

Original Article

## Derangement of metabolic homeostasis, detoxifying ability and CA 15-3 in young adult female rats by fructose (15%) drinking is akin to known carcinogens: A missed fiend?

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### ABSTRACT

**Objectives:** Breast cancer (BC) has been the bane of peri- and post-menopausal women, but is now increasingly incident in adolescent/young adult (AYA) females. *Pari-passu*, there has been a >1000% increase in consumption of fructose as a caloric sweetener in soft drinks, whose top consumers are AYAs. The link between fructose consumption and mammary gland (MG) carcinogenesis is not well-established and the same is investigated and compared against known carcinogens such as 7,12-dimethylbenz(a)anthracene (DMBA) and electromagnetic radiations from mobile phone (EMF-MP).

**Materials and Methods:** Weaned female Wistar rats were randomly grouped as normal control (NOR), fructose control (FRC), DMBA control (DMC), and exposure control (EXC). For 8 weeks, the NOR was provided chow and water, *ad libitum*, while FRC, DMC, and EXC additionally received 15% fructose drinking solution, *ad libitum*, DMBA (20 mg/Kg, p.o; at weaning) and EMF-MP (Global System for Mobile Communications [GSM]), 2 h/day daily, respectively. At the end of the study, the groups were compared for the biomarkers of insulin resistance (IR), carbohydrate and lipid metabolism, liver function, cardiometabolic function, oxidative stress, and MG carcinogenesis.

**Results:** The serum markers of MG carcinogenesis (CA 15-3), IR (homeostasis model assessment-IR, area under the curve – oral glucose tolerance test), and liver and cardiometabolic function (serum glutamic-oxaloacetic transaminase and homocysteine) were significantly raised ( $P < 0.05$ ) in FRC versus NOR. The metabolic homeostasis (leptin, ghrelin, triglyceride-Glucose index, glucose-6-phosphatase, triglyceride, high-density lipoprotein, serum glutamic pyruvic transaminase, alkaline phosphatase, and glycogen) and detoxifying ability (free-radical scavenging activity [%] and superoxide dismutase) in FRC were not different from DMC, or EXC.

**Conclusion:** The development of early indicators of MG carcinogenesis due to unhindered fructose drinking by AYA female rats is akin to exposure to DMBA or EMF-MPs that evidence the propensity of the former.

**Keywords:** Mammary gland carcinogenesis, Adolescent/young adults, Fructose, 7,12-dimethylbenz(a)anthracene, Electromagnetic field from a mobile phone, Insulin resistance

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## INTRODUCTION

Globally, breast cancer (BC) is the most frequently diagnosed cancer and a common cause of cancer-related death. It is no longer restricted to the peri- and post-menopausal age group, and in fact, 7% of the BC are seen in women who are adolescent/young adults (AYAs) under 40 years.<sup>[1,2]</sup>

The 7,12-dimethylbenz(a)anthracene (DMBA), a polyaromatic hydrocarbon, is tumour initiator (IARC group I carcinogen) and a risk factor for BC among women, and adolescents.<sup>[3,4]</sup> Exposure to electromagnetic field radiations from mobile phones (EMF-MP) has also been implicated in the pathogenesis of BC.<sup>[5]</sup> *Pari-passu*, there has been a >1000% increase in consumption of fructose as a caloric sweetener in soft drinks, whose top consumers are AYAs.<sup>[6,7]</sup>

Recently, preliminary reports indicate that a fructose-laden diet could be a risk factor, for triple-negative BC in AYAs, although concrete evidence is lacking.<sup>[8]</sup>

It is well-understood that impaired metabolism and insulin resistance (IR) are central to carcinogenesis, especially BC. Pre-clinical and clinical studies have implicated exposure to DMBA and EMF-MP with the pathogenesis of IR and metabolic derangements as demonstrated by impaired secretory actions of pancreatic beta cells, hepatic insulin signalling, triglyceride (TG) metabolism, *de novo* lipogenesis, hepatic inflammation, visceral adiposity, type-II-diabetes mellitus, fatty liver, and obesity.<sup>[9-11]</sup> Furthermore, obesity is known to promote DMBA-mediated mammary gland (MG) carcinogenesis in premenopausal women.<sup>[12]</sup>

Although fructose-rich diet is clearly correlated with the pathogenesis of IR, its role in MG carcinogenesis has neither been considered nor investigated. Natural fructose in fruits and honey has been consumed by humans since time immemorial. Alarming, in the last five decades, the mean fructose intake per person has surged by more than 32% in the guise of confectionaries, colas, beverages, juices, candies, pastries, etc.<sup>[13]</sup> On the one hand, the AYAs are the compulsive consumers of a fructose-rich diet, while, on the other hand, they are facing an increased incidence of BC.<sup>[14]</sup> A systematic investigation is warranted to understand the correlation between fructose consumption the MG carcinogenesis in AYAs, if any.

