IS THERE ASSOCIATION BETWEEN BISPHENOL A EXPOSURE AND GLUCOSE HOMEOSTASIS IN MALE AND FEMALE ALBINO RATS?

Basma Ahmed Ali El-Degwi [1], Mahmoud El-Sayed Awad[1], Wafaa Laimon [2], Samar A Askar[3], Doaa Abdel Wahab Ahmed El Morsi [1,4] Dalia Alsaied Moustafa Ahmed [1]

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Abstract:

Endocrine-disrupting chemical (EDC) bisphenol A (BPA), which is frequently used in a range of plastics made of polycarbonate as well as epoxy resins as (plastic bottles and the of food cans lining), was found in over ninety percent of urine samples from people. EDC exposure and type 1 diabetic mellitus (T1DM) have both increased globally. Animal studies have demonstrated that BPA can worsen T1DM, despite the fact that there are still questions about BPA's potential link to T1DM risk. To compare the effects of low and high doses of BPA on blood glucose levels and histological alterations in the pancreas of the tested rats to the control groups, thirty male and thirty female adult albino rats were subjected to 20 and 100 mg/kg of bisphenol A orally every day for six weeks. The findings revealed a dose-dependent significant rise in blood glucose levels in all tested groups of rats compared to the control group as well as numerous observed pathological alterations in the pancreas that were more pronounced in female than in male rats. Conclusion: The study found that BPA treatment at various doses can disrupt glucose hemostasis and lead to islet degeneration and pancreatic histological abnormalities, which can be used to explain why blood glucose levels are elevated.

Keywords: Bisphenol A, Type 1 Diabetes Mellitus, Langerhans islet degeneration

[1]Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Mansoura University
[2] Pediatric Endocrinology and Diabetes Unit, Department of Pediatrics, Mansoura Faculty of Medicine, Mansoura University Children Hospital (MUCH), Mansoura University
[3] Histology Department, Faculty of Medicine, Mansoura University
[4] Medical Education Department, Faculty of Medicine, Delta University for Science and Tecnology

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1. Introduction:

Humans are being exposed to a rising number of environmental contaminants, many of which lead to chronic diseases development ***(Woodruff, 2015).*** One of the most frequently produced compounds in the world is bisphenol A (BPA), and exposure to BPA is widespread in contemporary life. ***(Chu et al., 2021).***

The artificial substance known as bisphenol A is a member of the diphenylmethane derivatives and bisphenols group, which has two hydroxyphenyl groups and is carbon-based. Bisphenol A is present in the epoxy and polycarbonate resins used in a variety of applications, including packaging ***(Chu et al., 2021).*** While the epoxy resins are frequently used to cover metal cans that come into close contact with food, polycarbonate plastic is frequently utilized for reusable water bottles and containers used in food storage ***(Tkalec et al., 2021).***

Humans are exposed to BPA mostly through food because BPA migrates from the epoxy coatings on cans and from polycarbonate plastic that comes into contact with food and beverages ***(Geens et al., 2012).*** Food that has been canned has substantially greater amounts of BPA than food that has not been canned ***(Lorber et al., 2015).*** BPA is also present in teeth fillings, cash receipts, and children's plastic toys ***(Snoj Tratnik et al., 2019).*** Canned food consumption has been linked to increased BPA levels in urine ***(Russo et al., 2017).***

Bisphenol A can also be absorbed through the skin and respiratory tract in addition to the gastrointestinal tract **(Lakind and Naiman, 2011).** More than ninty percent of the population in the United States and other large countries has been exposed to BPA ***(Koch et al., 2012).*** Within 24 hours, the bulk of BPA is converted to glucuronide and almost entirely excreted from the body ***(Thayer et al., 2015).***

There is a link between bisphenol A exposure and obesity ***(Jiang et al., 2020),*** insulin resistance, diabetes mellitus type 2 (T2DM) ***(Duan et al., 2018),*** metabolic syndrome, hypertension ***(Shankar and Teppala, 2012)***, and cardiovascular

disorders. This is despite the convenience and widespread use of BPA in daily life ***(Moon et al., 2021; Zhang et al., 2020)***. BPA is known to harm the reproductive system, induce developmental toxicity, and have negative effects on the immune system, respiratory system, liver, kidneys, and mental health. It has also been shown a role in cancer growth ***(Ma et al., 2019)***. Individual vulnerability to these harmful health impacts is influenced by genetics ***(Tkalec et al, 2021).***

Because of worries about its toxicity and ongoing use, Manufacturers have begun removing BPA from their goods and replacing it with structurally identical alternatives. The most prevalent of them are bisphenol F (BPF) and S (BPS). In contrast to BPF, which is used in packaging of food, pipe linings, epoxy resins, dental materials, coatings, and adhesives ***(Usman et al., 2019)***. Bisphenol S is used to produce polycarbonate plastic, epoxy resins, and thermal paper ***(Skledar et al., 2016)***

Bisphenols are endocrine-disrupting chemicals (EDCs) present in many goods. Exogenous chemicals such as EDCs interfere with endocrinal sysytem such as hormone synthesis, secretion, function, and elimination, resulting in detrimental health effects ***(Darbre, 2019).***

The prevalence of type 1 diabetes Mellitus (T1DM) and EDC exposure have both increased globally. experimental studies demonstrated that Bisphenol A may enhance T1DM as well as autoimmunity in non obese diabetic female mice and streptozotocin-treated C57BL/6 male mice, even though the link between BPA and T1DM risk is still unclear ***(Bodin et al., 2013).*** Human studies also show a link between increasing exposure to bisphenol A and an increase in diabetes ***(Cetkovic-Cvrlje et al., 2017).*** Bisphenol A exposure can influence pancreatic beta cells and enhance autoimmunity, which in an animal model of T1DM speeds up the development of insulitis and diabetes ***(Bodin et al., 2014).***

The development of T1DM and immune system dysregulation in non-obese diabetic mice, a model with spontaneous T1DM similarities to human T1DM, have also been linked to sex ***(Scinicariello and Buser, 2016)***. Bisphenol A affects sex hormone levels in a way that is connected to sex, and in T1DM patients, different hormones can control the secretion of insulin and metabolic function ***(Fu et al., 2013)***.

The prevalence of type 1 diabetes is rising among US adults, and women with T1DM have a 40% higher mortality risk than men ***(Huxley et al., 2015)***. Different hormone levels in the two sexes have an impact on T1DM susceptibility. Human glucose homeostasis has been linked to sex-based alterations after exposure to bisphenol A ***(Rancière et al., 2015)***.

This study aims to evaluate whether exposure to bisphenol A affects the glucose homeostasis in adult male and female albino rats.

