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Effects of Sub-Chronic Exposure of the Food Dye Amaranth on the Biochemistry of Swiss Albino Mice (*Mus Musculus* L.)

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Abstract:- Amaranth is a colorant widely used in food products, drugs and cosmetics.Swiss albino-mice (*Mus musculus*) of age Mice are 6-8weeks old. The current study evaluates toxic effects of both (1200 mg /kg body weight) and high dose (2400mg/kg body weight) of Amaranth ongeneral health and biochemistry(Glucose, cholesterol, total protein) of Swiss albino mice exposed for 7,14,21,28 days throughamaranth were dissolved in ddH2O and administered through oral gavage.

Body weight showed that this change was positive for Group IIa while negative for Group IIb. It was also evident that substantial depreciation of body weight began early in amaranth exposed mice (i.e. on 7th day)and continued thereafter. More specifically, body weights in Group IIa was noted as20.37±0.23, 20.47±0.24, 20.74±0.25, and 21.66±0.22 g, on 7th, 14th, 21st, and 28th day,. Similarly, in Group IIb the followings were measured as 20.12±0.29, 20.20±0.27,20.14±0.22, and 19.87±0.13.

In comparison to control significant increase in the serum glucose was noticed in Group IIa and IIb. Highglucose was noted from 7th day of exposure and continued to elevate on 14th, 21st and 28th dayof administration. Maximum level of glucose was witnessed in Group IIb on 28th day, indicating direct influence of amaranth on glucose metabolism. Slight increase in level of cholesterol was apparent, despiteinsignificant alterations. In Group IIa level of cholesterol was noted as 133.28±1.74,124.60±1.99, 130.81±3.34, and 135.33±2.15 mg/dl on days 7, 14, 21, and 28, respectively.Likewise in Group IIb, the above were noted as 128.66±2.43, 133.05±1.45, 134.26±3.99, and129.26±1.58 mg/dl on respective days of investigation. Both groups ofanimals administered with amaranth indicated highly significant increase in the level of totalprotein. In Group IIa concentration of protein was evaluated as 5.67±0.10, 5.58±0.16,5.16±0.13, and 5.21±0.16 g/dl following 7th, 14th, 21st, and 28th day of administration.Similarly, Group IIb showed 5.76±0.11, 5.36±0.16, 5.33±0.19, and 5.04±0.24 g/dl on respective investigated days strangely, effect of higher dose ofamaranth (Group IIb) was nominal comparing to concentration of protein in Group IIa.

Keywords:- *Amaranth, sub-chronic toxicity, biochemistry, Glucose, cholesterol, Total protein, Swiss albino mice (Mus musculus L.).*

I. INTRODUCTION

Food colours are of extreme importance how one tastes and perceives food. There are many ways to introduce colours in the food, the natural way is to add more colourful ingredients in the dishes such as vegetables and herbs, or by adding food colours available in both forms natural and synthetic

Previous studies indicate that colour constituents impose sensory cues in the brain revealing personal expectations of taste and/or flavour (Clydesdale, 1984; Clydesdale, 1991; Clydesdale, 1993; Delwiche, 2012; Hall, 1958; Kanig, 1955; Kostyla and Clydesdale, 1978; Watson, 2014). In sense of judgment the 'taste' and the 'flavour' have different roles, where taste is defined by gustatory receptors found in oral cavity and flavour by olfactory component (Spence et al., 2015; Spence et al., 2010)

Colourants are according to the Codex Ailmentarius Commission a substance that colour food or compensate colours of food products (Demirağ et al., 2006; Aberoumand, 2011; Pandey and Upadhyay, 2012). Based on application food colourants have been divided into 3 separate groups: 1) Acceptable Daily Intake is defined and allowed to use, 2) Special instructions for use, and 3) Exclusive use in selected food products. Based on origin of food colorants they are again separated into, 1) Natural colourants, 2) Synthetic colourants.

Synthetic food colourants are chemical originated, and mostly belonging to azo group,can be divided in to two distinct categories based on permissibility (Zahra et al., 2015; Purba et al., 2015). permitted and non-permitted food colours. Most application of synthetic food colourants are used in bakery products, confectionaries, jellies, and many other beverages. Countries that does not follow strict regulation on food additives and adulteration, these ready to eat food products may contain some non-permitted synthetic colourants. Synthetic colours in food products are considered one of the major causes of food related toxicity, hyper immunogenic responses, mutation, organ failure, asthma, eczemas etc. (Bachalla, 2016; Nath et al., 2015; Shinde and Shinde, 2013; Rowe and Rowe, 1994; Tuormaa,1994).

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II. REVIEW OF LITERATURE

An article **by** Reutter (2004) for Illinois News Bureau wrote that food display and food colour affect on how much people eat. With example author writes that bowl containing mixed coloured jellybeans were eaten 69% more than bowls containing individual colour. A study by Elliot (2015) revealed that future research on colour psychology would provide measures for media to tempt consumers for predetermined conclusions. Same author also reported that colours are strongly associated with psychological functioning in humans (Elliot and Maier, 2014). Another study by Mentzel et al. (2017) examined red colour association to the concept of dominance. Authors found out that there is a strong association between colour and emotionality, where red colour elicit dominant emotional behaviour.

