossom that grows from the end of a bunch of bananas. Its sizable bracts, or leaves, snugly enclose delicate, sweetly scented male blossoms (Nelson et al., 2006). The female blossoms, which do not require fertilization to become fruit, grow farther up the stem from the male blossoms. The banana blossom grows at the end of a bunch of bananas. It is a leafy maroon coloured cone with cream coloured florets layered inside (Yang et al., 2003).

Banana flower has a huge nutritional value and healthy benefit. They represent a valuable source of potassium, vitamin A, vitamin C, vitamin E, fatty acid content, flavonoids, saponin, essential and non-essential amino acid, tannins, glycoside and steroid (Qiang jin et al., 2010). They are the good source of minerals such as magnesium, iron and copper. It also contains high quality protein because of its well-balanced essential amino acid in addition to high dietary fibre and flavonoid concentrations (J.J.Bhaskar, 2011). A research paper by Sheng et al (2010) showed "Banana flower has tremendous nutritional value" and these have been already stated by Oliveira et al. (2006). Banana flowers also have wonderful medicinal properties. It generally helps to cure stomach ulcers and also have useful effects in

treating throat ulcers. It can cure inflammation of eyes and eye afflictions. It can also help in treating vital diseases and nervous debilities. The blossoms contain a class of phytochemical known as saponins. Saponins lower LDL or bad cholesterol, boost our immunity against infection and are thought to inhibit the growth of cancer cells. They also have antioxidant activity and so can reduce our risk of chronic diseases including cardiovascular disease (Nataraj Loganayaki et al., 2010).

In West Bengal different varieties of banana tree are cultivated namely Martaman, Mohavog, Seed Banana, Giant Governor, Bagda Kathali. The flowers of the respective banana plant are also found in market. All of these are different in taste and nutritional point of view. The nutritional analyses of these flowers are not available in any kind of literature. Therefore the aim of this investigation is to evaluate and compare the nutritional composition of these five varieties of banana flowers commonly cultivated in west Bengal.

MATERIALS & METHODS:

Sample collection:

The samples (Banana Blossoms Of Martaman, Mohavog, Seed Banana, Giant Governor, & Bagda Kathali Variety) were collected from local market of Kolkata in sterile zip lock plastic bag and preserved in ice bag during transportation from land to the laboratory. For analysis flowers were used. Analysis :

i. Determination of moisture content :

Moisture content was determined by drying 10 g of samples at 105°C in a drying oven to a constant weight (AOAC, 1990).

ii. Determination of ash content :

Ash content was determined by weight difference method by using muffle furnace (5000°C). (Raghuramulu et al., 2003)

iii. Determination of pH : The pH of the sample was determined using pH meter (khosa et al., 2011).

iv. Determination of crude fibre content:

Crude fibre content of dry sample was determined by using AOAC, 2006 method. The sample is allowed to boil with 1.25% dilute H₂SO₄, washed with water, further boiled with 1.25% dilute sodium hydroxide and the remaining residue after digestion was taken as crude fibre.

v. Determination of total carbohydrate content:

The fresh sample was blended and used for the estimation of total carbohydrate content by using Anthrone Method (Mc.Cready et al., 1950). To the 0.1gm blended sample, 5ml 2.5(N) HCl was added and the mixture was hydrolyzed for 3 hours in a water bath. After cooling, the hydrolyzed mixture was neutralized with Na₂CO₃ and the volume was made up to 100 ml by distilled water and centrifuged at 3000 RPM for 15 minutes to remove any residues. To 1.0 ml of supernatant, 4ml of Anthrone reagent (0.2% in conc. H₂SO₄) was added and mixed thoroughly. The mixture was allowed to heat for 8 minutes in water bath and cooled. This was followed by recording absorbance in spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer) at 630 nm wavelengths against a blank. The blank was prepared by taking 1.0 ml distilled water instead of sample extract.

vi. Determination of protein content:

The Protein content was determined using BIS method (Bureau of Indian Standard, IS No. 7219:1973) based on Kjeldahl method. Once the nitrogen content has been determined, it is converted to a protein content using the appropriate conversion factor (N factor i.e. 6.25).

vii. Determination of tannin content:

Appropriate amount of grounded plant samples (dry weight basis) were boiled with distilled water for 30 minutes and cooled. It was then centrifuged and filtered with whatman 1 filter paper and the filtrate was taken for tannin estimation. Tannin content in

the plant extract was determined as described by (Schanderi, 1970), using tannic acid as the standard. The extract solution (1 ml) was mixed with the Folin denis reagent (5ml) and super saturated solution of Na₂CO₃(10 ml) and volume made up to 100ml by distilled water. After 30 minutes of incubation at room temperature, the absorbency of the reaction compound at 700 nm was measured spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer) The overall tannin content was expressed as mg of tannic acid equivalents / gm dry weight.

viii. Determination of antioxidant content:

Preparation of extracts :- Prior to extraction and analysis samples were washed and dried on paper towel and then subjected to size reduction using a knife and blended with a kitchen mixer to get a thick paste. Appropriate amount of plant paste sample was extracted with-----

•6 % metaphosphoric acid for vitamin C estimation and

•80% methanol and left it overnight. These were then centrifuged at 10,000 rpm for 15 minutes and the supernatants were decanted into polypropylene tubes and filtered through Whatman No.1 filter paper. The clear extracts were analyzed for determination of phenolic contents.