. **Materials and methods:**

1.1. Chemicals:

1. Bisphenol A (BPA) analytical standard (≥ 99.9%), (Sigma Aldrich Co., Germany).
2. β-Glucuronidase enzyme, Type HP-2, from Helix Promatia, aqueous solution, activity ≥ 100,000 units/ml, (Sigma Aldrich Co., Germany).
3. Acetonitrile (99.9%), (S.D Fine-Chem Limited., India).
4. Methanol (99.9%), *(*Honil Limited, London., United Kingdom).
5. Ethyl acetate (≥99.8%), (BDH Laboratory Supplies., England).
6. Hydrochloric acid (HCL), 36.5-38%, MW: 36.46, d: 1.2g/ml, (Sigma Aldrich Co., Germany).
7. Sodium acetate buffer solution; pH 5.2±1, 3M, (Sigma Aldrich Co., Germany).
8. Potassium phosphate monobasic; anhydrous (≥ 99%), MW: 136.09 g/mol, (Sigma Aldrich Co., Germany).

1.2. Study design:

An animal study was conducted at Medical Experimental Research Center (MERC), Mansoura University, Faculty of Medicine.

The study was conducted on 60 adult albino rats of the Wistar strain (30 males and 30 females) with an average body weight of 150-200 gm. They were kept in clean cages with standard environmental conditions at a temperature of 22°C to 2°C, soft wood chips for bedding, food, and water available at all times. Two weeks prior to the experiment, they were exposed to a 12h light/dark cycle for acclimatisation and to ensure normal growth and behaviour. All experimental animals in this study were handled following the guide for the care and use of laboratory animals.

Ethical statement: The study was approved by the Mansoura Faculty of Medicine Institutional Research Board (MFM-IRB) code: (MD:19.10.242).

Rats were randomly divided into three experimental groups (10 male and 10 female rats per group), as follows:

1. **Control group (20 rats):** received 0.5 ml of pure olive oil orally every day for six weeks. This group was subdivided into:

Control group (a): 10 male rats.

Control group (b): 10 female rats

2. **Group 1 (low dose BPA) (20 rats):** received a low dose of BPA (20 mg/kg/day) which represents 1/162 of LD50 of oral BPA administration in rats dissolved in pure olive oil orally every day for six weeks ***(Kamel et al., 2018).*** This group is subdivided into:

Low dose BPA Group (1a): includes 10 male rats.

Low dose BPA Group (1b): includes 10 female rats.

3. **Group 2 (High dose BPA) (20 rats):** received a high dose of BPA (100 mg/kg/day) which represents 1/32 of LD50 of oral BPA administration in rats dissolved in pure olive oil orally every day for six weeks ***(Kamel et al., 2018).*** This group is subdivided into:

High dose BPA Group (2a): includes 10 male rats.

High dose BPA Group (2b): includes 10 female rats.

N.B. LD50 of oral BPA intake in rats is 3250 mg/kg ***(Chapin et al., 2008)***.

The body weight of all rats was recorded on the first day of the study (before BPA intake) and at the end of the study. Also, fasting and 2 hours postprandial glucose levels were tested at the start and the end of the study.

At the end of six weeks, the animals were anaesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) ***(Zhuang, et al., 2018)*** and sacrificed, the pancreas was obtained from all rats and preserved for histopathological examination by Hematoxylin and Eosin (H&E) dye and measurement of islets of Langerhans diameter by image analysis.

1. Measurement of fasting and two hours postprandial blood glucose levels:

Before the measurement of fasting blood glucose level rats did not eat or drink anything but water for eight hours. Blood sample from the rat's tail (1 ml) was taken for measuring fasting and two hours postprandial blood glucose levels at the start and end of the six weeks. Blood was collected in a grey-top (Na fluoride) tube. It was allowed to clot and then centrifuged to separate serum or plasma.

Using a COBAS INTEGRA 400 Plus analyzer, the amount of glucose was calculated in milligrammes per deciliter. ***(Roche Diagnostics, Indianapolis, IN).***

The COBAS INTEGRA An in vitro diagnostic reagent system for the quantitative assessment of glucose in serum, plasma, urine, and cerebrospinal fluid (CSF) is contained in the glucose cassette. Hexokinase is used as the test principle in an enzymatic reference technique ***(Passey et al., 1977).***

N.B. The average fasting blood glucose (FBG) and postprandial blood glucose (PBG) of normal Wistar rats were 3.95 +/- 1.31 mmol/L (71.1+/-23.58 mg/dl) and 5.65 +/- 1.63 mmol/L (101.7+/-29.34 mg/dl) respectively ***(Wang et al., 2010).***

2. Histopathological study:

Each group's entire rat population underwent a histopathological analysis. After scarification, the pancreas was promptly removed from the animals, cleaned in ice-cold saline, and preserved in 10% formaldehyde for up to 12 hours. The tissues underwent conventional processing, including dehydration and cleaning, and were then embedded in paraffin wax. The anterior, middle, and posterior portions of the pancreas were divided. Five-micron thick paraffin slices were cut and mounted on a glass slide. After being deparaffinized twice for five minutes in xylene, they were rehydrated with graded alcohol, stained with hematoxylin, and then counterstained with eosin (H&E) dye ***(Suvarna et al., 2018).***

Comparing rats' pancreas given various treatments under a light microscope (Olympus Bx53, Olympus Corporation, Tokyo, Japan) equipped with Camera Model U-LHH Gand Imaging Software (Cell sense, Ver. 1.4.1) allowed for the morphological evaluation of the stained sections. ***(Zhang et al., 2011).*** The average area of islets diameter (4 islets in each section) have been evaluated as follows ***(Findlay and Thomas 1980, Jelodar et al., 2007)***. The software was used to automatically determine the volume of the islets, which totalled four for each pancreatic region in the field (n = 12 islets per rat) (the pancreas of each rat is sectioned at the posterior, anterior, and middle part, each section of these three sections show four islets of Langerhans) for 10 rats of each group was calculated. For ten rats from each group, four Langerhans islets are seen in each of these three areas. A systematic random pattern was used to sample the microscopic slide fields; the measurement progressed equally in both the X and Y directions starting at the corner of the slide. ***(Abdel-Rahman, Mohammed et al. 2019).***

1.3. Statistical analysis

Statistical social science software: (SPSS 27.0, IBM/SPSS Inc., Chicago, IL) was used to analyse the data. P-values below 0.05 are regarded as significant, and those over 0.01 are regarded as highly significant. Data was expressed as mean ± standard deviation (SD),

one-way ANOVA test was used for comparison between more than two independent groups.

2. Results:

2.1. Body weight measurements:

The mean of initial body weight values at the beginning of the study in all studied groups, reveal no statistically significant difference among all groups (p = 0.297) (Table 1).