To address the paucity of data, the present study was designed, wherein, weaned female Wistar rats were challenged with either fructose (15% as drinking solution), EMF-MP (GSM, 2 h/day × 8 weeks), or DMBA (20 mg/Kg p.o. single dose at weaning) and assessed for paradigms of carbohydrate and lipid metabolism and MG carcinogenesis. This pioneering pre-clinical study highlights that the markers of MG carcinogenesis in AYA female rats are skewed due to a fructose-rich diet akin to known carcinogens such as DMBA or EMF-MP, and paves the way for future clinical studies.

## MATERIALS AND METHODS

### Animals

Wistar albino rats (female, 28 days old, 45–55 g) were housed in a standard pathogen-free environment at an in-house Animal Facility. The study was approved by the Institutional Animal Ethics Committee (IAEC/2018-I/Prot.No. 02 dated October 29, 2018) on and conducted in compliance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals.

### Experimental design

The rats were allocated to four groups ( $n = 6$ ), that is, normal control (NOR), fructose control (FRC), DMBA control (DMC) and exposure control (EXC) using the method of minimisation based on sex, weight and age. The NOR was provided a standard pellet diet (RRD Enterprises, Pune) and filtered drinking water, *ad libitum*, whereas, FRC additionally received fructose (15%), *ad libitum*. In addition to standard conditions, DMC received DMBA (20 mg/Kg in sesame oil, p.o, single dose at weaning) and EXC was exposed to EMF-MP (GSM, 2 h/day, for 8 weeks).

### Exposure to EMF radiations from mobile phone

The exposure chamber consisted of a ventilated (2 mm diameter holes) plexiglas cage that could comfortably hold one animal at a time. On top of the chamber, a platform was tied to a pulley through string and its movement was controlled by a stepper motor which was programmed to undergo angular movement (45°). For the exposure duration (2 h/day × 8 weeks), an animal from the EXC group was placed in the chamber and the mobile phone (1760 MHz, 0.3988 mW/cm<sup>2</sup>, GSM, specific absorption rate 0.217 W/Kg) in 'switched-on mode' was placed on the platform that cyclically underwent angular motion.<sup>[15,16]</sup>

### Food, water/fructose solution (15%), body weight, and total calorie intake

Parameters such as food intake and water/fructose solution (15%) intake were recorded by weighing the leftover portion from the pre-weighed amount and body weight, which were recorded daily using the sensitive digital weighing balance over the period of 24 h. The total calorie (=Kcal metabolising energy from feed+Kcal metabolising energy from fructose solution (15%) intake) of each group over the study duration was calculated.<sup>[17]</sup>

### Fasting blood glucose (FBG)

Every week, following 12 h food but not water deprivation, the tail of the animals was pricked to measure the blood

glucose concentration using the digital glucometer (Accu Chek Active, Roche, USA).

### **Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)**

After study completion, the OGTT was conducted. The fasted rats (12 h) were force-fed glucose (2 g/kg, p.o.) and the blood glucose concentration was repeatedly recorded at 0, 30, 60, 90, 120, and 180 min post-administration.<sup>[18]</sup>

At the end of the study, the ITT was conducted by administering regular human insulin (0.75 IU/kg, s.c., Huminsulin R, Eli Lilly, and Company India Pvt., Ltd.) to 12 h fasted rats, as described elsewhere.<sup>[18]</sup>

### **Blood collection**

At the end of the study, the animals were anaesthetised (Ketamine hydrochloride 80 mg/kg, i.p.) and the blood was collected by cardiac puncture.

### **Visceral weight**

Still, under anaesthesia, the animals were perfused with phosphate-buffered saline (0.1 M, pH 7.4), and the viscera and MGs were excised, washed dried, weighed, and stored ( $-20^{\circ}\text{C}$ ).

