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RESEARCH ARTICLE
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OVARIAN DAMAGE CAUSED BY HIGH FRUCTOSE CORN SYRUP AND ITS MITIGATIVE EFFECCT WITH ALPHA LIPOIC ACID IN ALBINO MICE *(MUS MUSCULUS)*.

Hira Ashfaq[1], Asma Rashid Khan[2], Muhammad Farhan Nasir[3]*, Farah Yasmin[4], Alia Hussain[5], Tazeen Hidayat[6], Zeeshan Haider[7]

[1,4,5,7]Institute of Molecular biology and biotechnology, University of Lahore, Pakistan.
[1]Email: hiraashaq@yahoo.com, [4]Email: bafshal@gmail.com, [5]Email: aliahussain7770@gmail.com, [7]Email: zeehbhattimsc@gmail.com
[2]Govt. Graduate College for Women, Township Lahore Punjab, Pakistan.
Email: asmarashidalikhan@gmail.com,
[3]*Department of Zoology, Division of Science & Technology, University of Education Lahore, 54770 Punjab Pakistan Email: farhan.nasir@ue.edu.pk
[6]Gov't Girls Degree College, Pishin, Balochistan, Pakistan. Email: tazeenhidayat21@gmail.com

***Corresponding Author:** Muhammad Farhan Nasir
*Department of Zoology, Division of Science & Technology, University of Education Lahore, 54770 Punjab Pakistan Email: farhan.nasir@ue.edu.pk

Abstract:
Present study was based upon the toxic role of High Fructose Corn syrup (HFCS) on the female reproductive organ as well as it focused on the ameliorative role of Alpha lipoid acid (ALA), which ultimately minimizes the toxicity. Biochemical analysis was performed by measuring the levels of Luteinizing- hormone (LH), follicle-stimulating hormone (FSH), Malondialdehyde (MDA) and glutathione (GSH) in blood. Moreover, histopathological damage due to this chemical was also evaluated. There was a significant increase in body weight, reproductive and non-reproductive organ weight, Luteinizing hormone, follicle-stimulating hormone and Malondialdehyde (MDA) levels in dose group CS I in comparison with Control group. While GSH activities were reduced in the dose group CSI, when compared with the control- group. Administration of ALA significantly decreased ($p < 0.001$) body weight, reproductive and non reproductive organ weight, Luteinizing hormone, follicle-stimulating hormone and Malondialdehyde (MDA) levels in the dose group CSII, while GSH value increased in dose group CSII group in comparison with CSI Group. Histological studies showed following defects in the ovaries of dose group CS I mice i.e. oocytes with a loss of contact within the granulosa cells, denatured primordial follicles, Cysts development, degraded oocyte and a lower number of follicles and disarrangements of Tunica albuginea and ovarian surface epithelium. While treatment with ALA reduced the ovarian damage by improving the histopathological changes caused by HFCS. Our results showed that HFCS caused ovarian damages, while ALA may be used as a pharmacological agent against ovarian toxicity induced by HFCS. Key words: High Fructose Corn syrup (HFCS), Alpha lipoid acid (ALA).

INTRODUCTION

Sugar free foods are popular because of their low caloric content. As a result, the food industry uses lower calorie artificial sweeteners instead of sugars**.** The artificial sweeteners can break down into