Table (1): Comparison between the mean values of initial body weights (g) at the beginning of the study among all studied groups (n =60 rats)

	Control Group (a) (Males) (n=10)	Control Group (b) (Females) (n=10)	Low dose BPA Group(1a) (Males) (n=10)	Low dose BPA Group(1b) (Females) (n=10)	High dose BPA Group(2a) (Males) (n=10)	High dose BPA Group(2b) (Females) (n=10)	
Mean ± SD	161 ± 7.89	167.80 ± 11.18	166.60 ± 9.11	166.80 ± 8.73	167.80 ± 11.34	160 ± 11.06	F= 1.255 P = 0.297
P 1		0.651	0.808	0.784	0.651	0.996	
P 2			0.988	0.986	0.980	0.025*	
P 3				0.999	0.996	0.654	
P 4					0.995	0.651	
P 5						0.507	

n: number, SD: standard deviation, g: gram, BPA: bisphenol A, Low dose: 20 mg/kg/day of BPA
High dose: 100 mg/kg/day of BPA
∗ Statistical significant if p ≤0.05
∗∗ High statistical significant result if p ≤0.001
P_1: compared to control group (a)
P_2: comparedto control group(b)
P_3: compared to low dose BPA group (1a)
P_4: compared to low dose BPA group (1b)
P_5: compared to high dose BPA group (2a), F for ANOVA test

Comparison between the mean values of body weight in all studied groups after receiving low and high doses of BPA (at the end of the study) revealed no statistically significant difference between the control group (a) and group 1a (p1 = 0.999), also there is no statistically significant difference between the control group (b) and group 1b (p2 = 0.894) or between the control group (a) and group 2a (p1 = 0.987) and between group 1a and group 2a (p3 = 0.907). While a statistically significant difference is noticed between the control group (b) and group 2b (p2 = 0.005) and a high statistically significant difference also noticed between group 1b and group 2b (p4 < 0.001) (Table 2).

Table (2): Comparison between the mean values of final body weights (g) after BPA administration (at the end of the study) among all studied groups (n =60 rats)

	Control Group(a) (Males) (n=10)	Control Group(b) (Females) (n=10)	Low dose BPA Group(1a) (Males) (n=10)	Low dose BPA Group(1b) (Females) (n=10)	High dose BPA Group(2a) (Males) (n=10)	High dose BPA Group(2b) (Females) (n=10)	Test of significance
Mean ± SD	236.80 ± 8.98	246.80 ± 10.82	238.80 ± 11.73	252.40 ± 7.40	233.40 ± 15.51	226.80 ± 14.20	F= 6.133 p < 0.001 **
P 1		0.414	0.999	0.049*	0.987	0.414	
P 2			0.654	0.894	0.130	0.005 *	
P 3				0.119	0.907	0.221	
P 4					0.008*	< 0.001**	
P 5						0.809	

n: number, SD: standard deviation, g: gram, BPA: bisphenol A, Low dose: 20 mg/kg/day of BPA High dose: 100 mg/kg/day of BPA
∗ Statistical significant if p ≤0.05
∗∗ High statistical significant result if p ≤0.001
P_1: compared to control group (a)
P_2: compared to control group (b)
P_3: compared to low dose BPA group (1a)
P_4: compared to low dose BPA group (1b)
P_5: compared to high dose BPA group (2a)
F for ANOVA test

Comparing the mean of body weight values in each group at the beginning of the study and after BPA intake (at the end of the study) reveals a high statistically significant difference in all groups (p < 0.001) with the largest percentage of increase in group (1b) (Table 3).

Table (3): Comparison between the mean values of initial and final body weights (g) at the beginning of the study and after BPA administration (at the end of the study) among all studied groups (n = 60 rats)

	Control Group(a) (Males) (n=10)	Control Group(b) (Females) (n=10)	Low dose BPA Group(1a) (Males) (n=10)	Low dose BPA Group(1b) (Females) (n=10)	High dose BPA Group(2a) (Males) (n=10)	High dose BPA Group(2b) (Females) (n=10)
	Mean±SD					
At the beginning of the study	161 ± 7.89	167.80 ± 11.18	166.60 ± 9.11	166.80 ± 8.73	167.80 ± 11.34	160 ± 11.06
After BPA administration (at the end of the study)	236.80 ± 8.98	246.80 ± 10.82	238.80 ± 11.73	252.40 ± 7.40	233.40 ± 15.51	226.80 ± 14.20
Percent of change (%)	47.28± 6.61	47.29± 3.91	43.38±1.41	51.62±7.63	39.12±2.55	41.82±2.77
p-value (Paired samples t-test)	< 0.001 **	< 0.001 **	< 0.001 **	< 0.001 **	< 0.001 **	< 0.001 **

n: number, SD: standard deviation, g: gram, BPA: bisphenol A, Low dose: 20 mg/kg/day of BPA High dose: 100 mg/kg/day of BPA

∗ Statistical significant if p ≤0.05

∗∗ High statistical significant result if p ≤0.001

F for ANOVA test

2.2. Fasting blood glucose values:

The mean values of fasting blood glucose levels at the start of the study in all studied groups reveal no statistically significant difference between all studied groups (p = 0.702) (Table 4).

Table (4): Comparison of the mean values of fasting blood glucose levels (mg/dl) at the beginning of the study among all studied groups (n= 60 rats)

	Control Group(a) (Males) (n=10)	Control Group(b) (Females) (n=10)	Low dose BPA Group (1a) (Males) (n=10)	Low dose BPA Group (1b) (Females) (n=10)	High dose BPA Group (2a) (Males) (n=10)	High dose BPA Group(2b) (Females) (n=10)	
Mean ± SD	96.20 ± 12.71	88.20 ± 17.11	89.20 ± 19.56	89.20 ± 14.04	87 ± 14.98	96 ± 19.94	F= 0.579 P = 0.702
P_1		0.888	0.934	0.936	0.816	0.998	
P_2			0.996	0.998	0.964	0.899	
P_3				0.999	0.928	0.880	
P 4					0.930	0.914	
P 5						0.829	

n: number, SD: standard deviation, mg: milligram, dl: deciliter, BPA: bisphenol A

Low dose: 20 mg/kg/day of BPA, High dose: 100 mg/kg/day of BPA

∗ Statistical significant if p ≤0.05

∗∗ High statistical significant result if p ≤0.001

P_1: compared to control group (a)

P_2: compared to control group(b)

P_3: compared to low dose BPA group (1a)

P_4: compared to low dose BPA group (1b)

P_5: compared to high dose BPA group (2a)

F for ANOVA test

The mean values of fasting blood glucose in all studied groups after receiving low and high doses of BPA (at the end of the study), show no statistically significant difference between control groups (a & b) and groups 1a &1b (Low dose BPA) respectively. While a high statistically significant difference is noticed between the control group (a) and group 2a (p1< 0.001) and a statistically significant difference also noticed between group 1a and group 2a (p3 = 0.011). Furthermore, a statistical

significant difference is noticed between group 2b and control group (b) (p2 = 0.025) and no statistically significant difference between group 1b and group 2b (p4 = 0.985) (Table 5).