### **Biochemical assessment**

#### ***Insulin, homeostatic model assessment- estimated insulin resistance (HOMA-IR) index, leptin, ghrelin, and homocysteine (Hcy)***

The serum levels of insulin, leptin, ghrelin, and Hcy were measured using the commercially available kit (Elabscience, USA), and the HOMA-IR was calculated ( $= \text{glucose level (mg/dL)}^{-1} \times \text{insulin}/405$ ).<sup>[17]</sup>

#### ***Tumour marker-CA 15-3***

The tumour marker CA 15-3 was measured in the serum using a solid-phase enzyme-linked immunosorbent (MyBioSource, USA) assay.

### **Lipid profile**

The lipid profile, that is, total cholesterol, TGs, and high-density lipoprotein (HDL) was measured using commercially available kits in accordance with the manufacturer's method (Diaspertz, Microlyn Healthcare Pvt. Ltd. India).

### **Hepatic enzymes**

The hepatic enzymes, that is, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase

(SGPT), and alkaline phosphatase (ALP) were measured using commercially available colorimetric kits in accordance with the manufacturer's method (Diaspertz, Microlyn Healthcare private Ltd. India).

### **Triglyceride-glucose index (TyG Index) measurements**

TyG index was calculated using the formula:  $\text{Ln (fasting TG [mg/dL]} \times \text{fasting serum glucose [mg/dL]}/2)$ . The index value of 4.49 or greater is suggestive of IR with a likely diagnosis of non-alcoholic fatty liver disease (NAFLD) and proportionally increased risk of BC in female rats.<sup>[19]</sup>

### **Glycogen and carbohydrate metabolism enzymes**

The hepatic glycogen was estimated as per the standard anthrone test.<sup>[20]</sup> The activities of the enzymes of the carbohydrate metabolism, that is hexokinase, phosphofructokinase (PFK), and glucose-6-phosphatase (G6Pase) were estimated in the MG using ultraviolet-spectrophotometric biochemical assays.<sup>[21-23]</sup>

### **Oxidative stress**

The sections of hepatic and MG were homogenised (10% w/v in potassium phosphate buffer, 0.1 M, pH 7.4) to estimate the concentration of the reduced glutathione.<sup>[24]</sup> The homogenate thus obtained was centrifuged at 15,000 rpm and the debris-free clear supernatant was used for the estimation of superoxide dismutase (SOD), catalase, and protein using a UV spectrophotometer.<sup>[24]</sup>

### **Histopathological analysis**

The MG from the right inguinal regions was formalin-fixed (10%), paraffin-embedded, sectioned (5 mm), and stained (H&E) for morphological evaluation. The glands were evaluated to analyse any neoplastic changes in histoarchitecture such as terminal duct lobule, fatty tissue (F), terminal ducts, ductal epithelial cells (DEC), and myoepithelial cells (MC) surrounding basement membrane (BM).

### **Statistical analysis**

All the values are expressed as mean $\pm$ SEM; ( $n = 6$ ), two-way analysis of variance (ANOVA) followed by Bonferroni post-test is applied for comparison between groups in case of physiological parameters, that is, body weight, water/fructose, and food intake over 8 weeks study. The biochemical data were analysed using one-way ANOVA followed by Dunnett's multiple comparison tests versus NOR and FRC.  $P \leq 0.05$  was considered statistically significant. The statistical analysis was done using GraphPad Prism *ver* 5.0.

## RESULTS

### Effect of fructose intake, DMBA, and EMF-MP on, food, water/fructose intake body weight, and total calorie intake

The food, water/fructose intake, and body weight were measured daily and the weekly average was calculated for analysis. The food intake by FRC was significantly less ( $P < 0.001$ ) than NOR, DMC, and EXC. The body weight of FRC was significantly lower ( $P < 0.05$ ) than NOR, DMC, and EXC in the 3<sup>rd</sup> and 4<sup>th</sup> week, but increased thereafter. The FRC derived about 24% of its total calories by drinking fructose and 62% from the standard chow diet [Figure 1].

### Effect of fructose intake, DMBA, and EMF-MP on FBG, OGTT, and ITT

The weekly FBG of DMC was  $83.83 \pm 4.19$ ,  $95.50 \pm 2.14$ ,  $79.17 \pm 6.34$ , and  $88.00 \pm 2.96$  mg/dL, during 2<sup>nd</sup>, the 3<sup>rd</sup> and 4<sup>th</sup> week of the study, respectively, significantly higher than NOR, FRC and EXC [Figure 2a].