A study by Martins et al. (2016) claimed that colours are one of the most important reasons why consumer make a choice based on eating desires. Likewise, Demirağ and Uysal (2006), revealed that colour of food follows the same principle of light when it interacts with matter and visible wavelength 380-770 nm. These colourants are used for processing of food products such as; candies, margarine, cheese, soft drinks etc (FDA, 2017).

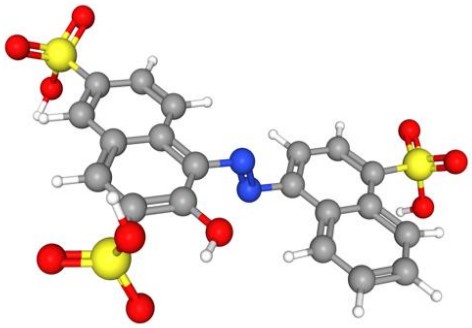
A review by Sigurdson et al. (2017) reported that although natural food colours are considered safe, it has lower stability, weaker tinctorial strength, interaction with food ingredients, and limited availability of hues. Another study by Brudzyńska et al. (2021) reported that colours are the most attractive feature of food and developing them through botanical agents enhance its applicability in consumer. Authors also convey that since multiple materials and compounds are required to make herbal colourants, it is considerably safe when compared with synthetic colours. Authors further mentioned that history of each natural food colours provides information on the civilization and how developed they were at that time.

III. MATERIAL AND METHODS

Test compounds: food colourant :Amaranth ($C_{20}H_{11}N_2Na_3O_{10}S_3$) were purchased from Sigma-Aldrich. Structural and chemical properties

E number : E123

Structure:



Molecular weight: 604.47
CAS No.: 0915-67-3

A. Animal model:

Swiss albino-mice (*Mus musculus*) of age 6-8 weeks were used in this study. Randomlybred mice were maintained in polypropylene cages of size 43×27×15 cm. These animals were maintained in departmental animal facilities with appropriate ratio of light and dark (12 h: 12 h) schedule. Mice were fed with pellet diet and provided water *ad libitum*. These animals were kept under strict observation of veterinary expert. All experiments were carried out according to the guidelines explained in Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, 2010).

B. Ethical approval:

The guidelines of Indian National Science Academy (INSA), New Delhi for Care and Use of Animals were followed in all experiments. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC).

C. Determination of LD50:

Groups of six healthy mice were treated with amaranth individually viaoral administration following overnight fasting. Food colourants were solubilized in ddH2O and administered with doses 50, 100, 1000, 5000, 10000 mg/kg body weight. Control animals were administered with ddH2O alone. Mice were observed for 14 days. Dead and/or moribund mice were removed swiftly and autopsied for inspection. Dead animals were counted every day.

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$LD50 = LD100 - ((a \times b)/n$
a = Difference between two successive doses
b = Average number of dead animals
LD100 = Lethal dose causing 100% death

D. Experimental design:

Based on results of LD50 experiments safe doses with confirmed 'no mortality' during14 days of observation, were utilised to study acute and sub-acute effects both food colourants. Experiments were divided into two experiments i.e. Experiment I and Experiment II, where, Exp I designated for treatment with azorubine, and Exp II represented amaranth treatment. Groups of animals in Exp I and Exp II were termed as Group II and III, respectively. Randomlyselected mice were distributed into 4 treatment groups, that was based on dose intervals, i.e. 7days, 14 days, 21 days, and 28 days. These groups were further bifurcated into dose dependentsub-groups, 'a' and 'b' for doses 1/4LD50 and 1/2LD50, respectively. Table 2 lists out groupsand their specifications. Five animals for each dose interval (7, 14, 21, and 28 days) were used,making 20 animals in each sub-group. Likewise, 20 healthy animals were sham treated withvehicle for parallel comparison, the control group was designated as Group I. Control groupwas common for both food colourants, as all experiments were running parallel to each other.Doses of amaranth was dissolved in ddH2O and administered through oralgavage.

Groups	Specification
Groups	Control (vehicle administered)
Experiment I	
Group IIa	Administered daily with 1/4LD50 dose of azorubine. Five animals each were terminated from treatment and sacrificed at 7th, 14th, 21st, and 28th day of administration.
Group IIa	Administered daily with 1/2LD50 dose of azorubine. Five animals each were terminated from treatment and sacrificed at 7th, 14th, 21st, and 28th day of administration.
Experiment II	
Group IIIa	Administered daily with 1/4LD50 dose of amaranth. Five animals each were terminated from treatment and sacrificed at 7th, 14th, 21st, and 28th day of administration.
Group IIIb	Administered daily with 1/2LD50 dose of amaranth. Five animals each were terminated from treatment and sacrificed at 7th, 14th, 21st, and 28th day of administration.