Table (5): Comparison of the mean values of fasting blood glucose (mg/dl) after administration of low and high doses of BPA (at the end of the study) among all studied groups (n = 60 rats)

	Control Group(a) (Males) (n=10)	Control Group(b) (Females) (n=10)	Low dose BPA Group(1a) (Males) (n=10)	Low dose BPA Group(1b) (Females) (n=10)	High dose BPA Group(2a) (Males) (n=10)	High dose BPA Group(2b) (Females) (n=10)	Test of significance
Mean ± SD	98.40 ± 9.97	103.60 ± 13.29	108 ± 13.35	123 ± 17.13	134.60 ± 12.48	128 ± 28.48	F= 7.464 p < 0.001 **
P 1		0.983	0.800	0.023*	< 0.001 **	0.003*	
P 2			0.992	0.124	0.002 *	0.025*	
P 3				0.365	0.011*	0.104	
P 4					0.644	0.985	
P 5						0.951	

n: number, SD: standard deviation, mg: milligram, dl: deciliter, BPA: bisphenol A
Low dose: 20 mg/kg/day of BPA, High dose: 100 mg/kg/day of BPA
∗ Statistical significant if p ≤0.05
∗∗ High statistical significant result if p ≤0.001
P_1: compared to control group (a)
P_2: compared to control group(b)
P_3: compared to low dose BPA group (1a)
P_4: compared to low dose BPA group (1b)
P_5: compared to high dose BPA group (2a)
F for ANOVA test

Comparing the mean of fasting blood glucose values in each group at the beginning of the study and after administration of BPA (at the end of the study) reveals no statistically significant difference in the control group (a) (p = 0.719), but reveals a statistically significant difference in the control group (b) and group 1a (p = 0.009 and 0.002 respectively) and reveals also, a high statistically significant difference in groups 1b, 2a and 2b (p < 0.001) (Table 6).

Table (6): Comparison between the mean values of fasting blood glucose (mg/dl) at the beginning of the study and after BPA administration (at the end of the study) among all studied groups (n = 60 rats)

	Control Group(a) (Males) (n=10)	Control Group(b) (Females) (n=10)	Low dose BPA Group(1a) (Males) (n=10)	Low dose BPA Group(1b) (Females) (n=10)	High dose BPA Group(2a) (Males) (n=10)	High dose BPA Group(2b) (Females) (n=10)
			Mean±SD			
At the beginning of the study	96.20 ± 12.71	88.20 ± 17.11	89.20 ± 19.56	89.20 ± 14.04	87 ± 14.98	96 ± 19.94
After BPA administration (at the end of the study)	98.40 ± 9.97	103.60 ± 13.29	108 ± 13.35	123 ± 17.13	134.60 ± 12.48	128 ± 28.48
p-value (Paired samples t-test)	0.719	0.009*	0.002*	< 0.001 **	< 0.001 **	< 0.001 **

n: number, SD: standard deviation, mg: milligram, dl: deciliter, BPA: bisphenol A
Low dose: 20 mg/kg/day of BPA, High dose: 100 mg/kg/day of BPA
∗ Statistical significant if p ≤0.05
∗∗ High statistical significant result if p ≤0.001
F for ANOVA test

2.3. Two hours postprandial blood glucose values:

The mean values of 2-hours postprandial blood glucose at the beginning of the study in all studied groups reveal no significant statistical difference between all groups (p = 0.452) (Table 7).

Table (7): Comparison between the mean values of two hours postprandial blood glucose (mg/dl) at the beginning of the study among all studied groups (n= 60 rats)

	Control Group(a) (Males) (n=10)	Control Group(b) (Females) (n=10)	Low dose BPA Group(1a) (Males) (n=10)	Low dose BPA Group(1b) (Females) (n=10)	High dose BPA Group(2a) (Males) (n=10)	High dose BPA Group(2b) (Females) (n=10)	Test of significance
Mean ± SD	106 ± 11.47	97.40 ± 22.06	105.60 ± 19.20	95.60 ± 13.93	102.60 ± 27.59	110.80 ± 10.01	F= 0.957 P = 0.452
P 1		0.901	0.999	0.805	0.992	0.990	
P 2			0.918	0.986	0.968	0.587	
P 3				0.829	0.991	0.982	
P 4					0.957	0.448	
P 5						0.918	

n: number, SD: standard deviation, mg: milligram, dl: deciliter, BPA: bisphenol A
Low dose: 20 mg/kg/day of BPA, High dose: 100 mg/kg/day of BPA
∗ Statistical significant if p ≤0.05
∗∗ High statistical significant result if p ≤0.001
P_1: compared to control group (a)
P_2: compared to control group(b)
P_3: compared to low dose BPA group (1a)
P_4: compared to low dose BPA group (1b)
P_5: compared to high dose BPA group (2a)
F for ANOVA test

The mean values of 2-hours postprandial blood glucose in all studied groups after receiving low and high doses of BPA (at the end of the study), show no statistically significant difference between the control group (a) and group 1a (p1 = 0.268), while there is a statistically significant difference between the control group (b) and group 1b (p2 = 0.019). There is also, a high statistically significant difference between the control group (a) and group 2a (p1 < 0.001) with no statistically significant difference between group 1a and group 2a (p3 = 0.162). Furthermore, there is a high statistically significant difference between group 2b and control group (b) (p2 < 0.001) and no statistically significant difference between group 1b and group 2b (p4 = 0.731) (Table 8)

Table (8): Comparison between the mean values of two hours postprandial blood glucose (mg/dl) after BPA administration (at the end of the study) among all studied groups (n= 60 rats)

	Control Group(a) (Males) (n=10)	Control Group(b) (Females) (n=10)	Low dose BPA (Males) Group(1a) (n=10)	Low dose BPA (Females) Group(1b) (n=10)	High dose BPA (Males) Group(2a) (n=10)	High dose BPA (Females) Group(2b) (n=10)	Test of significance
Mean ± SD	112.60 ± 10.51	119 ± 18.22	133.80 ± 18.52	151.40 ± 24.22	157.60 ± 24.68	165 ± 29.63	F= 9.633 p < 0.001 **
P 1		0.986	0.268	0.003*	< 0.001 **	< 0.001 **	
P 2			0.656	0.019*	0.003 *	< 0.001 **	
P 3				0.473	0.162	0.027*	
P 4					0.988	0.731	
P 5						0.973	

n: number, SD: standard deviation, mg: milligram, dl: deciliter, BPA: bisphenol A
Low dose: 20 mg/kg/day of BPA, High dose: 100 mg/kg/day of BPA
∗ Statistical significant if p ≤0.05
∗∗ High statistical significant result if p ≤0.001
P_1: compared to control group (a)
P_2: compared to control group(b)
P_3: compared to low dose BPA group (1a)
P_4: compared to low dose BPA group (1b)
P_5: compared to high dose BPA group (2a)
F for ANOVA test,

By comparing the mean values of 2-hours postprandial blood glucose in all studied groups at the beginning of the study and after BPA administration (at the end of the study), there is no statistically significant difference in the control group (a) (p = 0.320). On the contrary, there is a statistically significant difference in the control group (b) (p = 0.002) and a high statistically significant difference in groups 1a, 1b, and 2b (p < 0.001). There is also, a high statistically significant difference in group 2a (p = 0.001) (Table 9).