In the OGTT, the mean blood glucose level at T = 30 min, of DMC and EXC was  $137 \pm 3.64$  and  $142.25 \pm 6.80$  mg/dL, respectively, that was significantly ( $P < 0.05$ ) higher than NOR and FRC [Figure 2b]. The OGTT area under the curve

(AUC) of FRC, DMC, and EXC was significantly greater ( $P < 0.001$ ) than that of NOR [Figure 2c].

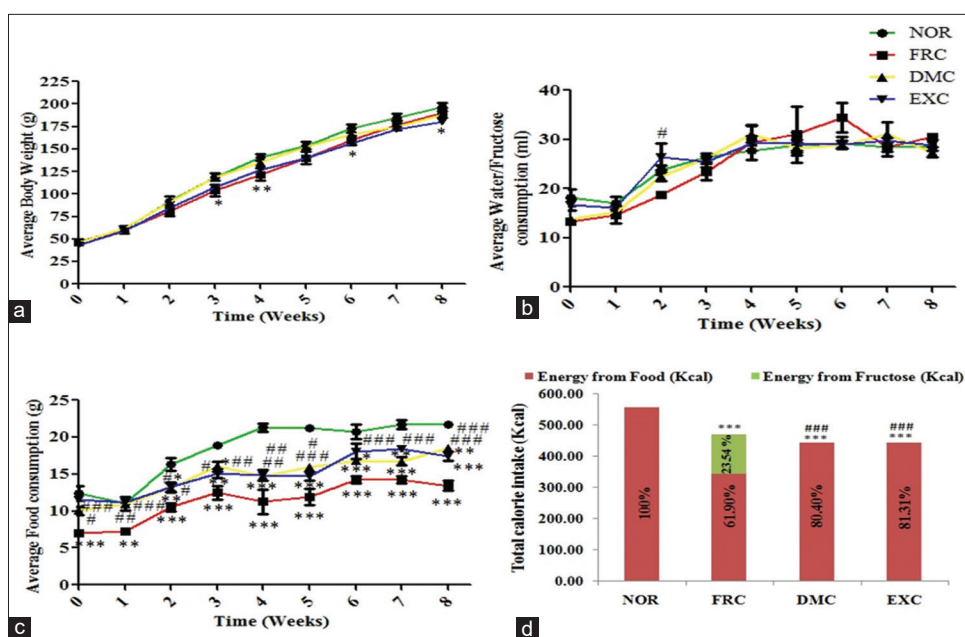
In ITT, the mean blood glucose level at T = 0 min, of DMC was  $31.67 \pm 2.16$  mg/dL that was significantly lower ( $P < 0.05$ ) than NOR, FRC, and EXC [Figure 2d]. The AUC-ITT of FRC was significantly lower ( $P < 0.05$ ) than that of NOR, DMC, and EXC [Figure 2e].

### Effect of fructose intake, DMBA, and EMF-MP on biochemical parameters

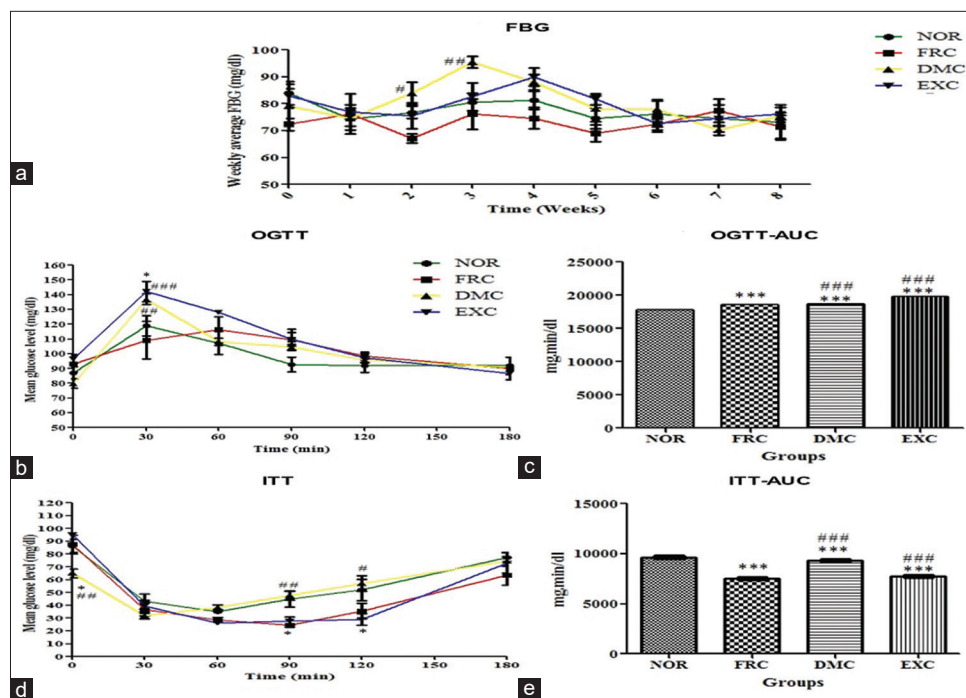
At the end of the study, the FRC serum levels of insulin were raised. The values of HOMA-IR, Hcy, and SGOT were significantly higher in FRC versus NOR ( $P < 0.05$ ). The SGPT, ALP, leptin, ghrelin, CA 15-3, and TG were raised and the serum HDL levels were significantly less ( $P < 0.001$ ) in FRC versus NOR [Table 1].