(a) Determination of total phenols

Total phenol content in the plant extract (extracted in 80% methanol, kept overnight) was determined as described by Singlaton and Rossi (1965), using gallic acid as the standard. The extract solution in 80% methanol (1 ml, 50 mg ml⁻¹) was mixed with the FC reagent (10%, 1 ml) and an aqueous solution of Na₂CO₃(7.5% , 0.8 ml) and volume made upto 10ml by distilled water. After 30 minutes of incubation at room temperature, the absorbance of the reaction compound at 765 nm was measured spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer) The overall phenol content was expressed as mg of gallic acid equivalents (GAE)/ gm dry weight.

(b) Estimation of ascorbic acids:

Ascorbic acid content in the plant extract (extracted in 6% metaphosphoric acid) was determined as described by Joseph et al., (1944) using ascorbic acid as the standard (1mg/ml). The extract solution (2 ml) was mixed with equal amount of acetate buffer (ph 4.0) and dye (sodium salt of 2,6 dichlorophenol indophenol)solution in the separating funnel. The content was mixed well and 10ml xylene was added . This was then mixed well and allowed to stand for 6 seconds for separating the layers and then the water layer was removed and the colour in xylene was measured in a spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer) at 500 nm. The ascorbic acid content was calculated as..

$$= \frac{0.1 \times \text{Blank- Sample}}{\text{Blank- Standard}} \times 100 \text{ (mg \%)}$$

Amount of the sample taken

ix. Determination of minerals content

Sodium, potassium, calcium and iron content were measured by using wet digestion method (AOAC,1975).

STATISTICAL ANALYSIS:

Each experiment was repeated three times and the results are presented with their means, and standard deviation using Microsoft Office Excel 2010. The statistical analysis was done using one way ANOVA with the help of the software SPSS 16.0. $P < 0.05$ indicates significant variation exists at 95% confidence level.

RESULTS

The results of analysis of nutrients , anti-nutrients and antioxidants and minerals content are given in Table 1, Table 2, and Table 3 respectively.

Table 1 : Moisture, Ash, pH, Crude Fibre, Total Carbohydrate, and Protein contents (gm %) of Banana blossoms-

SAMPLES	MOISTURE CONTENT (gm%) N = 3 *	ASH CONTENT (gm%) N = 3 *	PH N = 3 *	CRUDE FIBRE CONTENT (gm%) N = 3 *	CARBOHYDRATE (gm%) N = 3 *	PROTEIN CONTENT (gm%) N = 3 *
Martaman	90.80 ± 0.200	5.36 ± 0.140	5.600 ± 0.050	2.20 ± 0.050	6.80 ± 0.318	1.52 ± 0.027
Mohavog	91.30 ± 0.200	2.64 ± 0.111	5.870 ± 0.020	2.52 ± 0.075	4.40 ± 0.105	1.58 ± 0.020
Seed Banana	89.40 ± 0.655	1.22 ± 0.125	6.405 ± 0.064	0.40 ± 0.026	5.80 ± 0.070	1.23 ± 0.027
Giant Governor	91.00 ± 0.500	1.28 ± 0.040	6.970 ± 0.020	3.50 ± 0.082	5.09 ± 0.201	1.64 ± 0.046
Bagda Kanthali	90.70 ± 0.721	1.78 ± 0.075	6.330 ± 0.050	7.00 ± 0.447	4.20 ± 0.265	1.06 ± 0.060

Table 2 : Tannin, Total phenols And Ascorbic acids content (mg%) of Banana blossoms-

SAMPLES	TANNIN CONTENT (mg%) N = 3*	TOTAL PHENOL CONTENT (mg%) N = 3*	ASCORBIC ACID CONTENT (mg%) N = 3*
Martaman	32.38 ± 0.437	80.98 ± 0.666	1.40 ± 0.020
Mohavog	127.02 ± 0.126	331.30 ± 0.431	2.42 ± 0.104
Seed Banana	81.98 ± 0.186	37.38 ± 0.486	2.27 ± 0.076
Giant Governor	120.45 ± 0.577	31.10 ± 0.180	1.80 ± 0.050
Bagda Kanthali	112.53 ± 0.301	26.84 ± 0.760	1.60 ± 0.050