Table (9): Comparison between the mean values of 2-hours postprandial blood glucose (mg/dl) at the beginning of the study and after BPA administration (at the end of the study) among all studied groups (n = 60 rats)

	Control Group(a) (Males) (n=10)	Control Group(b) (Females) (n=10)	Low dose BPA Group(1a) (Males) (n=10)	Low dose BPA Group(1b) (Females) (n=10)	High dose BPA Group(2a) (Males) (n=10)	High dose BPA Group(2b) (Females) (n=10)
	Mean±SD					
At the beginning of the study	106 ± 11.47	97.40 ± 22.06	105.60 ± 19.20	95.60 ± 13.93	102.60 ± 27.59	110.80 ± 10.01
After BPA administration (at the end of the study)	112.60 ± 10.51	119 ± 18.22	133.80 ± 18.52	151.40 ± 24.22	157.60 ± 24.68	165 ± 29.63
p-value (Paired samples t-test)	0.320	0.002*	< 0.001 **	< 0.001 **	0.001 **	< 0.001 **

n: number, SD: standard deviation, mg: milligram, dl: deciliter, BPA: bisphenol A

Low dose: 20 mg/kg/day of BPA, High dose: 100 mg/kg/day of BPA

∗ Statistical significant if p ≤0.05

∗∗ High statistical significant result if p ≤0.001

F for ANOVA test

2.4. Imaging study:

About the mean values of the diameter of islets of Langerhans (µm) after BPA administration (at the end of the study) in all studied groups, there is a high statistically significant difference between control groups (a &b) and each of the low dose BPA groups (1a & 2a) respectively. There is a highly statistically significant difference between high-dose BPA groups (2a & 2b) compared to control groups (a & b) respectively (p < 0.001) and also compared to low-dose BPA groups (1a & 1b) respectively (p < 0.001) (Table 10).

Table (10): Comparison between the mean values of the diameter of islets of Langerhans (µm) among all studied groups after BPA administration (at the end of the study) (n = 60 rats)

	Control Group(a) (Males) (n=10)	Control Group(b) (Females) (n=10)	Low dose BPA Group(1a) (Males) (n=10)	Low dose BPA Group(1b) (Females) (n=10)	High dose BPA Group(2a) (Males) (n=10)	High dose BPA Group(2b) (Females) (n=10)	Test of significance
Mean ± SD	478 ± 18.05	469.13 ± 16.00	368.33 ± 21.06	370 ± 29.02	249.20 ± 12.53	240.20 ± 15.97	F= 132.15 p < 0.001 **
P 1		0.239	< 0.001 **	< 0.001 **	< 0.001 **	< 0.001 **	
P 2			< 0.001 **	< 0.001 **	< 0.001 **	< 0.001 **	
P 3				0.998	< 0.001 **	< 0.001 **	
P 4					< 0.001 **	< 0.001 **	
P 5						0.244	

n: number, SD: standard deviation, µm: micrometer, BPA: Bisphenol A

Low dose: 20 mg/kg/day of BPA, High dose: 100 mg/kg/day of BPA

∗ Statistical significant if p ≤0.05

∗∗ High statistical significant result if p ≤0.001

P_1: compared to control group (a)

P_2: compared to control group(b)

P_3: compared to low dose BPA group (1a)

P_4: compared to low dose BPA group (1b)

P_5: compared to high dose BPA group (2a)

F for ANOVA test

2.5. Histopathological examination:

Histopathological examination of the pancreas of both control groups (a & b) reveals the normal architecture of the endocrine part (islet of Langerhans) surrounded by the exocrine part which shows non-congested blood vessels. The exocrine part is formed of acini with basal basophilic cytoplasm and apical acidophilic cytoplasm. The islet shows β cells in the center and small α cells at the periphery (Photo 1).

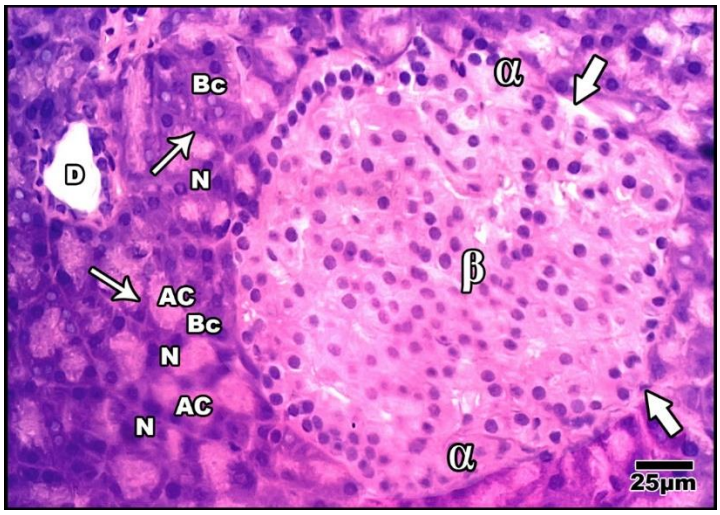

Photo (1): photomicrograph of a section of the pancreas of control groups shows the endocrine part islet of Langerhans (thick arrows) surrounded by the exocrine part (thin arrows). The exocrine part is formed of acini with basal basophilic cytoplasm (Bc), rounded nuclei (N), and apical acidophilic cytoplasm (Ac). Some interlobular ducts (D) are seen. The islet shows β cells in the center (β) and small cells (α) at the periphery that are most probably α cells.

Male rats who received low dose BPA (20 mg/kg) (group 1a) show some histopathological changes in the pancreas in the form of distorted cells in the center and small cells at the periphery of islets of Langerhans. An acidophilic material and empty space are seen between the cells. Many dark cells most probably lymphocytes are detected at the periphery of the islet (Photo 2 (a & c)).

Female rats received low dose BPA (20 mg/kg) (group 1b) show more changes in the islet; distorted cells in the center and small cells at the periphery. An acidophilic material and empty space are seen between the cells. Many dark cells most probably lymphocytes are detected at the periphery of the islet and dilated congested blood vessels are detected (Photo 2 (b)). Also, shrunken degenerated islet displaying cells with degenerative and necrotic changes are detected in some sections (Photo 2(d)).