### Effect of fructose intake, DMBA, and EMF-MP on TyG index

At the end of the study, the calculated TyG Index in NOR, FRC, DMC, and EXC was  $4.117 \pm 0.164$ ,  $4.740 \pm 0.149$ ,  $4.693 \pm 0.111$  and  $4.550 \pm 0.203$ , respectively.



**Figure 1:** Physiological parameters (a) body weight, (b) water/fructose intake, (c) food intake, and (d) calorie intake. The average body weight and food intake were significantly less in FRC versus NOR, DMC, and EXC and fructose consumption increased from the 5<sup>th</sup> week onwards. Values are expressed as mean $\pm$ SEM, ( $n = 6$ ), analysed using two-way repeated measures analysis of variance (RM ANOVA) followed by Bonferroni post-test to compare replicate means by row. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  versus NOR, # $P < 0.05$ , ## $P < 0.01$  and ### $P < 0.001$  versus FRC was considered as significant. NOR: Normal control, FRC: Fructose control, DMC: 7,12-dimethylbenz(a)anthracene (DMBA) control, EXC: Exposure control, SEM: Standard error of the mean, RM ANOVA: Repeated measures analysis of variance, \*: Sample size.



**Figure 2:** Blood glucose levels (a) FBG, (b) OGTT, (c) OGTT-AUC, (d) ITT and (e) ITT-AUC. The AUC-OGTT was higher in FRC, DMC, and EXC groups vs NOR and *vice versa* in the case of AUC-ITT. Values are expressed as mean±SEM, ( $n^* = 6$ ) where  $*P < 0.05$ ,  $**P < 0.01$  and  $***P < 0.001$  versus NOR,  $^{\#}P < 0.05$ ,  $^{\#\#}P < 0.01$  and  $^{\#\#\#}P < 0.001$  versus FRC, was considered significant. AUC was calculated using XY analysis and time interval comparison was analysed using two-way repeated measures analysis of variance (RM ANOVA). FBG: Fasting blood glucose, OGTT: Oral glucose tolerance test, ITT: Insulin tolerance test, AUC: Area under the curve, NOR: Normal control, FRC: Fructose control, DMC: 7,12-dimethylbenz(a)anthracene (DMBA) control, EXC: Exposure control, SEM: Standard error of the mean, RM ANOVA: Repeated measures analysis of variance, \*:sample size.

### Effect of fructose intake, DMBA, and EMF-MP on tissue glycogen and carbohydrate metabolism enzymes

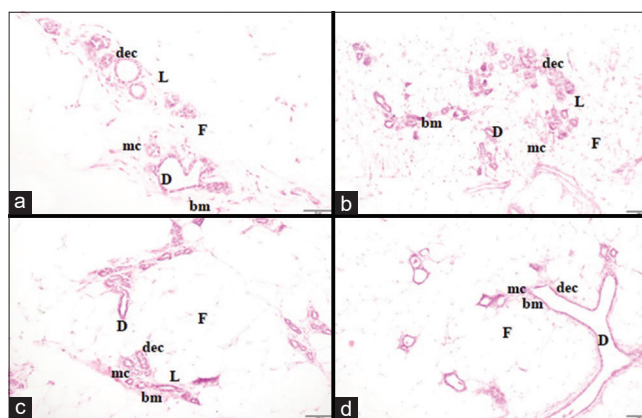
The hepatic glycogen and G6Pase activity in the MG of DMC was significantly greater ( $P < 0.05$ ) versus NOR [Table 1]. The PFK activity in FRC was not different from DMC and EXC.