toxic chemical byproducts. U.S. Food and Drug Administration has approved different artificial sweeteners, such as High Fructose Corn Syrup (HFCS) (Chattopadhyay *et al.*, 2014). High fructose corn syrup(HFCS) is an important artificial sweetener, which is used in the food industry, such as caloric beverages, soft drinks and colas and in different bakery products. Due to its lower price and easier handling it is a better choice for beverage and baking companies, canned food, jam and jelly, candy and dairy products (Sun, *et al.*, 2013; Sinir *et al.*, 2013). HFCS is made up of corn starch. Starch is a polymer which is made up of glucose molecules that are linked together. Firstly the corn starch is treated with alpha amylase and glucoamylase enzymes, by which the starch molecules are broken down into glucose. Glucose isomerase enzyme reversibly converts glucose in to fructose (Parker *et al.*, 2010) There are three types of high fructose corn syrup. HFCS 90 comprising 90% fructose & 10% glucose utilized in special applications but, more importantly in formation of HFCS 42 when, mixed with glucose syrups to make HFCS42 (58% glucose + 42% fructose) and HFCS55 which contains 55% fructose and 45% glucose (Koseler *et al.*, 2018) There was a positive correlation between weight gain and HFCS intake. It was reported that HFCS play an important role in the development of obesity and obesity is the major cause of metabolic disease, lipid metabolism, kidney stones, gout, cardiovascular disease and the risk of cancer (Meyers *et al.*, 2017; Sadowska *et al.*, 2019). HFCS also increases inflammatory biomarkers and oxidative stress (OS), risk of obesity and other problems such as high blood pressure. OS led to a number of reproductive diseases, such as polycystic ovarian syndrome, endometriosis, and infertility. OS also induces complications of pregnancy such as pregnancy loss and miscarriage (Ahmed *et al.*, 2016; Sohrabi *et al.*, 2019). The main drivers of the progression of metabolic syndrome (MS) is the increase in consumption of HFCS. Metabolic syndrome causes several risk factors, including insulin resistance (IR), obesity, hypertension, and dyslipidemia and hypogonadism, which can alter the reproductive functions and as a result leads towards infertility (Meydanli *et al.*, 2018). HFCS is usually associated with obesity and poor fertility. It induces estradiol, progesterone and altered luteinizing hormone (LH) before ovulation, changes in ovarian morphology and follicle arrest, morphology of germ cells, sperm count and serum hormones; testosterone, luteinizing hormone, follicle stimulating hormone (Volk *et al.*, 2017; Tkachenko *et al.*, 2020). Alpha lipoic acid (ALA) is an antioxidant. Utilizing its effects on free radical elimination, chelation of metal ions, increased levels of cytosolic glutathione and vitamin C (Polat et al., 2020). ALA is synthesized in the mitochondria of plants and animals in the presence of lipoic acid synthase enzyme. The involvement of ALA in oxidative metabolism is very important. A number of studies had shown that ALA has antioxidant, anti-inflammatory and blood sugar lowering properties and it is synthesized de novo in the body from fatty acids and a small amount of cysteine (Salehi *et al.*, 2020; Votano *et al.*, 2021). The ALA molecule exist in oxidized and reduced form. The oxidized form is called ALA, or simply lipoic acid, and the reduced form is called dihydrolipoic acid (DHLA). Both oxidized and reduced forms are antioxidants in the body (Karaarslan *et al.*, 2016). ALA exerted a significant protective effect on ischemia reperfusion injury by decreasing peroxidation of lipids and by regulating malondialdehyde (MDA) levels in a rat. The efficiency of ALA in protecting against reperfusion injury makes it an attractive clinical applications product. ALA treatment also increases the total number of follicles (Cosar *et al.*, 2007; Karapinar *et al.*, 2017). ALA was reduced to dihydrolipoic acid (DHLA), which can neutralize active oxygen, chelate metal ions Fe2+, Cu2+ and Cd2+, and regenerate the body's own antioxidants, such as glutathione (GSH), Vitamin E and Promotes vitamin C, which was reported to inhibit the release of pro-inflammatory cytokines. ALA also increased the maturation speed of egg cells and the antioxidant status of the ovaries (Nair *et al.*, 2020). ALA is a natural antioxidant that plays an important role to increase the overall antioxidant capacity of follicles, reduce the level of ROS, prevent premature membrane rupture, and treat endometriosis. By improving histology, immunohistochemistry, hormones and oxidative stress markers, the treatment of rats with ALA had a positive effect on reducing ovarian damage (Ozel *et al.*, 2020).

STUDY DESIGN AND METHODOLOGY

A group of 30 female mice (Mus musculus) attained for the research from the Veterinary Research Institute, Lahore to observe the effect of High fructose corn syrup. These mice were placed under the well-managed and controlled conditions, e.g., 24hour in iron cages having size 24 inches long and 12 inches wide. Minerals, proteins and multivitamins rich feed called chick Feed No. 14 was given to mice. Animals were also administrated with clean water in glass bottles having capillary tubes fixed. Mice were divided into three groups. Control group was labeled as (C), while experimental group was divided into, experimental group I labeled as CS I, and experimental group II labeled as CS II.

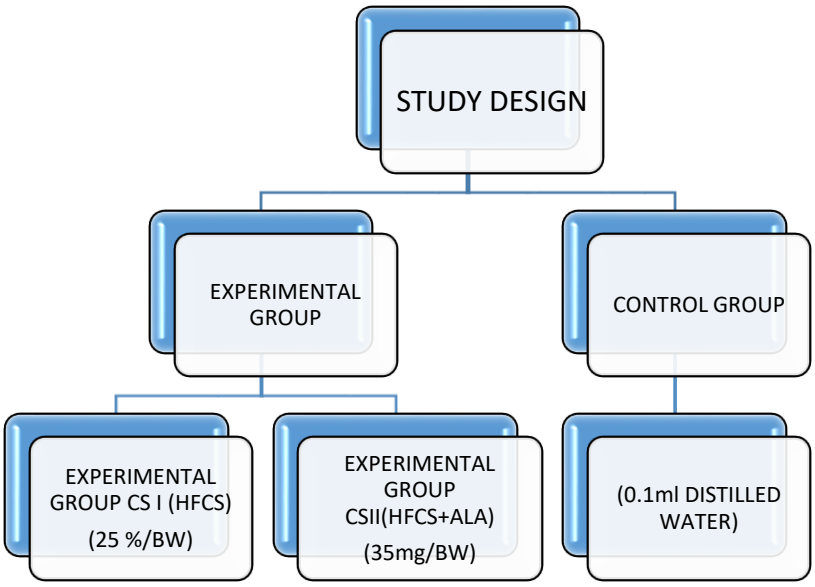

Fig 1.: Scheme representing the administration of different doses

HORMONAL ANALYSIS

The blood samples were collected from eyes of female mice after dose administration of 30 days in order to analyze the values of (FSH, LH) reproductive hormones, biomarkers of oxidative stress (MDA) and antioxidants, Glutathione.

1: Follicle stimulating hormone (FSH) and Luteinizing hormone (LH)

The FSH and LH level is measured by using RIA (Radioimmunoassay) method. ELISA kit was used to measure the level of hormones.

2: Glutathione-Peroxidase-5(G-Px5)

The glutathione was assessed by using Routine spectroscopic technique.

3: Malondealdehyde (MDA)

The MDA value was also determined using the strategy of Ohkawa *et al.*, (1979)

Morphological Evaluation

Under a binocular stereoscopic microscope, selected ovaries were observed. Morphological abnormalities were noticed under microscope. Then with the help of digital camera, these malformed ovaries were macro photographed.