Table 3 : Sodium, Potassium, Calcium And Iron content (mg%) of Banana blossoms-

SAMPLES	SODIUM CONTENT (mg%) N = 3*	POTASSIUM CONTENT (mg%) N = 3*	CALCIUM CONTENT (mg%) N = 3*	IRON CONTENT (mg%) N = 3*
Martaman	39.13 ± 0.170	654.70 ± 0.360	46.04 ± 0.072	8.97 ± 0.044
Mohavog	22.32 ± 0.104	374.39 ± 0.923	69.15 ± 0.064	2.49 ± 0.035
Seed Banana	25.89 ± 0.265	415.46 ± 0.380	34.80 ± 0.295	3.21 ± 0.032
Giant Governor	20.61 ± 0.240	274.97 ± 0.202	22.98 ± 0.086	0.79 ± 0.030
Bagda Kanthali	27.51 ± 0.273	369.32 ± 0.570	57.73 ± 0.335	5.51 ± 0.036

All results are expressed in MEAN ± SD

* significant variation exists among different varieties of samples (P < 0.05);

** no significant variation exists among different varieties of samples (P > 0.05).

DISCUSSIONS:

TABLE 1 : NUTRIENTS CONTENT

Table 1 represents the results of nutrients content in gm/100gm. The result of analysis showed significant differences (P < 0.05) in proportions of nutrients (Moisture, Ash, pH, Crude Fibre, Total Carbohydrate & Protein Content) among five different varieties of Banana Blossoms.

Moisture – The range of moisture content among five varieties of banana blossoms was between 89.40–91.30 gm%. Highest level of moisture was found in blossoms of Mohavog followed by, Giant governor, Martaman, Bagda kanthali, and finally Seed Banana.

Ash – Among the five varieties of banana blossoms, Martaman variety has highest level of ash content

(5.36 gm%) while seed banana variety has the lowest amount of ash (1.22 gm%).

pH – pH level of all five varieties of banana blossoms fall under neutral to slightly acidic zone, ranges between 5.36 to 6.97.

Crude Fibre - Among the five varieties of banana blossoms, crude fibre content was found to be highest in Bagda kanthali variety (7.00 gm%) while seed banana variety has the lowest amount (0.40 gm%).

Carbohydrate - The range of Carbohydrate content among five varieties of banana blossoms was between 4.20–6.80 gm%. Blossoms of Martaman variety has highest level while the blossoms of seed banana variety has the lowest level.

Protein – The protein content of five varieties of banana blossoms are very close to each other ranges

between 1.06 gm% (Bagda kanthali) to 1.58gm% (Giant governor).

TABLE 2 : ANTI-NUTRIENT AND ANTIOXIDANTS CONTENT

Table 2 reveals the results of anti-nutrient and antioxidant content of five different varieties of banana blossoms in mg%. Data showed significant differences exist among five different varieties of banana blossoms ($P < 0.05$) in terms of anti-nutrient and antioxidant content. Tannin and Total Phenol content of all five varieties of banana blossoms vary significantly, ranges between 32.38 – 127.02mg% and 26 – 331.30mg% respectively. Ascorbic acid content of all these varieties was found to be close to each other ranges between 1.40 – 2.42 mg%. Moreover the blossoms of Mohavog variety has the highest level of tannin, total phenol and also ascorbic acid content among all varieties of banana blossoms while least level was found in Blossoms of Martaman variety in case of both tannin & ascorbic acid content and in Bagda kanthali variety in case of total phenol content.

TABLE 3 : MINERALS CONTENT

The results of table 3 showed the amount of different minerals (sodium, potassium, calcium & iron) present in five different varieties of banana blossoms. The results are expressed in mg%. Data reveals that the sodium, potassium, calcium & iron content differ significantly between the five selected varieties of banana blossoms ($P < 0.05$). Moreover analysis of minerals content showed that the Blossoms of Martaman variety has the significant highest amount of Sodium (39.13 mg%), Potassium (654.70 mg%), & Iron (8.97mg%) content, while the Calcium content was found to be highest in the blossoms of Mohavog variety (69.15 mg%) as compared to the other varieties. Among all varieties, blossoms of Giant governor has the lowest level of Sodium (20.61mg%), Potassium (274.97mg%), Calcium (22.98mg%) and also Iron (0.79mg%).

CONCLUSION:

This study has comprehensively investigated the nutritional quality of five different varieties of banana

flowers cultivated in West Bengal. The results indicate that these banana flowers are good sources of both nutrients and antioxidants. The observations of the present study suggest that there were significant differences exist in the Nutrients, Anti-Nutrients and Antioxidant content ($P < 0.05$) of five different varieties of banana blossoms. Among all varieties, banana blossoms of Martaman and Mohavog are more nutritious because of their increased ash, minerals and antioxidant content as compared to others. Crude fibre content was found to be highest in Bagda kanthali variety. Banana blossoms are the poor sources of protein but rich sources of fibre, total phenols and minerals except the blossoms of Giant governor variety which is less nutritious as compared to others.