Table 1: Experimental groups and specifications

Biochemical analysis of serum was assessed by semi-atomic photometer analyser. The analyser was used according instruction manual for estimation of blood glucose, cholesterol, total protein, using providedreagents kits.

E. Total cholesterol:

Total cholesterol homogenate (liver/testis) was suspended in distilled water. Therefore, it was pipetted out into a glass stoppered centrifuge tube, later evaporated to dryness. A 5 ml of ethanolic KOH whichcontained, 6 ml 33% KOH to 100 ml with absolute ethanol, was added and mixed nicely. Itwas then warmed in a water bath at 37-41 °C for 55 minutes. After the solution cooled downto room temperature, 10 ml of petroleum ether was added and mixed. A 5 ml of water was thenadded to this and was mixed for 1 minute. This mixture was then centrifuged at low speed for5 minutes. A 4 ml of petroleum ether layer was pipetted out into a test tube and evaporated at60 °C. A 6 ml of colour reagent containing 20 ml acetic anhydride and 1 ml concentrated H2S04along with 10 ml of glacial acetic acid, was added to each tube and kept at 25 °C. Therefore, a6 ml of colour reagent was taken as blank. Following 30-35 minutes of resting OD was read at 620 nm.

F. Protein estimation:

Briefly, tissue homogenate was prepared in ddH2O. Further to this TCA was added and centrifuged for 10 min at 2000 rpm, precipitate will be collected and 1N NaOH added and kept in boiling water for 5 min. The solution was incubated with reagent D (0.1 NNaOH, sodium tartrate and CuSO4) for 10 min. Finally, FC reagent added and absorbance wasestimated on spectrophotometer.

G. Statistical analysis:

Mean of all numeric values were calculated and represented with their respectivestandard error (SE). One way Analysis of Variance (ANOVA) was applied to compare multiple parameters for the assessment of variables in conjunction with Tukey's multiple comparison test with

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95% CI (MINITAB, US). Paired analysis was performed by Student *t* test and respective significance level was analysed for scrutiny of variance. For all quantitative variation $p<0.05$, 0.01, and 0.001 were considered significant, highly significant and extremely significant.

IV. RESULTS

Lethal doses were estimated for amaranth. Likewise, most animals were dead by doses ofamaranth over 6000 mg/kg. Low number of animals were dead by the doses below 4000 mg/kgbody weight. Observed LD50 for amaranth was 6000 mg/kg body weight.

- **Body weight** : **Group I control**:

	0 days	7 days	14 days	21days	28 days
M-1	20.14	21.43	21.45	23.29	23.73
M-2	19.61	21.64	21.55	21.95	26.39
M-3	21.13	20.85	23.61	23.85	26.09
M-4	20.17	20.46	22.09	24.76	24.81
M-5	20.09	21.59	21.47	23.39	24.35
mean	20.23	21.19	22.03	23.45	25.07
SD	0.55	0.52	0.92	1.02	1.14
SE	0.25	0.23	0.41	0.46	0.51

Table 1

- **Group IIa (1200 mg/kg b.wt.)**

	0 days	7 days	14 days	21 days	28 days
M-1	19.35	20.01	20.09	20.14	21.09
M-2	20.05	20.08	20.11	20.19	21.18
M-3	20.21	20.3	20.43	21.06	21.75
M-4	19.47	20.21	20.36	20.86	22.11
M-5	21.17	21.25	21.38	21.46	22.15
Mean	20.05	20.37	20.47	20.74	21.66
SD	0.73	0.50	0.53	0.57	0.50
SE	0.32	0.23	0.24	0.25	0.22

Table 2

- **Group IIa (2400 mg/kg b.wt.)**

	0 days	7 days	14 days	21 days	28 days
M-1	21.09	21.09	21.13	20.85	20.3
M-2	20.14	20.1	20.11	20.05	19.84
M-3	20.35	20.29	20.35	20.21	20.01
M-4	19.11	19.61	19.88	20.09	19.71
M-5	19.61	19.49	19.55	19.5	19.51
Mean	20.06	20.12	20.20	20.14	19.87
SD	0.75	0.64	0.60	0.48	0.30
SE	0.34	0.29	0.27	0.22	0.13

Table 3

- **T- Test:**

GroupI - IIa	7 days	14 days	21days	28 days
7 days	0.040			
14 days		0.034		
21 days			0.002	
28 days				0.004
GroupI - IIb	7 days	14days	21days	28 days
7 days	0.02			
14 days		0.019		
21 days			0.0027	
28 days				0.0006

Table 4

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Body weights of animals exposed to amaranth showed slightly aggravated decline inweight. Results showed that during period of investigation closeto 1 g change in weight was observed in mice administered with amaranth. Results showed that this change was positive for Group IIa while negative for Group IIb. It was also evident that substantial depreciation of body weight began early in amaranth exposed mice (i.e. on 7th day) and continued thereafter. More specifically, body weights in Group IIa was noted as20.37±0.23, 20.47±0.24, 20.74±0.25, and 21.66±0.22 g, on 7th, 14th, 21st, and 28th day,respectively. Similarly, in Group IIb the followings were measured as 20.12±0.29, 20.20±0.27, 20.14±0.22, and 19.87±0.13, respectively. Through box plot it appeared that both higher doses of amaranth (Group Ib and Group IIb) displayed decline in weight gain .