Morphometric Evaluation

During the experimental period, the body weight of mice of all groups was measured by using digital weighing scale. Digital weighing scale was used to determine the weight of the ovaries, liver and kidneys, in the both control and dose groups. Morphometric abnormalities were observed in both groups.

HISTOLOGICAL EVALUATION

Ovaries were chemically treated for histological examination. During this examination, tissues were dehydrated in different concentration of alcohols. After that, the embedding tissues were cut at microtome. When the ribbon appeared perfectly clear, it was mounted to clean slides covered with Mayer glue, this glue helped to stick the ribbon to the slides. Harris-hematoxylin and Eosin stains were used. Selected histological slides were macro-photographed.

Statistical investigation

SPSS software, being one of the most suitable statistical data analysis technique, was used in the research. The analysis was carried out on the basis of the values acquired through computer base. Independent Sample T- Test was used for data analysis. Probability level of $p < 0.001$ was reckoned significant.

RESULTS

Morphological Analysis

Control Group: The control group showed regular appearance of ovaries. The ovaries were round with smooth and shiny surface and are vascular, giving them pinkish appearance. The ovary showed a smooth pink-white surface. The capsular cortex of ovary were in normal shape.

Experimental Groups: After morphological examination of experimental groups different, deformities appeared.

I. **Dose group CSI (25 % HFCS):** Ovaries from the dose group CSI (25 % HFCS) were found to be irregular in shape. They showed malformations like, wrinkles and color change of ovaries. The capsular cortex gave the ovary nodular appearance. Moreover, fat deposition was also very clearly visible on the external surface of ovaries.

II. **Dose group CSII (25%HFCS +35mg/kg BW ALA):** On the contrary, ovaries recovered from dose group CSII (25%HFCS +35mg/kg BW ALA) showed comparatively normal shape, more or less like control group. Their surfaces were found to have smooth appearance. They showed no wrinkles on their surfaces, which were seen in dose group CSI (25 % HFCS). Nodular cortex was also not found. The fat bodies were also not visible and the ovarian surface epithelium was going to take on a typical appearance, when treated with ALA after the administration of 25% HFCS.

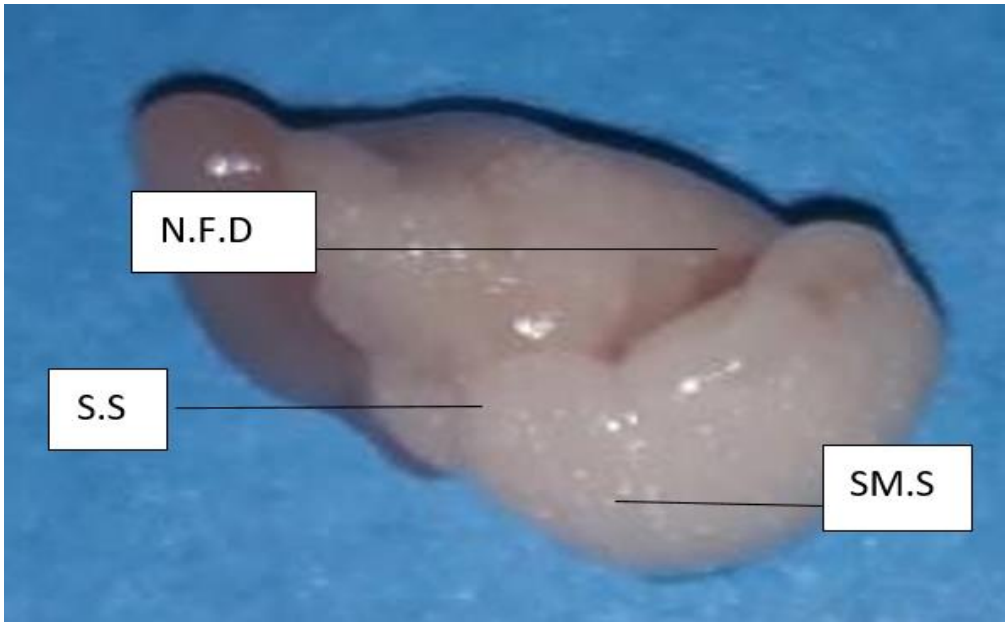

Figure No. 1.1: Macro-photograph of ovary of eight-week-old albino mice of Control Group. S.S: Shiny- surface SM.S: Smooth-surface N.F.D: No Fat Deposition.