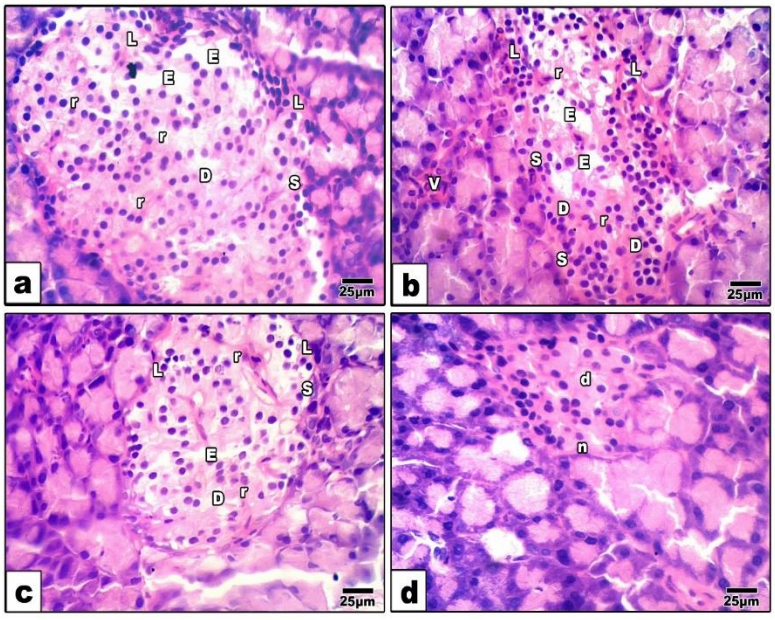

Photo (2) (a-d): (a & c): photomicrograph of sections of the pancreas of group 1a (male rats received low dose BPA) showing some islet with distorted cells in the center (D) and small cells (S) at the periphery. An acidophilic red material (r) and empty space (E) are seen between the cells. Many dark cells most probably lymphocytes (L) are detected at the periphery of the islet. (b): a section of the pancreas of group 1b (female rats received low dose BPA) showing more changes in the islet; distorted cells in the center (D) and small cells (S) at the periphery. An acidophilic red material (r) and

empty space (E) are seen between the cells. Many lymphocytes (L) are detected at the periphery of the islet. Also, dilated congested blood vessels (bl) are seen. (d): a section of the pancreas of group 1b (female rats received low dose BPA) showing some shrunken degenerated islet displaying cells with degenerative (d) and necrotic (n) changes are detected **(H&E X 400).**

Histopathological examination of pancreatic sections of male rats received high dose BPA (100 mg/kg) (group 2a) show small degenerated islets displaying empty space, degenerated central cells and many lymphocytes, degenerated and necrotic cells are also detected (Photo 3 (a)), while, sections of the pancreas of female rats received high dose BPA (100 mg/kg) (group 2b) show more obvious changes in the form of small, degenerated islet displaying more empty spaces, degenerated and necrotic cells and few lymphocytes (Photo 3 (b)).

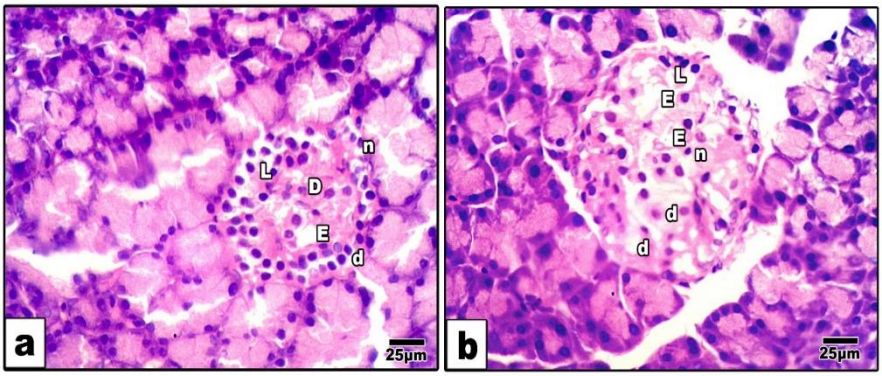

Photo (3) (a & b): (a): photomicrograph of a section of the pancreas of group 2a (male rats received high dose BPA) shows small, degenerated islet displaying empty space (E), degenerated central cells (D) and many lymphocytes (L). degenerated (d) and necrotic (n) cells are detected. (b): a section of the pancreas of group 2b (female rats received high dose BPA) showing more obvious changes in the form of small, degenerated islets displaying many empty spaces (E), degenerated (d) and necrotic cells (n) and few lymphocytes (L) **(H&E X 400).**

3. **Discussion:**

Bisphenol A (BPA) is the molecular building block for epoxy resins and polycarbonate plastics. These days, it may be found almost anywhere in the environment, and it is frequently discovered in drinking water and dust particles. It is now found almost everywhere in the environment and is detected commonly in drinking water and dust particles ***(Mohamed and Rateb, 2019)***. Numerous experimental investigations show that BPA exposure causes changes in lipid synthesis or metabolism, glucose intolerance, insulin resistance, impaired glucose homeostasis, and insulin resistance. **(*Angle et al., 2013; García-Arevalo et al., 2014; Marmugi et al., 2014;Moon et al., 2015)***.

Insulin shortage brought on by the death of pancreatic beta-cells characterises Type 1 diabetes mellitus (T1DM), a chronic autoimmune condition that causes hyperglycemia. Although symptoms typically begin in childhood or adolescence, they can occasionally appear much later in life. T cell-mediated death of -cells is thought to have a role in the pathogenesis of T1DM, despite the fact that its etiology is not fully known. ***(Katsarou et al., 2017)***.

Exposure to bisphenol A can affect the pancreatic β-cells of and enhance autoimmunity, which leads to insulitis and diabetes in an animal model of T1DM ***(Bodin et al., 2014)***. According to certain research, exposure to bisphenol A in maturity or throughout life may raise the risk of TIDM. ***(Bodin et al., 2014; Cetkovic-Cvrlje et al., 2017).***

This study aimed to evaluate if there is an association between bisphenol A exposure and glucose homeostasis in adult albino rats.

The body weight of rats was measured at the beginning of the study and after BPA intake for 6 weeks (at the end of the study). The following investigations were done; fasting blood glucose level, two hours post prandial level, imaging study of islets of Langerhans and histopathological examination.

Regarding body weight of rats, comparison between the mean values of body weight in all studied groups after receiving low and high doses of BPA, revealed a statistically significant difference between control group (b) and high dose BPA group (2b) and a high statistically significant difference between low dose BPA group (1b) and high dose BPA group (2b) (Table 2). body weight of rats at the beginning of the study and after BPA administration (at the end of the study) revealed that there was a high statistically significant increase in the body weight after BPA administration (at the end of the study) in all groups with the largest percent of increase was detected in low dose BPA female group (1b) (Table 3).