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A SURVEY ON THE ARSENIC AFFECTED AREAS OF BENGAL

Dr. Sutripta Sarkar¹ and Debapriya Roy²

Abstract

Water is vital to the existence of living organisms however, heavy metal contamination of drinking water sources is a major problem faced by humanity. The objective of the study was to assess the quality of the underground water and to study its impact on the communities living in the arsenic affected areas. Underground water samples were collected from 7 districts and arsenic, iron and phosphate content was measured. An Interview Schedule Method was used as a tool for the survey. More than 100 people were surveyed in 3 districts. The Survey was conducted at- Belur (near Ganga), {Semi urban area}, Salkia {Semi urban area}, Kudghat {urban area}, Habra (Simulpur, Upnae, Salka) {rural

areas} to estimate the impact of intake of arsenic laden water in terms of manifestation of disease symptoms.

Around 19% of the water samples from North 24 Parganas district were found to contain arsenic higher than the WHO recommended level (10µg/ml) which was highest amongst the districts surveyed. Liver diseases, improper digestion and other gastrointestinal problems were predominant in the people surveyed in the 3 districts. People surveyed in Habra of North 24 Parganas showed skin pigmentation, dermatitis and hyperkeratinisation which are diagnostic of arsenic toxicity.

Keywords: Water pollution, Arsenic toxicity, skin pigmentation, dermatitis

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Introduction

Arsenic toxicity is a major health and environmental problem which is affecting millions of people across the world. Argentina, Mexico, Chile, Ghana, south-west parts of USA, China, Taiwan, Nepal are among the countries where ground water level of arsenic is higher than the WHO standards (i.e. 10µg/litre). The worst affected areas are perhaps the Gangetic delta region which spans across West Bengal, India and Bangladesh. The problem arises in delta regions because of the relatively young age of the sediments deposition made by the Ganga-Bramhaputra-

Meghna river system. The sediments containing minerals like biotite undergo slow "diagenetic" reactions as the sediments become compacted, and which, under the reducing conditions of the groundwater, release arsenic in the form of toxic As³⁺ (Edmunds et al, 2012). The problem is restricted to sediments of Holocene age and groundwater of a certain depth (mainly 30-150m), which coincides with the optimum well depth (Edmunds et al, 2012). Acute arsenic poisoning causes symptoms like severe abdominal pain, nausea, vomiting and acute diarrhea. Chronic arsenic toxicity affects several

organs of the body and is one of the leading causes of cancer. Several Studies have been conducted on the impact of arsenic toxicity on health and its mitigation. A constant monitoring of the ground water aquifers is required to warn people against its consumption. The current study aims at exploring the extent of ground water contamination by arsenic and how it has impacted the people dwelling in these areas.

Review of Literature:

High concentration of arsenic in underground water is a major problem in India, especially the state of West Bengal (Mukherjee et al, 2006). Arsenicosis, caused due to long term consumption of arsenic contaminated ground water is fast becoming a major epidemiological problem (Saha et al, 2010). It is now well established that inorganic arsenic is extremely toxic, both acute and chronic. Inorganic arsenic (Arsenate and arsenite) is 500 times more harmful than organic form (Smedly et al, 2005). It can be absorbed by intestine, skin or inhaled by lungs. Arsenic trioxide causes detrimental effect on liver and kidney (Charles, 2014), lungs and skin. Inorganic arsenic is carcinogenic and its exposure may cause DNA hypo-methylation due to continuous methyl depletion, facilitate in aberrant gene expression, that results in carcinogenesis (Roy and Saha, 2002). Arsenic affects and alters cellular pathways and causes increased oxidative stress to disrupt the pro/anti oxidant balance (Flora, 2011). Research has also revealed that arsenic can replace phosphates in important biomolecules (Twafik and Viola, 2011). It is very difficult to diagnose early symptoms of arsenicosis because such non-specific symptoms may also be present in many other diseases (Saha, 2010). The burden of diseases due to arsenic is primarily because of lack of awareness and not taking proper precautions. Deficiency of proteins, folate, vitamin B in diet aggravates arsenicosis. These deficiencies negatively affect the mechanism of biomethylation or biotransformation, which helps in excretion of arsenic from the body (Chakrabarty, 2015).

Antioxidants/ chelating agents help in the reducing arsenic induced oxidative stress. N-acetylcysteine, α -lipoic acid, vitamin E, vitamin C, quercetin etc. show prophylactic activity against arsenic mediated injuries (Flora, 2011). These components are abundant in citrus fruits, garlic, nuts etc. A clinical trial conducted in Bangladesh showed that incorporation of high selenium lentils in diet counteracted the toxic effects of arsenic successfully (Krohn et al. 2016). Proteins are effective in mitigating arsenic toxicity to a large extent (Chakrabarty, 2015).

Though it is well established that surveyed areas are arsenic prone, attempt has been made to add to the existing data on arsenic contamination. A monitoring of the health status of the people living in these regions is also very essential.

Objective and Scope:

The research carried out was an exploratory study conducted to assess the quality of groundwater and to determine the effect of arsenic poisoning in people living in the affected areas. A survey was conducted on more than 100 people living in the arsenic affected areas (rural, semi-urban and urban) to determine the extent of manifestation of arsenicosis.