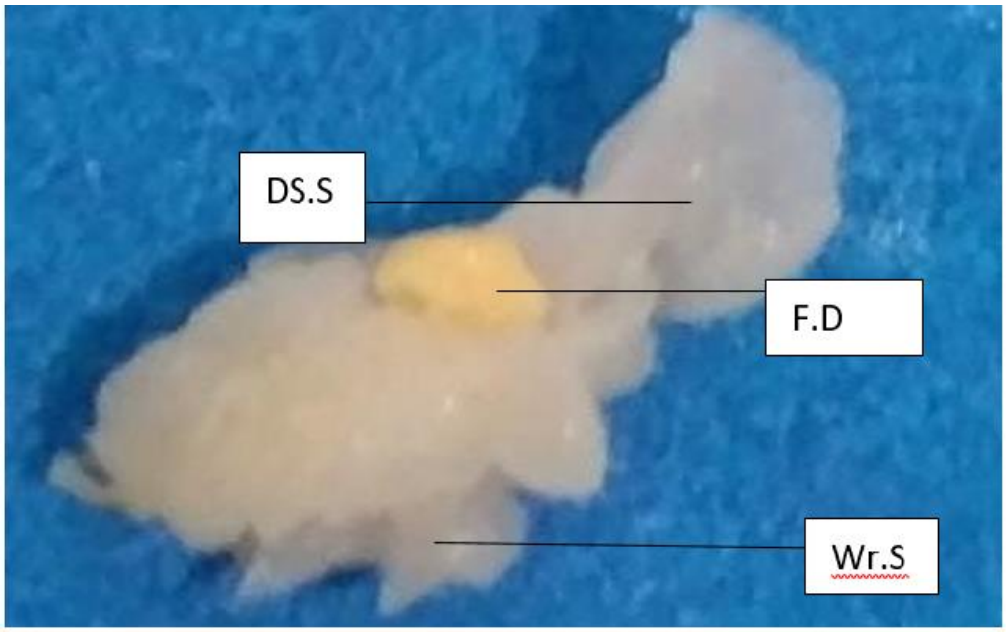

Figure No. 1.2: Macro-photograph of ovary of eight-week-old albino mice of Dose group CSI (25%HFCS). DS.S: Deshaped Structure Wr.S: wrinkled Surface F.D:Fat Deposition

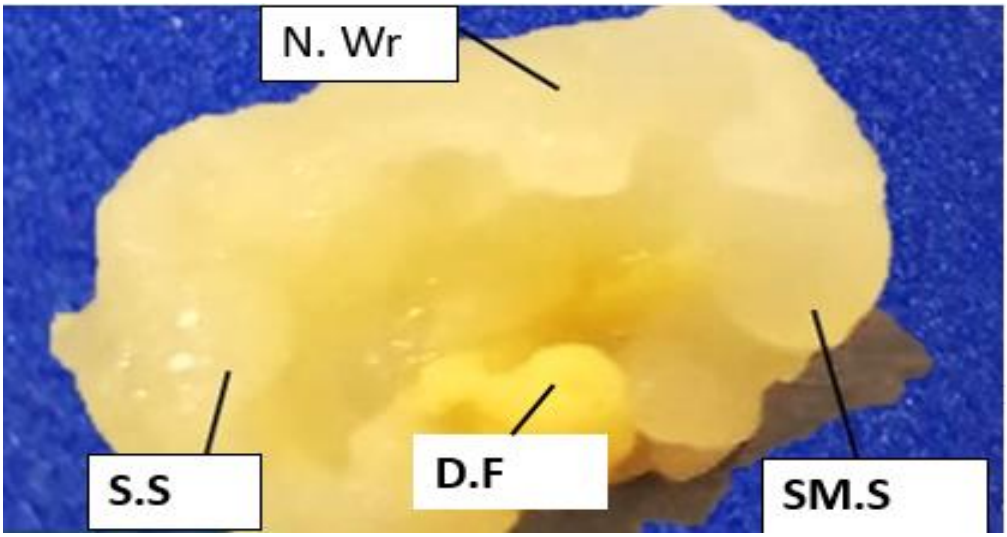

Figure No. 1.3: Macro-photograph of ovary eight-week-old albino mice of Dose group CSII (25%HFCS + 35mg/kg BW ALA).S.S: Shiny Surface SM.S: Smooth Surface D.F: Decreased Fat N.Wr No wrinkle surface

Morphometric analysis
Body weight Analysis
Control group
Mice of control group exhibited normal weight. The average weight (g ± SEM) of control group mice was 16.1±0.314. (Table 1)

Experimental Groups
The dose group CSI (25 % HFCS) showed an increase in body weight as compared to control group. (Control vs CS I: 16.1±0.314 vs 27.5 ± 1.13 g ± SEM). It was significantly ($p < 0.001$) greater than control mice. (Table no1). While dose group CS II (25%HFCS +35mg/kg BW ALA) showed decrease in body weight as compared to the dose group CSI (25 % HFCS) (CS I vs CS II: 27.5 ± 1.13 vs 18.9 ± 0.378 g ± SEM. (Table No 2)

Ovarian weight Analysis

Control group

Ovaries from Control group were subjected to morphometric analysis. The average weight (g ± SEM) of ovaries of control group was found to be 0.04±0.03. (Table No. 1)

Experimental Groups

Ovaries from Dose group CSI (25 % HFCS) were also analysed. Weight of ovaries of Dose group CSI (25 % HFCS) showed significant increase as compared to Control group (Control vs HFCS:0.04±0.003 vs 0.112 ± 0.002 g ± SEM) (Table No.1). Ovaries obtained from the Dose group CSII (25%HFCS +35mg/kg BW ALA) showed decline in average weight. It was found to be 0.09 ±0.002 g ±SEM. i.e significantly ($p < 0.001$) below than Dose group CSI (25 % HFCS). (CS I VS CS II: 0.112 ± 0.002 vs 0.09 ±0.002 g ± SEM). (Table No.2)

Table 1. Effect of HFCS on the body and ovarian weight of female albino mice. (g±SEM)

Weight (g)	Control group (Weight)	HFCS (Weight)
Body (g)	16.1± 0.314	27.5 ±1.13***
Ovary (g)	0.04 ± 0.003	0.112 ± 0.002***

Table .2. Effect of HFCS +ALA on the body and ovarian weight of female albino mice. (g ±SEM)

Weight (g)	HFCS (Weight)	HFCS + ALA (Weight)
Body (g)	27.5 ±1.13	18.9 ± 0.378***
Ovary (g)	0.112 ± 0.002	0.08 ± 0.002***

Reproductive hormonal Analysis

Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) level

Control group

The average LH level of control group was 7.44 ±0.010 (IU/L±SEM) and the average FSH level of Control group mice was 2.44 ±0.020 (IU/L±SEM). (Table No3).