- **Glucose(mg/dl)GroupI (Control)**

	7 days	14 days	21 days	28 days
M-1	84.39	80.56	89.45	103.15
M-2	88.61	94.46	90.34	87.46
M-3	94.44	99.01	96.48	88.29
M-4	93.64	84.53	99.01	94.61
M-5	81.81	89.61	94.35	97.05
Mean	88.58	89.63	93.93	94.11
SD	5.55	7.41	4.04	6.49
SE	2.48	3.31	1.81	2.90

Table 5

- **GroupII A (1200mg/kgb.wt.)**

	7 days	14 days	21 days	28 days
M-1	103.45	105.48	111.38	118.31
M-2	105.61	113.09	117.45	114.05
M-3	111.23	115.83	110.5	105.23
M-4	109.5	112.05	119.34	109.73
M-5	101.24	101.01	103.29	113.08
Mean	106.21	109.49	112.39	112.08
SD	4.14	6.08	6.35	4.90
SE	1.85	2.72	2.84	2.19

Table 6

- **Group IiB (2400mg/kg b.wt.)**

	7 days	14 days	21 days	28 days
M-1	117.35	115.05	123.3	129.27
M-2	119.33	119.29	129.04	120.65
M-3	109.35	111.02	127.13	121.44
M-4	125.09	120.37	115.59	125.61
M-5	119.15	119.49	125.47	118.18
Mean	118.05	117.04	124.11	123.03
SD	5.67	3.95	5.21	4.40
SE	2.54	1.77	2.33	1.97

Table 7

- **T.Test:**

GroupI-IIa	7 days	14 days	21 days	28 days
7- days	0.000013	0.000037	0.00047	0.0059
14- days		0.00233	0.00766	0.0114
21 - days			0.00431	0.00118
28 -days				0.00118

Table 8

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GroupI-IIb	7- days	14 - days	21 - days	28- days
7- days	0.0015	0.00118	0.00075	0.00032
14 - days		0.00312	0.00015	0.0025
21- days			0.0012	0.00055
28- days				0.00024

Table 9

GroupIIa-IIb	7-days	14-days	21-days	28 – days
7-days	0.027	0.023	0.005	0.002
14-days		0.113	0.013	0.014
21-days			0.053	0.0087
28- days				0.0087

Table 10

Results were observed in mice administered with amaranth. In comparison tocontrol significant increase in the serum glucose was noticed in Group IIa and IIb. It appearedthat both doses of amaranth had dose dependent impact on the glucose metabolism. Highglucose was noted from 7th day of exposure and continued to elevate on 14th, 21st and 28th day of administration. Maximum level of glucose was witnessed in Group IIb on 28th day,indicating direct influence of amaranth on glucose metabolism .

- **Cholesterol (mg/dl)**

Group I (control):	7-days	14-days	21-days	28-days
M-1	133.25	125.35	128.15	133.62
M-2	127.45	129.61S	137.39	138.61
M-3	125.64	130.55	130.9	127.44
M-4	135.49	137.12	135.61	129.25
M-5	124.75	133.2	129.64	135.76
Mean	129.32	131.17	132.28	132.86
SD	4.78	4.37	4.03	4.47
SE	2.14	1.95	1.80	2.00

Table 11

- **GroupIIa (1200 mg/kg b.wt.)**

	7- days	14 - days	21-days	28- days
M-1	134.13	121.74	129.37	141.62
M-2	129.45	125.45	133.98	129.46
M-3	138.37	119.39	137.14	138.17
M-4	129.32	131.12	118.45	132.09
M-5	135.12	125.29	135.09	135.29
Mean	133.28	124.60	130.81	135.33
SD	3.88	4.45	7.47	4.81
SE	1.74	1.99	3.34	2.15

Table 12

- **Group IIb (2400mg/kg b.wt.):**

	7-days	14-days	21-days	28-days
M-1	129.45	135.55	134.42	129.43
M-2	129.05	130.06	149.22	135.09
M-3	133.24	132.64	131.45	128.43
M-4	132.11	129.88	125.82	125.75
M-5	119.45	137.13	130.39	127.61
Mean	128.66	133.05	134.26	129.26
SD	5.44	3.24	8.92	3.53
SE	2.43	1.45	3.99	1.58

Table 13

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- **T.Test:**

Group I-IIa	7 days	14 days	21 days	28 days
7 days	0.3	0.08	0.79	0.11
14 days		0.008	0.94	0.31
21 days			0.74	0.50
28 days				0.50