### Effect of fructose intake, DMBA, and EMF-MP on oxidative stress

The levels of SOD and % FRS were significantly less ( $P < 0.05$ ) in FRC versus NOR [Table 2].

### Effect of fructose intake, DMBA and EMF-MP on histopathology of MG

The histoarchitecture of sections of MG from NOR, FRC, DMC and EXC showed normal lobular structure (L), and ducts (D) lined by DEC and surrounded by MC in BM and F. In FRC, the sections of MGs were in the lobular stage while that of EXC appeared to be in the ductal stage as compared to the alveolar bud stage in NOR. There was no evidence of



**Figure 3:** Histopathology (a) NOR, (b) FRC, (c) DMC, and (d) EXC group. The mammary tissue of (a) NOR (alveolar bud stage), (b) FRC (lobular stage), (c) DMC (alveolar bud stage), and (d) EXC (ductal stage) group shows the normal lobular structure (L), ducts (D) and fatty tissue (F) with no evidence of neoplasm ( $\times 10$ ). NOR: Normal control, FRC: Fructose control, DMC: DMBA control, EXC: Exposure control, DEC: Ductal epithelial cells, MC: myoepithelial cells.

neoplasm in the histopathology of either NOR, FRC, DMC or EXC ( $\times 10$ ) [Figure 3].

**Table 1:** Changes in biochemical parameters in serum and tissue sample of animals of NOR, FRC, DMC and EXC group.

| Parameters   | NOR         | FRC            | DMC                     | EXC                     |
|--|-------------|----------------|-------------------------|-------------------------|
| Insulin (ng/mL)                                      | 0.68±0.004  | 0.70±0.004     | 0.60±0.01****           | 0.61±0.004****          |
| HOMA-IR  | 0.12±0.01   | 0.14±0.01*     | 0.11±0.002 <sup>#</sup> | 0.12±0.001 <sup>#</sup> |
| LEP (ng/mL)  | 2.50±0.08   | 2.92±0.12      | 2.10±0.12 <sup>#</sup>  | 2.31±0.30               |
| GHR (pg/mL)  | 0.33±0.02   | 0.40±0.01      | 0.47±0.03*              | 0.40±0.04               |
| Hcy (pg/mL)  | 0.86±0.01   | 0.92±0.01*     | 1.05±0.02****           | 0.95±0.02**             |
| CA 15-3 U/mL   | 1.21±0.05   | 1.48±0.10      | 0.64±0.32               | 0.22±0.02 <sup>#</sup>  |
| TC (mg/dL)   | 134.51±6.78 | 122.70±5.52    | 86.40±2.42****          | 72.60±7.48****          |
| TG (mg/dL)   | 64.75±13.34 | 182.85±61.00   | 168.07±34.28            | 139.70±59.36            |
| HDL (mg/dL)  | 26.42±0.82  | 3.96±0.93***   | 11.18±1.04****          | 5.97±0.40***            |
| SGOT (IU/L)  | 94.30±29.47 | 218.05±21.25** | 200.37±15.60*           | 194.48±17.70*           |
| SGPT (IU/L)  | 64.83±11.80 | 88.40±10.21    | 94.30±15.60             | 94.30±5.90              |
| ALP (IU/L)   | 73.44±9.80  | 98.46±16.17    | 130.02±12.13            | 139.81±24.54*           |
| Glycogen (ng/mg)                                     | 0.98±0.01   | 1.14±0.02      | 0.92±0.08 <sup>#</sup>  | 1.01±0.02               |
| HK (µmol of NADH formed/min/mg protein)              | 0.28±0.05   | 0.25±0.04      | 0.25±0.02               | 0.23±0.01               |
| PFK (µmol of NADH formed/min/mg protein)             | 1.44±0.14   | 1.40±0.11      | 1.42±0.11               | 1.60±0.26               |
| G-6Pase (µmol of phosphate liberated/min/mg protein) | 1.60±0.26   | 2.64±0.45      | 4.22±0.67*              | 3.55±0.54               |