Experimental Groups

Dose group CSI (25 % HFCS) showed significant increase ($p < 0.001$) in the level of LH and FSH as compared to Control group (Table No3). While Dose group CS II (25%HFCS +35mg/kg BW ALA) showed decline in the level of LH and FSH (Table No.4).

Biochemical analysis

Malondealdehyde (MDA) and Glutathione (GSH)

Control group

The value of MDA was found to be 0.8265±0.008. It was taken as normal in all control group mice. The average GSH level in the blood sample of control group was found to be 4.409±0.063 (mg/dl±SEM). (Table No.3).

Experimental Group

The level of MDA assessed from blood sample of Dose group CSI (HFCS 25%) was essentially ($p<0.001$) higher than Control group. While the typical degree of GSH assessed from blood sample of dose group CSI (HFCS 25%) was significantly ($p<0.001$) lower than control group esteem. (Table No.3). The level of of dose group CSII (25%HFCS +35mg/kg BW ALA) was significantly ($p<0.001$) below than dose group CSI esteem. Whereas, dose group CSII (25%HFCS +35mg/kg BW ALA) showed an increase in antioxidant level, i.e. GSH. (Table 4).

Table 3: Effect of HFCS on stress markers and Reproductive hormones in female albino mice.

Parameters	Control group	HFCS
MDA (µmol/ml)	0.8265 ± 0.008	0.9721 ±0.009***
GSH (mg/dl)	4.409 ± 0.063	3.549 ± 0.0268***
LH (IU/L)	7.44 ± 0.010	10.46 ± 0.040***
FSH (IU/L)	2.44 ± 0.020	3.59 ± 0.067***

Note: Asterisks show significant difference against*** ($p < 0.001$)

Table 4: Effect of HFCS +ALA on stress markers and Reproductive hormones in female albino mice.

Parameters	HFCS	HFCS+ALA
MDA (µmol/ml)	0.9721 ±0.009	0.8090 ±0.002***
GSH (mg/dl)	3.549 ± 0.0268	4.630 ± 0.039***
LH (IU/L)	10.46 ± 0.040	7.80 ± 0.068***
FSH (IU/L)	3.59 ± 0.067	2.70 ±0.036***

Note: Asterisks show significant difference against***($p < 0.001$)

Histological Analysis of Ovaries

Control group

During the histological examination, we observed that the cortex of ovary showed normal ovarian follicles. Follicles showed the presence of granulosa cells, zona pellucida and primary oocytes. Normal follicles were found to be, round shaped. Ovarian surface epithelium and theca follicular were in proper arrangements. (Fig No. 1.4, 1.5)

Experimental groups

In dose group CS I, histological abnormalities were observed. It revealed follicles with a muddled shape, oocytes with a loss of contact within the granulosa cells, and denatured primordial follicles. Cysts and a lower number of follicles were also seen in some areas. Around the ovary, fatty tissues were also apparent (Fig No. 1.6, 1.7)

In mitigation dose group, CSII sections appeared to be closer to the normal. Oocytes acquired normal shape. Number of follicles also showed a rise. Regeneration of granulosa cells, zona pellucida and primordial follicle were noted. Cystic development were absent in this group. (Fig No. 1.8, 1.9).

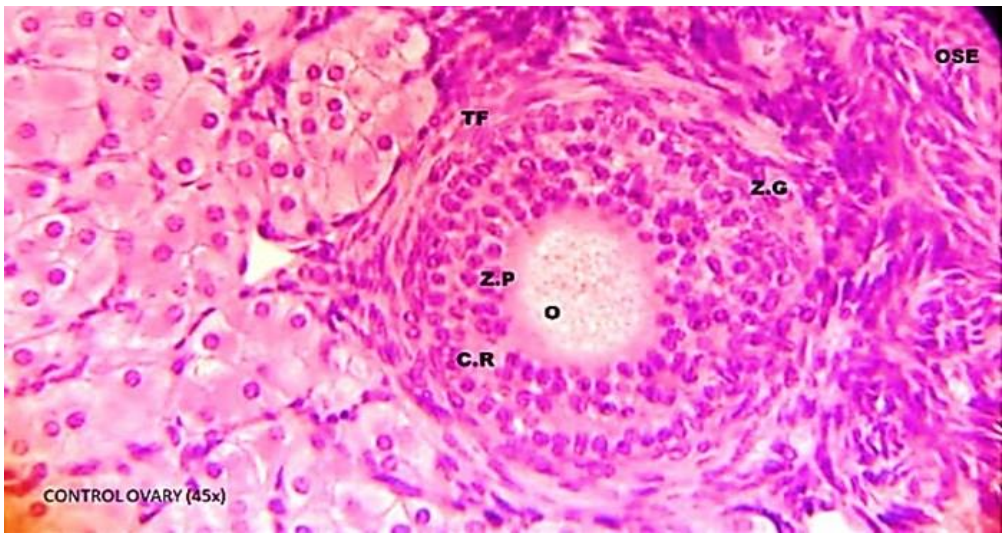

Figure No.1. 4. A Macro-photograph showing histology of Ovary of eight-week-old albino mice of Control-group (45X).