Material and Methods:

In the current study, two experiments were conducted.

Experiment 1. Samples were collected from arsenic prone areas to find the current status of water quality in these areas. Random samples were collected from these areas to assess the arsenic level, and also iron level, phosphate level, pH level & the total hardness. The samples were collected from the deep tube wells, of the 45 areas. The samples were collected in triplicate in 50 ml sterilized container and kept at -20°C till analysed. All analysis were done within seven days of collection.

Table no.1:- Districts from where the water samples are collected are collected.

A Survey On The Arsenic Affected Areas Of Bengal

DISTRICTS	AREAS
Howrah	Bally, Bally Jaw Gacha, Salkia, Belanagar, Bagnan, Belur (Ganga Water), Chandmari (Belur), Dharmatola Road (Belur), Belur (near Ganga)
Nadia	Chakdaha Town Chakdaha Interior
South 24 paraganas	Sonarpur, Rajpur
Kolkata (North)	M.G. Road, DumDum Cantonment, Sodepur, Saltlake (Bidhan nagar, Near no. tank 9), Saltlake Block A, Tobin Road (Borahonagar)
Kolkata (South)	Jadavpur, Kosba, Kudghat
Hooghly	Hooghly, Nalikul, Begampur, Tarakeswar, Uttarpara
Burdwan	Burdwan Town, Memari, Asansol

Detection of arsenic, iron, phosphate and total hardness:-

Arsenic, iron, phosphate and total hardness was detected by water analysis field kits manufactured by Nice Chemicals Pvt. Ltd., India. Arsenic was detected by using the classical Gutzeit method (1891) based on the reaction of arsine gas with mercuric bromide.

Statistical Analysis:-

Tests were carried out in triplicate. Standard deviation was calculated on triplicate values. Relative abundance (%) were formulated by-

$$\frac{\text{No. of samples containing arsenic/ phosphate/ iron}}{\text{Total sample}} \times 100$$

Experiment 2: A survey was conducted among those people who lived in the arsenic prone areas to determine the extent of manifestation of arsenicosis. A total no. of 112 people (from rural, semi urban and urban areas) was selected as samples for the survey. The survey was conducted in areas where arsenic was detected in ground water - Belur (near Ganga), {Semi urban area}, Salkia {Semi urban area}, Kudghat

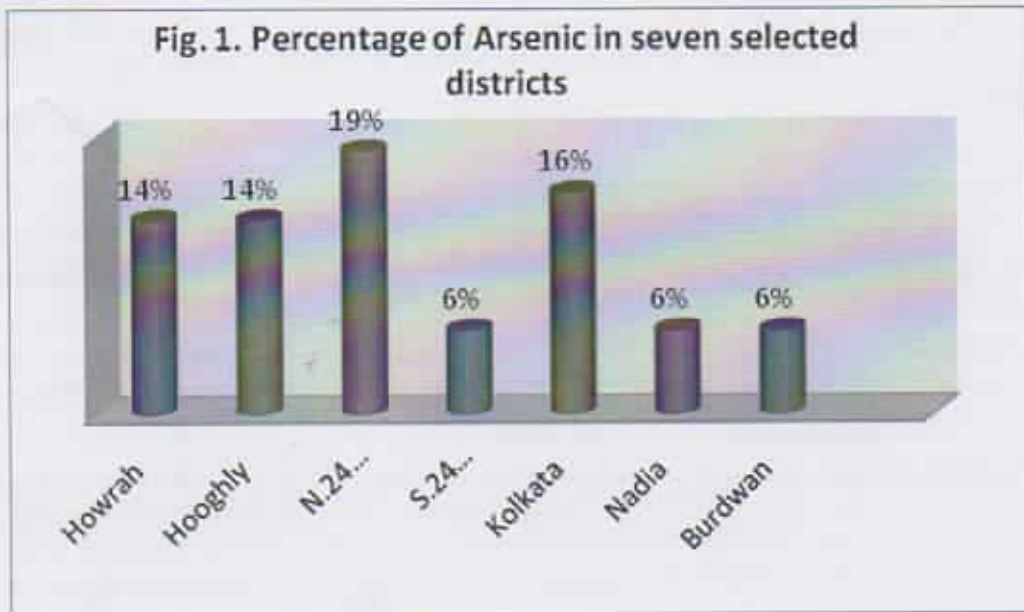
{urban area}, Habra (Simulpur, Upnae, Salka) {rural areas}. Rural, semi-urban and urban areas where intentionally surveyed to understand the impact of standard of living on the extent of the symptoms shown by the population living there. Though all the seven districts in the study were covered in the survey, area wise data is shown to highlight the urban - rural divide. An Interview Schedule Method had been used as a tool of the survey. The Questioner Form is given as annexure 1. Name, age, height, weight, BMR, education level, income etc. was inquired. Disease history, any skin lesions, pigmentation or patches, dysentery, nausea etc. were also taken into account. A 24 hr. diet recall was done in the study areas to determine the quality and quantity of food intake by the people inhabiting the arsenic prone areas. In this study the symptoms caused due to arsenic is the dependant variable and dietary intake is the independent variable.