Values are expressed as mean±standard error mean (SEM), (*n*\*=3), \**P*<0.05, \*\**P*<0.01 and \*\*\**P*<0.001 versus NOR, <sup>#</sup>*P*<0.05, <sup>#</sup>*P*<0.01 and <sup>#</sup>*P*<0.001 versus FRC, analysed using One-way RM ANOVA followed by Dunnett's multiple comparison test. Glycogen values are expressed as glucose equivalents, NOR: Normal control, DMC: DMBA control, FRC: Fructose control, EXC: Exposure control, LEP: Leptin, GHR: Ghrelin, Hcy: Homocysteine, CA: Cancer antigen, TC: Total cholesterol, TG: Triglyceride, HDL: High-density lipoprotein SGOT: Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase, ALP: Alkaline phosphatase, HK: Hexokinase, PFK: Phosphofructokinase, HOMA-IR: Homeostatic model assessment-estimated insulin resistance index, NADH: Nicotinamide adenine dinucleotide reduced, G-6Pase: Glucose 6-phosphatase, \*sample size, RM ANOVA: Repeated measures analysis of variance

**Table 2:** Changes in oxidative stress parameters in NOR, FRC, DMC, and EXC groups.

| Parameters  | Liver        |              |               |                           | Mammary gland |              |               |                           |
|---|--------------|--------------|---------------|---------------------------|---------------|--------------|---------------|---------------------------|
|   | NOR          | FRC          | DMC           | EXC                       | NOR           | FRC          | DMC           | EXC                       |
| Tissue weight (g)   | 8.30±0.70    | 7.33±0.28    | 5.47±0.34**** | 5.97±0.33**               | 2.15±0.11     | 1.55±0.03**  | 1.90±0.16     | 1.41±0.18**               |
| GSH (µmol GSH/g liver tissue)                                   | 306.03±65.76 | 222.03±5.66  | 325.60±59.38  | 428.17±25.41 <sup>#</sup> | 423.37±60.20  | 354.90±7.81  | 478.05±75.60  | 564.17±19.91 <sup>#</sup> |
| CAT(mM of H <sub>2</sub> O <sub>2</sub> degraded/mg of protein) | 33.31±8.40   | 18.94±7.12   | 40.85±24.78   | 40.87±10.31               | 165.10±1.92   | 143.90±6.98  | 130.15±2.62** | 113.51±7.90****           |
| SOD (IU/mg protein)   | 8.80±0.37    | 4.93±0.75*   | 6.50±0.98     | 5.65±0.58*                | 162.10±33.50  | 62.77±17.10* | 66.57±4.70*   | 40.56±9.63**              |
| %Free radical scavenging  | 72.90±1.96   | 39.55±3.44** | 53.67±7.35*   | 48.60±3.96*               | 38.33±6.67    | 23.33±4.41   | 23.33±3.33    | 13.33±3.33*               |

Values are expressed as mean±standard error mean (SEM), (*n*\*=3), \**P*<0.05, \*\**P*<0.01 and \*\*\**P*<0.001 versus NOR, <sup>#</sup>*P*<0.05, <sup>#</sup>*P*<0.01 and <sup>#</sup>*P*<0.001 versus FRC, analysed using one-way RM ANOVA followed by Dunnett's Multiple comparison test. NOR: Normal control, FRC: Fructose control, DMC: DMBA control, EXC: Exposure control, GSH: Glutathione reduced, CAT: Catalase, SOD: Superoxide dismutase, H<sub>2</sub>O<sub>2</sub>: Hydrogen peroxide, \*sample size, RM ANOVA: Repeated measures analysis of variance

## DISCUSSION

The present-day AYAs are oblivious to the menace posed by the unrestricted consumption of fructose-based diet on their health and the present study evidences that its deleterious effects are no less than those posed by either DMBA or EMF-MP. To dispel the paucity of substantive correlative data, the present study simulated the condition in developing female rats and compared the propensity of ingesting unlimited fructose (15%)

to derange the mechanisms involved in the MG carcinogenesis and its risk stratification against DMBA and EMF-MP.

Here, we report that ingesting unlimited fructose (15%) during adolescence had a greater propensity than pubertal exposure to either DMBA or EMF-MP to raise circulating levels of CA15-3, leptin, ghrelin, Hcy, and insulin. On the other hand, the food intake, calorie intake, body weight, glucose tolerance, and insulin sensitivity were reduced by

ingesting fructose (15%) akin to DMBA and EMF-MP. Further, the raised activities of G6Pase, glycogen destocking, and hypertriglyceridemia proportionately reduce the HDL levels and perturb the liver function. The development of the state of IR was confirmed by raised indices of HOMA-IR in FRC and TyG in all three FRC, DMC, and EXC.