Table 14

Group I-IIb	7 days	14 days	21 days	28 days
7 days	0.79	0.27	0.38	0.98
14 days		0.53	0.58	0.57
21 days			0.60	0.17
28 days				0.06

Table 15

GroupIIa-IIb	7 days	14 days	21 days	28 days
7 days	0.21	0.87	0.84	0.20
14 days		0.04	0.11	0.17
21 days			0.42	0.12
28 days				0.12

Table 16

Overall, a slight increase in level of cholesterol was apparent, despite insignificant alterations. In Group IIa level of cholesterol was noted as 133.28±1.74, 124.60±1.99, 130.81±3.34, and 135.33±2.15 mg/dl on days 7, 14, 21, and 28, respectively. Likewise in Group IIb, the above were noted as 128.66±2.43, 133.05±1.45, 134.26±3.99, and129.26±1.58 mg/dl on respective days of investigation.

- **Total Protein (g/dl):**

Group I (Control)	7 days	14 days	21 days	28 days
M-1	4.34	4.55	4.09	4.4
M-2	4.57	4.81	4.13	4.39
M-3	4.21	4.52	4.35	4.75
M-4	4.1	4.88	4.01	4.5
M-5	4.64	4.23	4.34	4.81
Mean	4.37	4.60	4.18	4.57
SD	0.23	0.26	0.15	0.20
SE	0.10	0.12	0.07	0.09

Table 17

- **Group IIa (1200 mg/kg b.wt) :**

	7 days	14 days	21 days	28 days
M-1	5.34	5.39	4.99	5.43
M-2	5.78	6.01	5.38	4.69
M-3	5.81	5.34	5.02	5.64
M-4	5.89	5.91	4.88	5.21
M-5	5.55	5.23	5.55	5.09
Mean	5.67	5.58	5.16	5.21
SD	0.23	0.36	0.29	0.36
SE	0.10	0.16	0.13	0.16

Table 18

- **Group IIb (2400 mg/kg bwt.)**

	7 days	14 days	21 days	28 days
M-1	6.09	5.09	5.34	5.29
M-2	5.59	5.48	4.71	4.41
M-3	5.68	5.92	5.89	4.56
M-4	5.92	5.32	5.47	5.66
M-5	5.53	5.01	5.25	5.29
Mean	5.76	5.36	5.33	5.04
SD	0.24	0.36	0.43	0.53
SE	0.10	0.16	0.19	0.24

Table 19

- **T.Test**

Group I-IIa	7- days	14- days	21-days	28-days
7-days	0.0015	0.004	0.00004	0.024
14-days		0.00014	0.056	0.052
21-days			0.0008	0.013
28-days				0.013

Table 20

Group I-IIb	7-days	14-days	21-days	28-days
7-days	0.0017	0.0117	0.02	0.080
14-days		0.010	0.040	0.175
21-days			0.003	0.035
28-days				0.136

Table 21

Group IIa-IIb	7-days	14-days	21-days	28-days
7-days	0.63	0.064	0.169	0.0867
14-days		0.369	0.47	0.151
21-days			0.58	0.550
28-dayss				0.550

Table 22

Both groups ofanimals administered with amaranth indicated highly significant increase in the level of total protein. In Group IIa concentration of protein was evaluated as 5.67±0.10, 5.58±0.16,5.16±0.13, and 5.21±0.16 g/dl following 7th, 14th, 21st, and 28th day of administration.Similarly, Group IIb showed 5.76±0.11, 5.36±0.16, 5.33±0.19, and 5.04±0.24 g/dl onrespective investigated days (Table 3.17 and Figure 3.18). Strangely, effect of higher dose ofamaranth (Group IIb) was nominal comparing to concentration of protein in Group IIa.

V. DISCUSSION

Colours communicate through visual language. Think for instance flowers of variouscolours have unique capability to preferentially select its pollinator (Fenster et al., 2004; Rosas-Guerrero et al., 2014). Can you just by looking at food, taste the flavour, examine the freshness, judge whether you would eat it or not? Without any doubt, most of us would have some insight on the importance of colours in food. Sharp appealing colours fascinate people without any particular significance. Previous studies claimed that colour is the first cue that encourages oneto make choices while selecting food. Interestingly, colours in/of food have shown noassociation with taste and flavour (Zampini et al. 2007).

A study by Oo et al. (2019**)** reported that use of artificial coloursbeyond local FDA regulation in Union Territory of Myanmar was extremely prevalent.Although there are regulations at place in many countries but these measures are hardlyfunctional at ground level. Indian regulation on uses of food colours though work effectivelyon big brands, local vendors for candyfloss, sugar toys, and bakery are beyond reach of theseregulations. According to a study by Dixit et al. (2011) use of non-permitted colours is most prevalent in locally sold candies and sugar toys.