This is in agreement with ***Moghaddam et al. (2015)*** who reported weight gain in the tested groups which were exposed to 0.5 and 2 mg/kg/ day BPA intraperitoneally for four weeks compared with the normal healthy mice in a study conducted in Iran.

Angle et al. (2013) also conducted a study in Columbia looking at the consequences of fetal BPA exposure in male mice. They discovered a rise in body weight and belly fat mass, which correlated with an increase in adipocyte numbers and size. Additionally, they discovered that male progeny had lower levels of serum adiponectin and concurrently greater levels of leptin and insulin in the blood, providing strong evidence of metabolic dysfunction in white adipose tissue brought on by BPA exposure.

Miyawaki et al. (2007) in a study established in Ehime University, Japan observed an elevation in the adipose tissue mass as well as body weight in males and females offspring of pregnant mice exposed to BPA in drinking water at concentrations of 1 and 10 µg/mL from gestation day 10 and throughout the lactating period by comparison to control group offspring, Thus, it was established that obesity and hyperlipidemia in mice were brought on by BPA exposure that occurred continuously during the prenatal and postnatal periods. Many in vivo and in vitro investigations showed an increase in adipose tissue mass, showing a direct impact of BPA on adipocytes and affecting their metabolic processes, according to ***Vom Saal et al. (2012)..***

In contrast to our results, a study established in Brazil by ***Azevedo et al. (2019)*** showed that rats orally exposed to 50 µg/kg/day of BPA for 38 weeks did not exhibit significant changes in body weight gain when compared to the control group.

Moreover, ***Liu et al. (2013***) reported no alterations in body weight of rodents exposed to 100 µg/kg/day of BPA perinatally and postnatally (from day one pregnancy till weaning) through subcutaneous injection in a study conducted in China. Also, ***Marmugi et al. (2014)*** when exposed adult mice in a study done in France to 5, 50, 500 and 5000 µg /kg/day of BPA orally for 8 months, the weight measured during the study did not reveal any difference between mice exposed to BPA and controls.

In the current study, regarding the mean values of fasting blood glucose after receiving low and high doses of BPA (at the end of the study), they showed a high statistically significant difference between control group (a) and high dose BPA group (2a) and a statistically significant difference between low dose BPA group (1a) and high dose BPA group (2a). Furthermore, there was a statistically significant difference between control group (b) and high dose BPA group (2b), while there was no statistically significant difference between low dose BPA group (1b) and high dose BPA group (2b) (Table 5).

Comparing between fasting blood glucose values at the beginning of the study and after BPA administration (at the end of the study) revealed that there was a high statistically significant increase in fasting blood glucose levels in low dose BPA group (1b), high dose BPA group (2a) and high dose BPA group (2b). Additionally, there was a statistically significant increase in control (b) and low dose BPA (1a) groups. However, there was no statistically significant increase in control a group (Table 6).

About the mean values of 2 hours postprandial blood glucose after receiving low and high doses of BPA (at the end of the study), the current study results showed a statistically significant difference between control group (b) and low dose BPA group (1b). There was also, a high statistically significant difference between control group (a) and high dose BPA group (2a). Also, there was a high statistically significant difference between high dose BPA group (2b) and control group (b) (Table 8).

Moreover, comparing two hours post prandial blood glucose values at the beginning of the study and after BPA administration (at the end of the study) revealed a high statistically significant increase in low dose BPA group (1a), low dose BPA group (1b), high dose BPA group (2a) and high dose BPA group (2b) and a statistically significant increase in control (b) group (Table 9 and figure 3).

These results came in accordance with ***Marmugi et al. (2014)*** who conducted a study in France and reported that plasma glucose levels increased in rats treated with 500 and 5,000 µg/kg of BPA in drinking water for 8 months.

In ***(2015)***, ***Alonso-Magdalena et al.*** performed a study in Spain, they treated pregnant mice with 10 µg/kg/day of Bisphenol A by subcutaneously during gestational days 9–16; moms' metabolic health was compromised, as seen by elevated insulin and leptin levels, impaired lipid profiles, impaired glucose tolerance, during and after pregnancy until four months after delivery. Additionally, compared to children of women who were not treated, the six-month-old male offspring of mothers acquired insulin resistance and a reduction in glucose tolerance, which were associated with higher levels of plasma insulin.

Using pregnant mice exposed to 500 g/kg/day of BPA orally from gestational day 9 to gestational day 18, ***Angle et al. (2013)*** showed that The exposure of prenatal foetuses led to postnatal weight gain, impaired glucose tolerance and insulin resistance with hyperinsulinemia, a rise in adipocyte number and volume with a corresponding increase in abdominal fat, and an increase in adiponectin and leptin levels.

Furthermore, ***Song et al. (2014)*** investigated BPA effects on glucose metabolism in early and later life of male rat offspring in a study conducted in China. They found that, after perinatal exposure to 1 and 10 µg/mL of BPA orally, a reduction in adiponectin (regulating glucose levels, lipid metabolism and insulin sensitivity) gene expression and production were observed later in life and that at high BPA dose (10 µg/mL) while the metabolic dysfunction was detected earlier.

It's interesting to note that an in vitro study employing human adipocytes treated with 1 nM of BPA showed impairment of insulin-stimulated glucose utilisation and insulin signalling pathway, dysregulating adipocyte activity. ***(Valentino et al., 2013)***.

Rat insulinoma (INS-1) cells were used as a model by ***Lin et al. (2013)*** to examine the impact of BPA on insulin production. They demonstrated that INS-1 cells exposed to 0, 0.0020, 0.020, 0.20, or 2.0 μM of BPA underwent apoptosis, which resulted in mitochondrial abnormalities that reduced cell viability and decreased insulin secretion in response to glucose.

The elevated blood glucose level could be explained by over stimulation of the estrogen receptors alpha (ERα) in pancreatic B-cells by BPA which produced an excessive insulin signaling in the liver, endothelium and in fats, thus leading to obesity, glucose intolerance and dyslipidemia ***(Nadal et al., 2009)***.

Contradictory results were reported in a study conducted in Selcuk University, Turkey by ***Ozaydan et al. (2018)*** who concluded that there was no significant difference in plasma insulin and glucose levels between rats in control group and rats given different doses of BPA (5, 50 and 500 µg/kg body weights/day) orally for 8 weeks.

Moreover, ***Ke et al. (2016)*** reported that there was no significant difference in blood glucose level in 8-week-old rats received 0.5 µg/ kg/day BPA orally for 8 weeks, whereas in 10-month-old rats they found decreased high density lipoprotein (HDL) level and increased blood glucose.