The present study indicates that when the weanlings were provided unrestricted access to fructose (15%) during adolescence, they underwent a metabolic reprogramming toward IR and MG carcinogenesis, by early adulthood. The unrestricted intake of fructose (15%) solution by FRC significantly reduced ( $P < 0.001$ ) preference for conventional chow diet to 62%, but the total calories consumed were significantly higher ( $P < 0.001$ ) Kcal than that consumed by DMBA or EMF-MPs. As compared to DMBA and EMF-MPs, the HOMA-IR and Hcy of FRC were significantly higher ( $P < 0.05$ ). The impaired glucose tolerance in FRC, DMBA and EMF-MPs underlies IR as the common pathway for initiating MG carcinogenesis, irrespective of the causative insult. Golbahar *et al.* studied the association between Hcy levels and IR in an *in vivo* study and concluded that hyperhomocysteinaemia induces IR.<sup>[25]</sup> Kallamadi *et al.* concluded in their study that impaired glucose tolerance or pre-diabetes increase the risk of BC development.<sup>[26]</sup> According to similar findings by Emam *et al.*, higher levels of CA 15-3 with fructose (51%) consumption clearly associate the role of this natural sweetener in commencing metabolic events favouring the development of mammary carcinogenesis.<sup>[27]</sup> Hypertriglyceridemia and high liver enzymes (SGOT and SGPT) have been correlated with NAFLD and IR, which are in consonance with present findings.<sup>[28]</sup> In comparison to NOR, the DMBA, EMF-MPs, and FRC had high TyG indices. As previously established, the indices of IR, such as the TyG index, are closely associated with the risk of MG carcinogenesis in young females.<sup>[29]</sup>

Further, the fructose-drinking inflicted prominent oxidative damage generated ROS, and retarded the activities of antioxidant enzymes present in the liver and MG. It may be postulated that the metabolic reprogramming entailed insulin insufficiency, triggered incomplete  $\beta$  oxidation in mitochondria, and generated ROS that may have initiated the pathogenesis of BC.<sup>[30]</sup> Lewis *et al.* have reported that women with IR may have slightly elevated levels of circulating estradiol, which may raise their chance of developing BC. IR, on the other hand, heightens oxidative stress in breast tissue and may encourage carcinogenesis through oxidative DNA damage.<sup>[31]</sup> Studies are ongoing in our laboratory to further corroborate the underlying pathogenesis.

EMF-MP and DMBA are known genotoxic stressors that have also been reported to cause metabolic reprogramming, perturbations in health markers, and MG carcinogenesis but their propensity increases exponentially when the animals are

repeatedly exposed at mature age for chronic duration.<sup>[32-34]</sup> In sharp contrast, the evidence presented, here, indicates that the ingestion of fructose (15%) during pre-, peri- and post-puberty, derange homeostasis and initiates disease pathogenesis by early adulthood.

For the 1<sup>st</sup> time, this study pins the role of neuropeptidergic signal-leptin as an early, direct, and independent beacon that can be developed to call out potential cases of MG carcinogenesis. It is an adipocytokine, secreted by mammary epithelial tissue and positively associated with changes in body weight. Considerably higher levels of leptin and ghrelin but significantly lower ( $P < 0.05$ ) body weight until puberty in FRC need to be highlighted. The role of hyperinsulinaemia and hyperleptinaemia in MG carcinogenesis is being accepted globally.<sup>[35-37]</sup>

The study investigates the early metabolic events initiated in AYAs by known carcinogens such as DMBA and EMF-MP for a better understanding of the disease initiation. Interestingly, IR emerges as a common pathway towards MG carcinogenesis, and incessant consumption of fructose (15%) induced a tougher onslaught on metabolic milieu (TyG) than either DMBA or EMF-MP.

The study is the first of its type and shows how the IR and metabolic derangement caused by fructose (15%) administration increases the risk of MG carcinogenesis in AYAs. The findings, further, highlight the importance of profiling IR while screening BC in AYAs.

## CONCLUSION

This study provides evidence that fructose (15%) drinking by adolescent female rats-initiated insulin and leptin resistance, raised lipolysis, glycogen levels, gluconeogenesis, oxidative stress, TyG, and CA15-3 by early adulthood akin to DMBA and EMF-MP.

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## Declaration of patient consent

The authors certify that they have obtained all appropriate consent.

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## Conflicts of interest

There are no conflicts of interest.

## Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The author(s) confirms that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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