O: Oocyte **Z.P: Zona** pellucida **C.R: Corona** radiata **TF:** Theca folliculi **Z.G:** Zona granullosa **OSE:**Ovarian surface epithelium

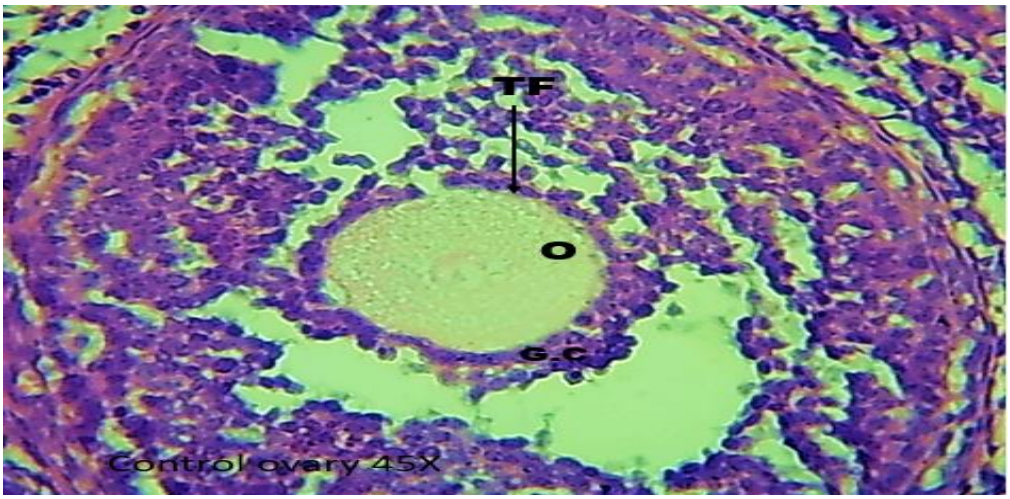

Figure No.1. 5. A Macro-photograph showing histology of Ovary of eight-week-old albino mice of Control-group (45X).
O: Oocyte **TF:** Theca folliculi **G.C:** Granulosa cells

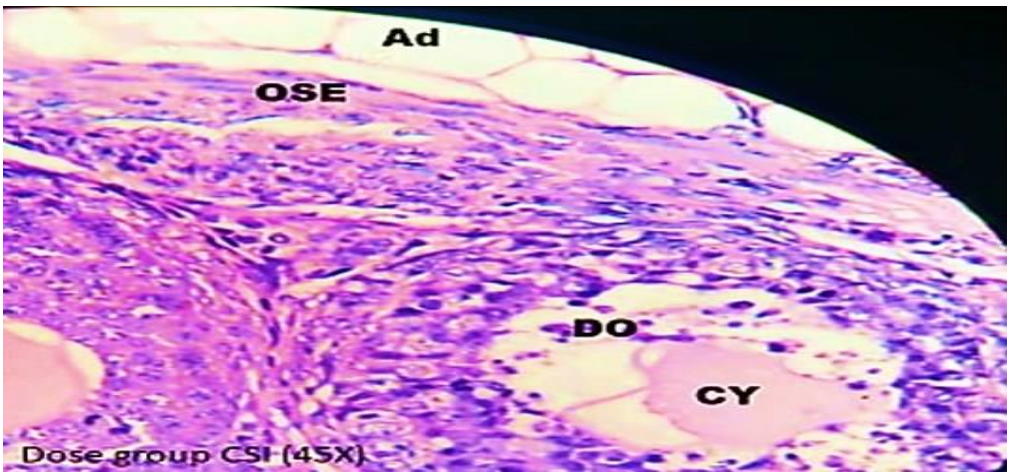

Figure No.1.6. A Macro-photograph showing histology of Ovary of eight-week-old albino mice of Dose- group CSI (25% HFCS).
OSE: Ovarian surface epithelium was not in proper arrangement. **CY:** Follicular cyst **Ad:** Adipose tissue **DO:** Degenerated oocytes due to cyst formation

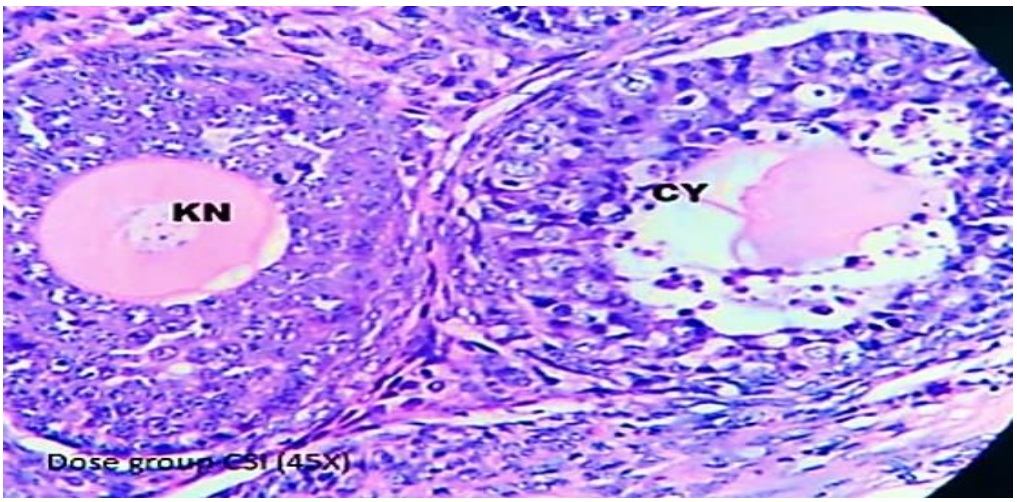

Figure No. 1.7. A Macro-photograph showing histology of Ovary of eight-week-old albino mice of Dose- group CSI (25% HFCS).
CY: Follicular cyst degraded oocyte **KN:** karyorrhexis of the nucleus of follicle cells