However, due to the use of various procedures, reports on the effects of BPA on metabolism are inconclusive; animal species, exposure time, dose of BPA, length of BPA exposure, and the route of administration employed in various research affect the results. ***(Ke et al., 2016)***.

In the present study, studying the diameter of islets of Langerhans by imaging, revealed that there was a high statistically significant decrease in the diameter of islets of Langerhans (µm) after BPA administration (at the end of the study) which was dose dependant. Low dose BPA groups (1a & 1b) showed high statistically significant decrease in diameter of islets of Langerhans compared to control (a) and (b) groups respectively. Furthermore, more decrease was detected in high dose BPA groups (2a & 2b) compared to both control (a) and (b) groups and low dose BPA groups (1a & 1b) (Table 10).

This was in line with a study by ***Alonso-Magdalena et al. (2015)*** conducted in Spain, which showed that female mice exposed to 10 and 100 µg/kg/day of BPA subcutaneously on days 9 to 16 of pregnancy showed that even seven months after delivery, still displayed impaired glucose tolerance, and had diminished pancreatic cell mass and function., possibly as a result of protracted insulin resistance.

On the other hand, ***Ozaydn et al. (2018)*** conducted a study in Turkey and discovered that there was no significant difference between the rats who received 5, 50, and 500 µg/kg body weights/day of BPA orally for 8 weeks and the control group in terms of the mean area of islets of Langerhans.

Regarding histopathological study by H&E, the present results stated that rats received low dose BPA (20 mg/kg) (group 1a & 1b) showed histopathological changes in the form of distorted cells in the center and small cells at the periphery of islets of Langerhans. An acidophilic material and empty spaces were seen between the cells. Many dark cells most probably lymphocytes were detected at the periphery of the islet (photo 2 a-d). Some pancreatic sections from female rats showed also dilated congested blood vessels and shrunken degenerated islet displaying cells with more obvious degenerative and necrotic changes (Photo 2 b & d).

While, histopathological examination of pancreatic sections of male and female rats received high dose BPA (100 mg/kg) (group 2a & 2b) showed small, degenerated islet displaying empty spaces, degenerated cells and many lymphocytes. Some sections showed shrunken degenerated islet displaying cells with degenerative and necrotic changes with more apparent changes in female rats' sections (Photo 3 a-b).

Xu et al. (2019) conducted a study in United States of America (USA) and reported that Sex significantly influences how BPA affects the risk of T1DM. However, it was delayed in male mice. BPA accelerated the development of T1DM in adult non-obese diabetic females. The most plausible mechanism for changing the risk of T1DM was discovered to be the modification of immunological homeostasis.

Mohamed and Bastwrous (2021) performed a study in Assiut university, Egypt and reported that rats received 50 mg/kg of BPA through a gastric tube for 30 days showed dilated congested blood vessels, destroyed acinar cells and the islets showed areas of focal degeneration.

In H&E-stained sections of BPA, there were dilated congested blood vessels. Dilatation of the blood vessels could be due to increased levels of nitric oxide production with chronic use of

bisphenol leading to smooth muscle relaxation with subsequent vasodilatation, this explanation was declared by ***Ramanlal and Gupta (2020)*** in an investigation carried out in the US. ***Eid et al. (2015)*** noted similar results. who conducted a study in Kingdom of Saudi Arabia and reported increased levels of nitric oxide production (with chronic use of bisphenol) which is a potent oxidant and nitrating agent can attack and modifying proteins, lipids, and DNA as well as depleting antioxidant defenses.

The recent findings showed that BPA induced a long-lasting inflammatory response that manifested as lymphocytic infiltration and blood vessel obstruction. Similar findings were observed by ***Moon et al. (2015)*** in a study conducted in Korea. High levels of inflammatory biomarkers such interleukin-1 (IL-1), interleukin-6 (IL-16), and tumour necrosis factor (TNF-) have been linked to exposure to bisphenol A. Pro-inflammatory cytokines mediate the inflammation and aid in cytotoxic damage. (***Arita et al., 2019)***, This study was conducted in the USA.

BPA also increased inflammatory cytokines, exacerbated oxidative stress, and increased apoptosis. Additionally, bisphenol A has the potential to harm vital biological elements like nucleic acids, genes, and gene repair processes, which might result in apoptosis. ***(Rahmani et al., 2020)***.

Bisphenol A may induce oxidative stress and apoptosis, these were confirmed by ***Faheem et al. (2021)*** conducted a study in Ain Shams University, Egypt and stated that BPA when administered orally to albino rats for 30 days at a dose of 50 mg/kg/day induced oxidative stress.

There is a link between Bisphenol A exposure and oxidative stress. Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) as (superoxide, hydrogen peroxide, hydroxyl radicals, singlet oxygen, nitric oxide, peroxynitrite) and the cellular defense mechanisms of the antioxidants. It has been shown that BPA reduces the production of antioxidant enzymes like superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase, and catalase, which in turn aids in the development of oxidative stress. As a result, there is an increase in ROS and/or a decrease in antioxidant defences ***(Gassman, 2017; Helal et al., 2018; Mokra et al., 2018; Fadda et al., 2019 Alfahdawi et al., 2022)***.

Additionally, BPA-induced apoptosis could be the cause of the cells' shrinkage, loss, and acidophilic cytoplasm.. The cell death might be triggered by cytotoxins. A similar explanation has been reported by ***Morsi et al. (2022)*** who reported cytotoxicity in the pancreatic islets in albino rats received 4.5 µg/L of BPA in drinking water for 45 days in a study conducted in Saudi Arabia.

Also, BPA increases the expression of genes involved in the stress response, which increases the expression of genes involved in oxidative stress and cellular damage. Lipids, nucleic acids, carbohydrates, proteins, and DNA especially mitochondrial DNA have all been damaged by high amounts of ROS. due to their high reactivity with these structures ***(Helal et al., 2018; Aboelhassan et al., 2022; Alfahdawi et al., 2022)***.

4. Conclusions:

In conclusion, the current study demonstrated that low and high dose BPA administration for six weeks in adult Wistar rats caused significant increase in body weight, fasting and 2-hour postprandial blood glucose levels compared to control groups. Also, the diameter of islets of Langerhans significantly decreased in all rats received BPA. Rats received BPA showed histopathological changes in the pancreatic tissues with more changes in rats received the high dose and females were more affected than males.

So, from this study, we can conclude that BPA has a significant effect on blood glucose homeostasis and pancreas thus it may be a risk factor for DM.

5. Recommendations:

Health programmes should be run to gather data and inform the public about BPA exposure sources and its harmful effects on human health.

By outlawing all non-essential applications of bisphenol A, such as in plastics and canned goods, exposure should be reduced as much as feasible.

More research are needed to illustrate whether the effect of BPA on pancreas is sex related or not.

Human studies are recommended to investigate the link between Bisphenol A and Diabetes mellitus and whether it is considered as a risk factor or not

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