Result and Discussion

Seven districts had been selected for assessing the iron, phosphate and most importantly arsenic levels

in underground water. The highest level of arsenic contamination in underground was found in the Samples of North 24 paragnas (Fig1). Around 19% of the samples contained high level of arsenic though the

highest quantity of arsenic in individual sample were found in Asansol (Burdwan district) and Salkia in Howrah district.

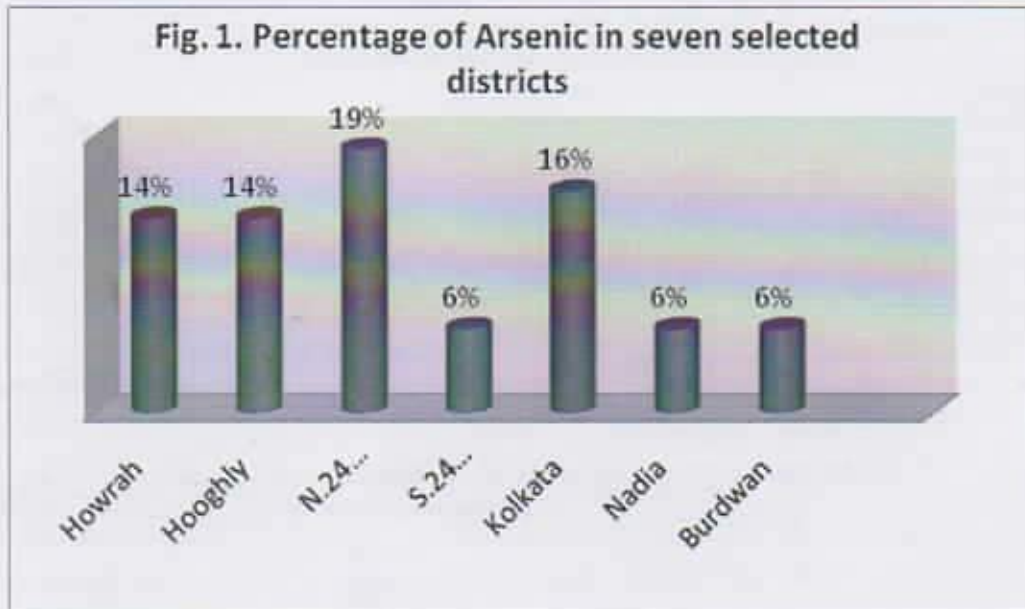


Iron, though not as toxic as arsenic, is found in large quantities in underground water. It increases the hardness of water and makes it very distasteful. The samples from North 24 paragnas were found to have

highest level of iron compared to other districts (Fig 2). 33% of the samples contained medium to high level of Iron.