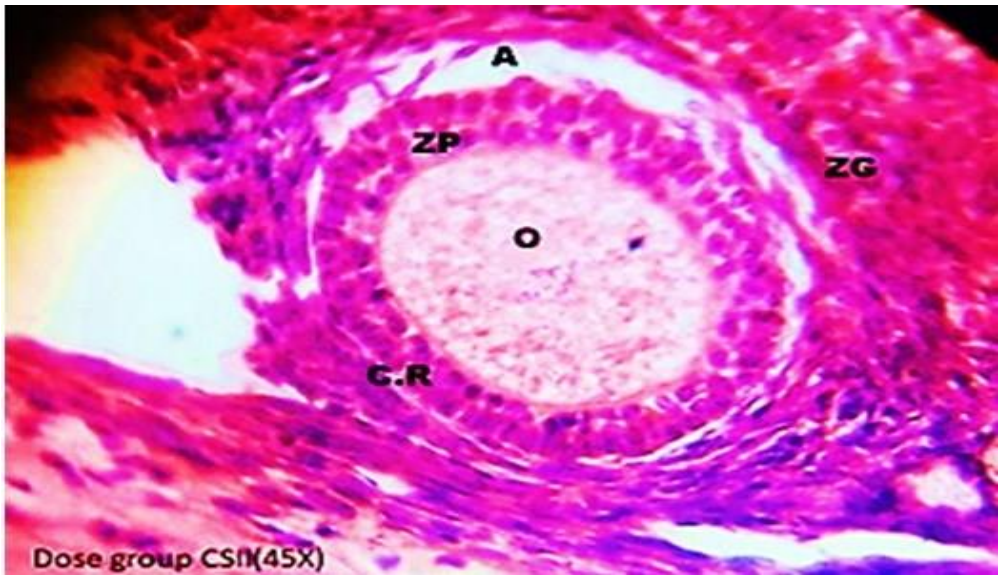

Figure No.1.8. A Macro-photograph showing histology of Ovary of eight-week-old albino mice of Dose- group CSII (25%HFCS +35mg/kg BW ALA).
O: Oocyte in proper shape **Z.P:** Zona pellucida clearly visible **C.R:** Corona radiata properly arranged **Z.G:** Zona granullosa recover its connection with oocyte **A:** Antrum

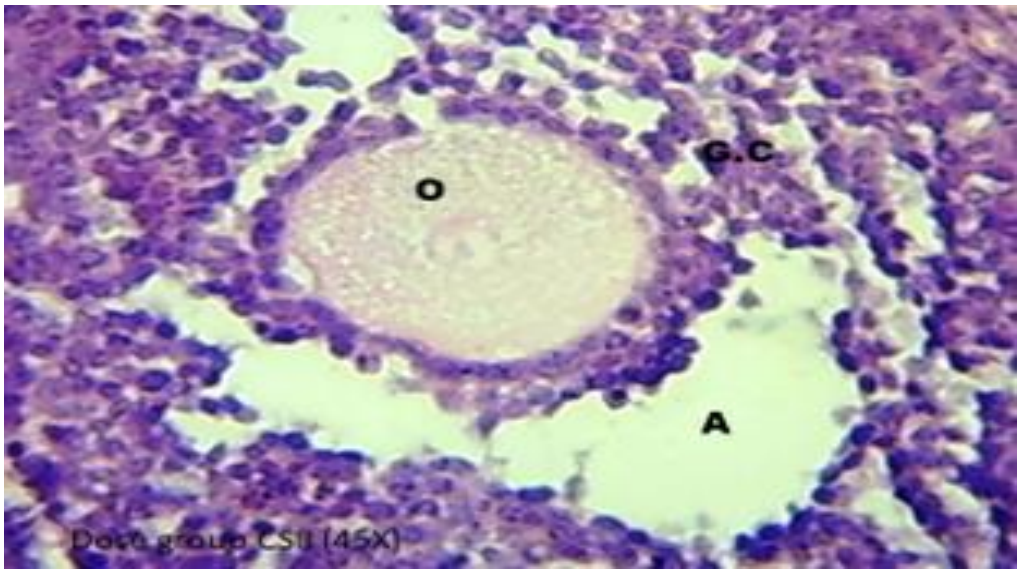

Figure No.1.9. A Macro-photograph showing histology of Ovary of eight-week-old albino mice of Dose- group CSII (25%HFCS +35mg/kg BW ALA).
O: Oocyte **G.C:** Granulosa cells **A:** Antrum

DISCUSSION

High fructose corn syrup has been used in almost all commercial foods, from bread to beverages. HFCS can contribute to the onset of obesity, which is the leading cause of reproductive dysfunction. Alpha-lipoic acid (ALA) is a sulfur containing antioxidant, which is synthesized by plants and animals. It is an effective micronutrient by having various organic and pharmacological properties. It interacts with destructive reactive-oxygen (RO) species and reactive-nitrogen (RN) species and results in stopping oxidative damage (Meyers *et al.,* 2017).