The present study observed a slightly higher LD50 for amaranth comparing to azorubine. Results showed that oral dose of 6000 mg/kg of amaranth was able to kill half ofthe animals. Thus, according to Teke and Kuete (2014**)** this dose was categorised as slightly toxic and practically nontoxic and both categories. According data published by Inchem (inchem.org) LD50 of amaranth was higher than 10 g/kg bodyweight. This indicated at least 40% lower LD50. It is interesting to note that both azorubine and amaranth showed similar pattern in percentage low in LD50, clearly this

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indicates deviation in factors affecting overall impact of amaranth.

Interestingly, decline in weight gain in animals administered with amaranth was more aggressive comparing to azorubine. Decline in weight gain was prompted immediately following 7 days of administration. Previous study revealed that at low dose amaranth had no effect on body weight (Hashem et al., 2010). Hashem et al. (2010) used doses as low as 50-200 mg/kg. However, studies that used higher doses (>1000 mg/kg) of amaranth indicated adversities in body weight and vital organ weights (Clode et al., 1987). There is not much information available on the effect of amaranth on body weight, nonetheless, since LD50 of amaranth was higher than azorubine (6 g/kg body weight) the amount of dye administered daily (1/4LD50 and 1/2LD50) to animals was also high. Which may have reduced appetite significantly causing steep decline in weight gain. Interestingly, box plot of weight change in animalsindicated that both dyes were extremely aggressive in high-dose groups. Indicating direct role of administration of food colours on body weight.

Glucose in blood comes from diet, blood carries glucose to cells as it is the main source of energy. Glucose is stored as glycogen in the body. According to previous studies uptake of glucose may vary under influence of ingested chemical compounds (Sakurai et al., 2004). The present study indicated significant increase in animals administered with azorubine and amaranth. There was a clear increase in the level of blood glucose as the days of administrationm progressed for both dyes. Maximum increase in blood glucose was observed in animals administered with amaranth. It seems somehow azo dyes improve glucose metabolism. There are chemical with oestrogenic properties that can increase cellular energy expenditure pushing cells to demand for mote glucose (Cederroth et al., 2007; Cederroth et al., 2008). It appeared that azorubine and amaranth could modulate glucose homeostasis in animals in a dose and time dependent pattern. This observation was further confirmed by decline in gain of body weight in those animals administered with azo dyes. We already showed earlier that there was decline in weight gain according dose and time of administration of amaranth.

Cholesterol serves as a precursor to steroids and vitamins. More importantly, it is the major constituent of cellular membrane (Hercberg et al., 2004). In the present study cholesterolwas measured in both serum and testicular tissues. Results were distinctly different in bothinvestigated parameters. Interestingly, this study observed significant decline in concentrationof cholesterol in serum, more specifically in animals administered with azorubine. However,no significant change was observed in those animals treated with amaranth.

This study also evaluated cholesterol in liver tissue of animals exposed to investigated azo dyes. It is important to note that most cholesterol absorbed from food end up in liver tissues.

Many human and animal studies have found that cholesterol in plasma can influence risk of cardiovascular disease (Krehl, 1977). Since plasma cholesterol is mostly contributed from diet,,any increase in serum cholesterol could be due to high cholesterol diet or high absorption ofcholesterol. This study noted significant increase in the level of cholesterol in liver tissues ofanimals administered with azo dyes during all investigated days. Administration of 1/4LD50dose of azorubine was an exception, although there was increase observed on individual daysbut it was not statistically significant. It reflects that azo dyes might increase absorption ofcholesterol from daily diet, as no atherogenic diet was given to the animals. Amin et al. (2010)reported remarkable increase in the level of total cholesterol, triglycerides, and low-densitylipoprotein in azorubine administered rats. One of the earlier study by Koch et al. (1929)recorded higher absorption of cholesterol and ergosterol in animals administered with azo dyes.

It was hypothesized that increase in plasma cholesterol of animals administered with azo dyes occur due to increase in absorption of dietary cholesterol, thus, azo dyes promote absorption of cholesterol and possibly other lipids.

Protein was estimated in serum and testicular tissues. This study noted significant difference between level of protein in serum and testicular tissue. In serum concentration of protein was significant high for both dyes, at least for the maximum dose. Whereas, in testicular tissues level of protein was not inadequately altered. It appeared to have declined the totalconcentration of protein in animals exposed to 1/2LD50 dose groups of azorubin and amaranth.

Any alteration in concentration of protein indicates underlying characteristics related to treatment (Ponten et al., 2009). A study by Wolfe (2017) reported that in absence of adequate dietary protein tissues undergo synthesis of endogenous protein. It appeared that due to administration of high doses of synthetic dyes animals must have lost good amount of diet, resulting into insufficient protein intake. Which was reflected in testicular tissues, on the other hand increase in serum protein could be due to endogenous synthesis of protein to accommodate cellular deprivation of protein.