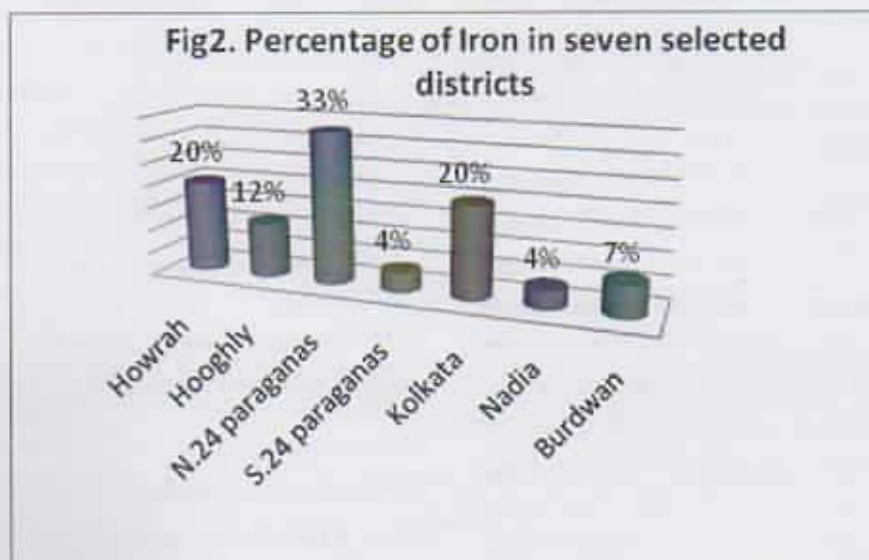
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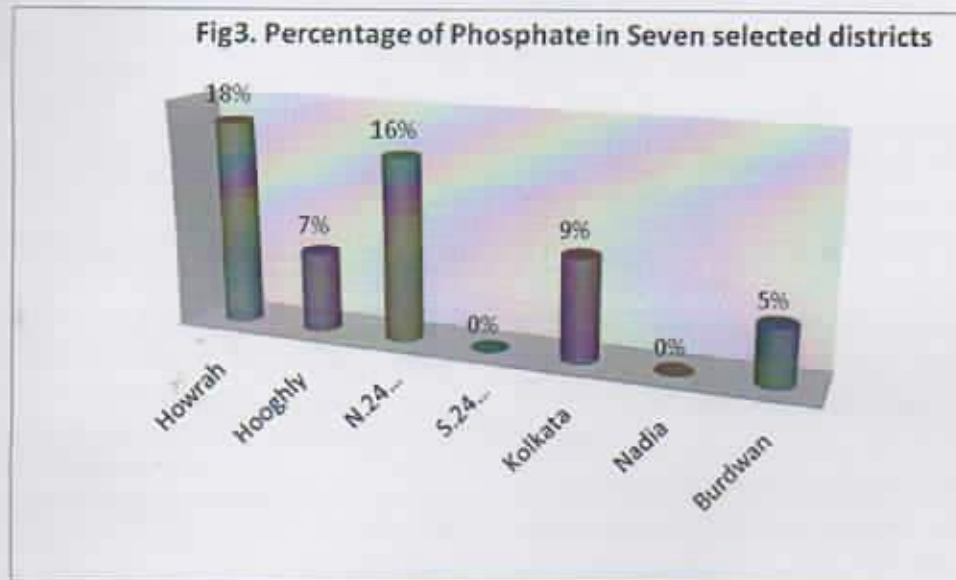
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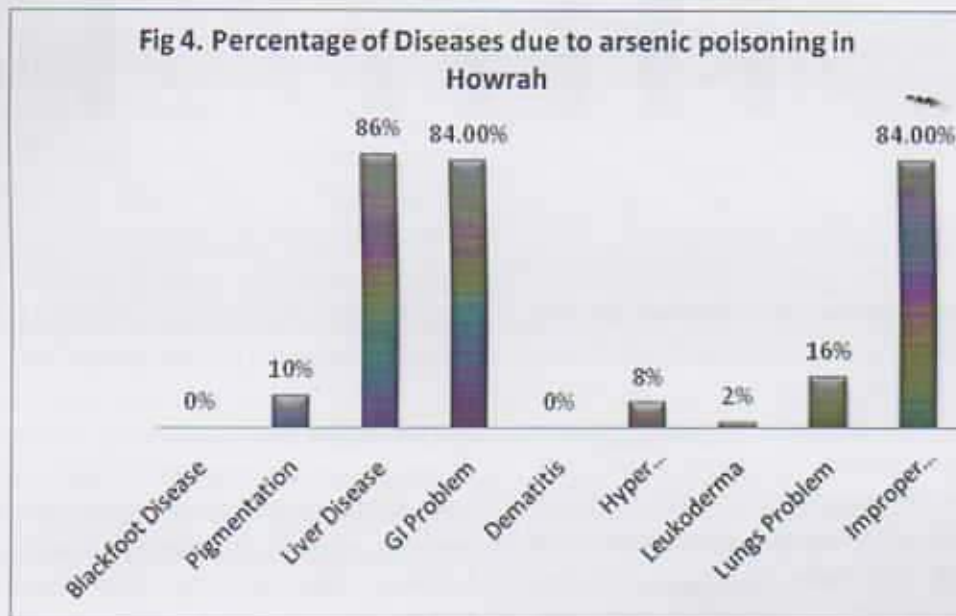
Howrah was found to have the highest level of phosphate (Fig 3) around 18% followed closely by North 24 paragnas (16%). High phosphate level

increases the hardness of water and it also interferes with the effective removal of arsenic from water.

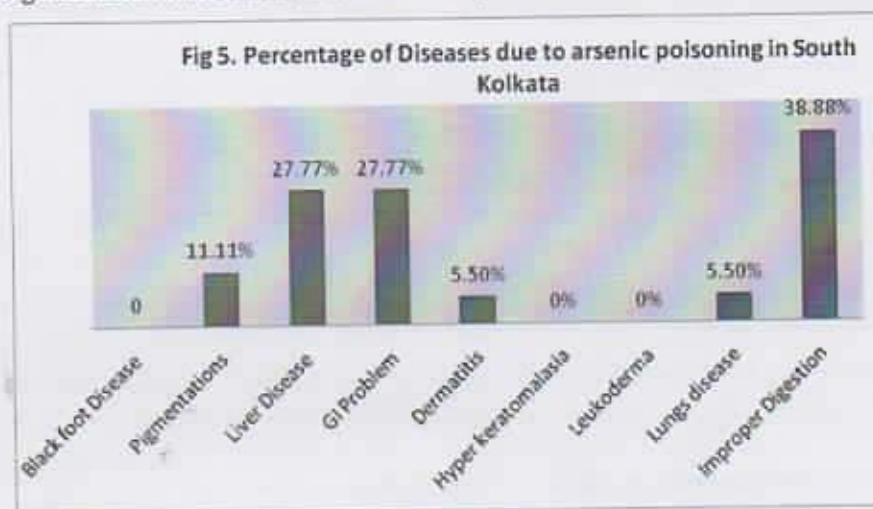


Investigators variously reported symptoms like nausea, diarrhoea, anorexia and abdominal pain in cases of chronic arsenic toxicity. Prolong drinking of arsenic contaminated water is associated with hepatomegaly, predominant lesion being hepatic fibrosis (Guha Mazumdar, 2008).