In present study, different morphological defects were observed in ovaries of dose group CS I (25%HFCS) after the administration of HFCS. Deformations observed were, wrinkled surface and increase in fat layer around ovary. (Fig No.1.1). Our results are supported by study, which clearly showed that high sugar diet caused cystic development that resulted with entirely altered ovarian

morphology or development of an abnormal reproductive phenotype (Volk *et al.,* 2017). In present study dose group CS II (25%HFCS +35mg/kg BW ALA) showed that extracted ovaries are found to be normal in the shape and appearance. Surface of ovaries appeared to be smooth and shiny to some extent and fat cells were not found, after administration of ALA. (Fig No.1.2). This is supported by previous study that, treatment with ALA in rats had a beneficial effect in reducing ovarian damage, improving histological, hormonal levels and markers of oxidative stress (Ozel, *et al.,* 2020).

Morphometric analysis in a recent study program indicates increase in the weight of body of mice of dose group CS I (25% HFCS). This increase in body weight was found to be highly significant ($p < 0.001$), when compared with the control group (Control vs CS I: 16.1±0.314 vs 27.5 ± 1.13 g ± SEM). (Table No.1). According to the previous, research the long-term administration of HFCS on the reproductive system both male and female rats, developed significant body weight gain than control groups. This increase was due to presence of fat, particularly in the abdomen as well as higher levels of circulating triglycerides. These findings, when applied to people, show that high-fructose corn syrup consumption may contribute to the onset of obesity being the major cause of reproductive dysfunction. Polycystic ovaries (PCO) are most commonly caused by belly obesity in women (Bocarsly, Powell, Avena and Hoebel 2010).
The altered values of reproductive hormones as compared to control group. The level of LH and FSH increased in dose group CS I (25 % HFCS). The dose group CS I(25 % HFCS) showed significant increase ($p <0.001$) in the level of LH as compared to Control group, which was measured as 10.41 ± 0.040 IU/L. (Table No.3). Furthermore, the average FSH level was measured as 3.59 ± 0.067 IU/L that was significantly ($p <0.001$) greater than the control- group. (Table No.3). While in CSII (25%HFCS +35mg/kg BW ALA) group the values of reproductive hormones decreased significantly ($p <0.001$) when compared with dose group CS I (25 % HFCS). The level of LH was 7.80 ± 0.068 IU/. (Table No.4). FSH level showed significant decline. It was found to be 2.70 ±0.036 IU/L. (Table No.4). These outcome appeared to be normal. Increased FSH an LH level relative to control value causes a follicular damage. Previously no work has done on female reproductive hormonal changes caused by HFCS. Further researches will be helpful in this field for understanding the effect of HFCS on reproductive hormones. Furthermore, another study had shown that animals administrated with a high fat and high sugar diet had higher number of cystic follicles, when it was compared to control group animals. It is still undefined how the high fat and high sugar diet lead to ovarian dysfunction; however, it might be due to hyperinsulinemia, and increased production of ovarian estrorogens and androgens along with the luteinizing hormone (Roberts *et al* 2017). Our results that ALA decreased the reproductive hormones, are supported through the previous study, which indicates that through the administration of ALA, the level of FSH and LH were significantly decreased (Nasir *et al.,* 2022). Recent research indicates that the level of serum glutathione (GSH) and Malondealdehyde (MDA) changes in female mice because of administration of HFCS. The average level of MDA in dose group CSI (25%HFCS) was measured as 0.9721 ± 0.009 µmol/ml, which was significantly ($p<0.001$) greater than control- group mice. (Table 3). While dose group CSII (25%HFCS +35mg/kg BW ALA) showed decline in MDA level which was 0.8090 ± 0.002 µmol/ml, i.e significantly ($p<0.001$) below than dose group CSI esteem. (Table No. 4). These results are asserted by another research, which showed that the diet riched in high fructose was significantly related with an increase levels of plasma MDA and GSH. MDA was increased ($p<0.0001$) in those dose group which were fed with high fructose diet compared with controls mice, and ALA treatment decreased the levels of MDA. In contrast, in high fructose diet group glutathione levels were decreased ($p<0.0001$) when compared with controls and ALA treatment increased the levels of this parameter (Ozdogan *et al.,* 2012)..

During histological analysis, various abnormalities were observed. The dose group CS I (HFCS 25%) showed follicles with a muddled shape, oocytes with a loss of contact within the granulosa cells, and denatured primordial follicles. Cysts and a lower number of follicles were also seen. Around the ovary, fatty tissues were also apparent. Tunica albuginea, ovarian surface epithelium, and primordial

follicles were not arranged properly. (Fig No.1.6.1.7). While in dose group CSII (25%HFCS +35mg/kg BW ALA) sections appeared to be closer to the normal. Oocytes acquired normal shape. Number of follicles also showed a rise. Regeneration of granulosa cells, zona pellucida and primordial follicle were noted. Cystic development were absent in this group. Tunica albuginea, ovarian surface epithelium, and primordial follicles were arranged properly. (Fig No.1.8,1.09). This finding is supported by research that a diet enriched with high carbohydrate was main cause of increasing the degenerated follicles number in rats. Another study found that a high sugar diet (HSD) causes ovarian dysfunction in rats, which may be linked to polycystic ovary syndrome (PCOS) in women. HSD animals also accumulated more adipose tissue. HSD animals' ovaries had a higher number of atretic antral follicles (degenerated follicles) and cystic follicles, Another investigation revealed that ALA improved the ovarian tissue, as it was able to control oxidative stress. In addition, ALA maintained follicular normality and promoted the development of primordial follicles (Naupas *et al.*, 2021).

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