VI. CONCLUSION

Food without colours is not appealing to many, due to this particular reason food colours have special importance in the food markets. It has a booming business around the globe with an expected value of USD 4288 million in year 2021 and projected to reach USD 5387 million by 2026. The business market is itself so large that it can have impact on small countries whole GDP. Let think it this way corrupt government may tweak the rules to allow adulteration at large scale. Consumers must be vigilant about what they are eating. There are increasing incidences of bladder cancer, intestinal and colon cancer, which has been associated with azo dyes. Based on rigorous studies around the globe selected number of food colourants have been allowed by the authorities. There are recommendations and prescription of accepted daily doses. But can that be assurance enough for consumers that it will not affect their health in a long

term. When drug is examined, it examined in a solo, controlled, and with a fixed protocol. Would that be the real-time experiment, would that replicate the real scenario where mixed-up toxicants are exposed simultaneously with unregistered, and unregulated pattern.

REFERENCES

[1.] Aberoumand , A.,(2011). A review article on edible pigments properties and sources as natural biocolorants in foodstuff and food industry. *World Journal of Dairy & Food Sciences. 6 (1):1-78.*

[2.] Amin, K.A, Abdel Hameid, H. (2010). 2nd, Abd Elsttar AH. Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. *Food and Chemical Toxicological* 48(10): 2994-9.

[3.] Bachalla, N.(2016). Identification of synthetic food colors adulteration by paper chromatography and spectrophotometric methods. IAIM. ;3(6):182–191.

[4.] Brudzyńska, P., Sionkowska, A., Grisel, M.(2021) Plant-Derived Colorants for Food, Cosmetic andTextile Industries: A Review. *Materials (Basel).;* 14(13):3484.

[5.] Cederroth, C.R., Vinciguerra, M., Kuhne, F., Madani, R., Doerge, D.R., Visser, T.J, et al. (2007).: Aphytoestrogen-rich diet increases energy expenditure and decreases adiposity in mice. *Environ.Health Perspect* 115: 1467–1473.

[6.] Cederroth, C.R., Vinciguerra, M., Gjinovci, A., Kuhne, F., Klein, M., Cederroth, M., et al. (2008):Dietary phytoestrogens activate AMP-activated protein kinase with improvement in lipid and glucose metabolism. Diabetes. *Environ.Health Perspect* 57: 1176–1185.

[7.] Clode, S.A., Hooson, J., Grant, D., Butler, W.H. (1987). Long-term toxicity study of amaranth in ratsusing animals exposed in utero. *Food Chemical Toxicological,* 25(12): 937-46.

[8.] Clydesdale ,F.M. (1991):Color perception and food quality. *Journal Food Qual.* ;14:61–74.

[9.] Clydesdale, F.M.(1984). The influence of colour on sensory perception and food choices. In: walford J, editor. Developments in food colours–2. London, UK: *Elsevier Appliedscience; . p. 75–112.*

[10.] Clydesdale, F.M.,(1993). Color as a factor in food choice. *Critical Review Food Science Nutrition.* 33:83–101.

[11.] Delwiche, J.F.(2012). You eat with your eyes first. *Physiol Behaviour.* ;107:502–4.

[12.] Demirağ K, Uysal V. Renklendiriciler (2006). In: Altuğ T, editor. Gıda katkı maddeleri. Meta Basım, İzmir; . p. 169-191.

[13.] Dixit, S., Purshottam, S..K, Khanna, S.K., Das, M. (2011). Usage pattern of synthetic food colours in different states of India and exposure assessment through commodities preferentially consumed by children. Food Addit Contam Part A *Chem Anal Control Expo Risk Assess. 2*8(8): 996-1005.

[14.] Elliot, A.J., Maier, M.A.,(2014). Color psychology: effects of perceiving color on psychological functioning in humans. *Annual Review Psychological.* ;65:95-120.

[15.] Elliot, A.J,.(2015). Color and psychological functioning: a review of theoretical and empirical work.*Front Psychological.* 6:368.

[16.] FDA. Food and Drug Administration, & US Department of Health and Human Services.(2010). Food ingredients and colors. International Food Information Council (IFIC) and US Food and Drug Administration.

[17.] Fenster, C.B., Armbruster, W.S., Wilson, P., Dudash, M.R., Thomson, J.D. (2004). Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics.* 35: 375–403.

[18.] Hall, R.L.,(1958). Flavor study approaches at McCormick and Company, Inc. In: In AD Little, Inc, editor. Flavor research and food acceptance: A survey of thescope of flavor and associated research, compiled from papers presented in a series of symposia given in 1956–1957. *New York, NY: Reinhold*,p. 224–240.

[19.] Hashem ,M.M., Atta, A.H., Arbid, M.S.., Nada, S.A., Asaad ,G.F., (2010). Immunological studies on Amaranth, Sunset Yellow and Curcumin as food colouring agents in albino rats. *Food Chemical Toxicological*. 48(6): 1581-6.

[20.] Hercberg, S., Galan, P., Preziosi, P., (2004). The SU. VI. MAX Study: a randomized, placebo controlled trial of the health effects of antioxidant vitamins and minerals. *Archial International Medicine.* 164:2335–2342.