The survey data reveals that around 85% of the people surveyed in Howrah district suffered from liver and gastrointestinal diseases. Only 8-10% of the subjects reported some skin related problem (Fig 4).

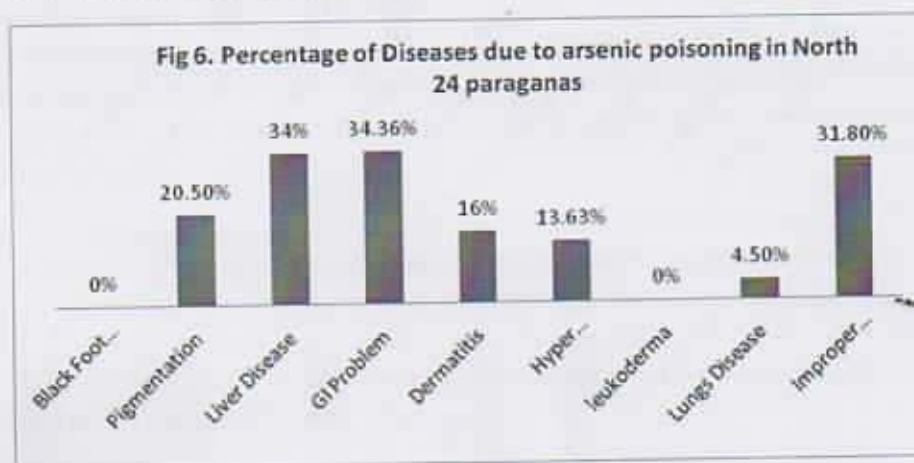


Around 30- 40% of the people surveyed in South Kolkata (Kudghat) reported problems due to improper digestion, liver and gastrointestinal related diseases and only a few cases of skin diseases were found (Fig 5).



Habra in North 24 paraganas was the worst affected area (Fig.6). Skin problem was very predominant in the local residents of the area. At least three families that were surveyed reported death of their akin due to arsenicosis. The survivors also showed skin lesion

characteristic of arsenic toxicity. Most of the people in this area were illiterate and, though they knew that underground water was arsenic contaminated, continued using it due to lack of other alternatives.



Conclusions: The current study reinforces the fact that ground water of several areas of West Bengal areas has high arsenic content. High phosphate content in water will probably interfere with the effective removal of arsenic. The survey reveals that people had some diseases like liver disease, lungs disease, skin problems etc which is the symptoms of arsenic poisoning. But these symptoms are also caused due to some nutrients disorders and other

toxicity. However, the data obtained from Habra (North 24 Paragnas) clearly reflects that people suffer from arsenic toxicity. The poor economic condition and diet of the inhabitants of this region could be the reason for the high manifestation of arsenicosis in this area. Creating awareness among people, banning used of arsenic containing pesticides, making available clean drinking water, incorporation of protein and antioxidant rich food in diet will help in

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containing this problem to a large extent.

Acknowledgements:

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A Survey on Arsenic Affected Areas of West Bengal

Annexure 1: Questionnaire

Name :- _____

Age :- _____ yr.

Sex :- _____

Height :- _____ (cm)

Weight :- _____ (kg)

BMI :- _____

BMR :- _____

Occupation :- _____

Working status :- Sedentary/Moderate/Heavy

Income :- Rs. _____ / (Month)

BP :- _____ (mmHg)

Body Age :- _____

Personal inquires:-

Water consumption :- 1lt / 2lt / 3lt / 4lt

Source of water intake and use for other needs:- Mineral water/Hand pump/Pond water/Purified water /other source.

Educational level:-

Do you have any addiction? :-

Do you take regular bath? :-

Symptoms of Arsenic

- | | | |
|-----|--|-----------|
| 1. | Black foot disease | :- yes/no |
| 2. | Pigmentation | :- yes/no |
| 3. | Gangrin(hand/foot/back) | :- yes/no |
| 4. | Nodulation | :- yes/no |
| 5. | Any liver problem | :- yes/no |
| 6. | GI problem(vomiting, severe diarrhoea) | :- yes/no |
| 7. | Anaemia/leucopenia | :- yes/no |
| 8. | Nasal mucosa | :- yes/no |
| 9. | Larynx and bronchi | :- yes/no |
| 10. | Conjunctivitis | :- yes/no |
| 11. | Dermatitis | :- yes/no |
| 12. | Hyperkeratosis | :- yes/no |
| 13. | Leukoderma | :- yes/no |

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