[21.] Jimenez-Gonzalez, O., Ruiz-Espinosa, H., Luna-Guevara, J.J., Ochoa-Velasco, C.E., Luna, Vital, D., Luna-Guevara, M.L.(2018). A potential natural coloring agent with antioxidant properties: Microencapsulates of Renealmia alpinia (Rottb.) Maas fruit pericarp, *National Food Science Journal*, Volume 13, Pages 1-9.

[22.] Kanig, J.L.(1955). Mental impact of colors in foods studied. *Food Field Reporter*;23:57.

[23.] Koch, E.M., Koch, F.C., Lemon, H.B. (1929). Absorption spectra studies on cholesterol andergosterol. *Journal of Biological Chemistry.* 85(1): 159-167.

[24.] Kostyla, A.S., Clydesdale ,F.M.(1978). The psychophysical relationships between color and flavor. CRC Critic *Rev Food Science Nutrition* ;10:303–19.

[25.] Krehl, W.A. (1977). The nutritional epidemiology of cardiovascular disease. *Annual National Y Academic Science* 300: 335–359.

[26.] Martins, N., Roriz, C.L, Morales, P., Barros, L., Ferreiral, ICFR(2016). Food colorants: challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices. *Trend in Food Science*. 1-72.

[27.] Mentzel ,S.V., Schucker, L., Hagemann, N., Strauss, B.(2017).Emotionality of Colors: An Implicit Link between Red and Dominance. *Front Psychological* ;8:317.

[28.] Nath, P..P, Sarkar, K., Tarafder, P., Mondal, M., Das, K., Paul, G.(2015) . Practice of using metanil yellow as food colour to process food in unorganized sector of

west bengal-a case study. *International Food Research Journal;22(4):*1424–1428.
[29.] Oo, N., Saw, Y.M., Aye, HNN., Aung, Z.Z., Kyaw, H.N., Tun, A.M., (2019). Consumption of foods containing prohibited artificial colors among middle-school children in Nay Pyi Taw union territory, Myanmar. BMC *Public Health*. 19(1): 344.
[30.] Pandey ,R.M., Upadhyay, S.K..(2012). Food additive. *InTechnical İndia*. ;5:1-31.
[31.] Pontén ,F., Gry, M., Fagerberg, L., Lundberg, E., Asplund, A., Berglund, L, et al. (2009). A global view of protein expression in human cells, tissues, and organs. *Molecular Systamatic Biological*. *5*: 337.
[32.] Purba, M.K., Agrawal, N., Shukla, S.K.(2015). Detection of nonpermitted food colors in edibles. *Journal of Forensic Research. ;*S4.
[33.] Reutter, M.,(2004). Food displays, food colors affect how much people eat, *researcher concludes.*
[34.] Rosas-Guerrero, V., Aguilar, R., Mart en-Rodr ıguez, S., Ashworth, L., Lopezaraiza- Mikel, M.,Bastida, JM., et al. (2014). A quantitative review of pollination syndromes: do floral traits predict effective pollinators? Ecology Letters 17: 388–400.
[35.] Rowe, K.S., Rowe, K.J.,(1994). Synthetic food coloring and behavior: a dose response effect in a double-blind, placebo controlled, repeated-measures study. *e Journal of Pediatrics*;125(5):691–698.
[36.] Sigurdson, G.T., Tang, P., Giusti, M.M.(2017). Natural colorants: food colorants from natural sources. Annual Review Food Science Technological ;8:261–280.
[37.] Shinde, A., Shinde, M.(2013). Adulteration of Food by Synthetic Colours and Safe Natural Colours.Search And Research ;1(1):21–23.
[38.] Spence ,C., Smith, B., Auvray, M.(2015). Confusing tastes and flavours. In: Stokes D, Matthen M, Biggs S, editors. Perception and its modalities. Oxford: *Oxford University Press*; . p. 247–74.
[39.] Teke, G.N., Kuete, V. (2014). 5 - Acute and Subacute Toxicities of African Medicinal Plants, Toxicological Survey of African Medicinal Plants, Elsevier. pp 63-98.
[40.] Tuormaa, T.E.(1994). The adverse effects of food additives on health: a review of the literature with a special emphasis on childhood hyperactivity. *Journal of Orthomolecular Medicine*;9(4):225–243.
[41.] Watson ,E.(2014). We eat with our eyes: flavor perception strongly influenced by food color.
[42.] Wolfe, R.R (2017): Branched-chain amino acids and muscle protein synthesis in humans: myth or reality? Journal International *Soc Sports Nutrition* 14: 30.
[43.] Zampin,i M.,, Sanabria D., Phillips, N., Spence, C. (2007): The multisensory perception of flavor: assessing the influence of color cues on flavor discrimination responses. *Food Qual Prefer*. 18: 975–84.
[44.] Zahra, N., Alim-un-Nisa, Z.F, Kalim, I., Saeed ,K.(2015). Identification of synthetic food dyes in beverages by thin layer chromatography. Pakistan Journal of Food Sciences ;25(4): 178–181.