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Morphological Study of the Effects of Caffeine Beverages Can Cause Birth Defects on Swiss White Mice Embryos

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ABSTRACT

The current study was conducted to identify the effects of different doses of Arabic coffee, black tea and Coca-Cola syrup on the possibility of birth defects in Swiss white mice embryos. (25) pregnant female mice were used in the study, divided into five experimental groups, including the control group dosed with distilled water, and four experimental groups were dosed with concentrations (4000-2500-2000-1000) mg/kg body weight for Arabic coffee and black tea and (2,3,4,6 ml/kg) for Coca-Cola syrup; once a day, from the seventh day until the eighteenth day of pregnancy.

The results of the current study showed cases of abortion and fetal death at varying degrees and many abnormalities in white mouse embryos due to the different concentrations of the beverages used were characterized by the appearance of elongated and enlarged embryos. The elongation and deformation of the fetus, And the embryos mutated. Small embryos folded like a ball and curved in the shape of letter C, and the severity of the deformities increased by the appearance of mutated embryos resembling the body of a fish and deformed embryos similar to a water mermaid and not fully developed and the appearance of the head in the shape of a blurred triangle, as well as forward with a pointed end resembling a bird's beak, and brain deformation through depression in the posterior cranial region of the skull, and for the first time a new deformation is a cerebral Meningomyelocele in the form of a cystic tumor in the posterior brain region.

1 Introduction

In most countries around the world, social beverages are now one of the most popular drinks, but how does it look when consumed in large amounts during pregnancy, causing miscarriages, damages, deformities, and birth defects (AL-Rasheedi,2009). Currently, commercial advertisements in various media outlets are widely observed by companies and factories that produce such drinks strikingly in order to highlight their nutritional importance, which has caused a large number of people to over-consume them without any reference to the negative effects of unusually using them. Caffeine increases the amount of energy in the body and stimulates the muscles to oxidize fat and protein (Yen *et al*.,2005). Caffeine crosses the placental wall to the fetus and increases the likelihood of spontaneous abortion, as well as a lack of fetal growth and congenital malformations within the womb, which sometimes leads to fetal loss and death (Weng and Odouli,2008). There are very few studies on the effect of caffeine beverages on fetus levels and fetal development, especially during pregnancy (Bech *et al*.,2005). Three types of coffee beverages, Arabic coffee, black tea and Coca-Cola syrup, have been selected in the current study. Many studies have focused on the benefits and importance of these drinks in the first place (Greenberg *et al*.,2006). Very small studies (Bech *et al*.,2007).

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This matter has stopped embryologists from paying attention to studying the various deformities that occur during the embryonic development process, their types, causes and mechanisms of formation under the name of Congenital malformations. Teratology (Ashmead,2003), that many pregnant women frequently drink coffee and tea and over-drink various soft drinks without concern. The health damage caused by these high doses of used drinks. The fact that women who drank four or more cups of coffee increased the function of the placenta and thus the death of the fetus (CARE,2008) (Bierrnacki *et al*.,2000). Given the lack of studies to clarify the effects of caffeine beverages on birth defects, this study was conducted. Therefore, the aim of study to identify the teratogenic effects of caffeinated beverages on the fetuses of pregnant albino mice

2. Materials and Methods

Mice were selected at (9-12) weeks of age for both sexes, their average weight was (2 ± 23) g, and the mice were healthy and healthy. The mice were placed under standard laboratory conditions throughout the study period. Females ready for fertilization have been placed with males at the rate of one male with three females in each cage. And the fertilization was confirmed by the observation of the vaginal plug in the morning of the following day. The date of mating is day zero of pregnancy, and the next day is the first day of mating (10).

2.1 Drinks Used

I used Arabic coffee that was bought from the local market. The aqueous solution was prepared for coffee by dissolving (1-4) grams of coffee in (50) ml of distilled water, boiling the solution for two minutes and stirring continuously to simulate what is being prepared and used by humans, use caffeine black tea purchased from the local market and prepare the aqueous solution of black tea by boiling (1-4) grams of black tea in (50) ml of distilled water And boiling the solution for three minutes, as a simulation of what is prepared and used by humans, as for the Coca-Cola drink, is to use a ready-made drink packed in 330 ml metal cans.

2.2 Experimental Design

Table 1 Shows the experimental design

Dosing period	Groups	Arabic coffee concentrate: mg / kg	Black tea concentrate: mg / kg	Coca-Cola syrup concentrate ml / kg	The number of potions available	The number of mice
An oral dose of The seventh day to the eighteenth day of pregnancy	**The control**	**Distilled water**	**Distilled water**	**Distilled water**	**11**	**5**
	Experimental (1)	1000	1000	2	11	5
	Experimental (2)	2000	2000	3	11	5
	Experimental (3)	2500	2500	4	11	5
	Experimental (4)	4000	4000	6	11	5

2.3 Concentrations used

(25) pregnant female mice, which were divided into five groups and included in the control group and were dosed with distilled water, and four experimental groups were dosed with an aqueous solution of Arabica coffee at an increase in body weight (4000,2500,2000,1000) mg/kg In comparison to the study's median lethal dose (LD50) of (5 g / kg body weight) orally (43.5 mg caffeine / kg) which was reached through this study, Black tea with progressive concentrations (4000,2500,2000,1000) mg/kg body weight in comparison to (LD50) and its amount (6 g/kg body weight) orally (39 mg caffeine / kg), which was achieved in this study and Coca-Cola syrup, the concentrations used are (2 ml, 3 ml, 4 ml, 6 ml) In comparison to the median lethal dose (LD50) of (200 ml/kg body weight) orally (0.86 mg caffeine/kg) obtained in this research, daily and once orally with a gavage absorbent needle starting on the seventh day of pregnancy (Organogenesis stage) until the 18th day of pregnancy (The day of the autopsy).

3. Results

The results of the study showed the incidence of partial and total abortion to varying degrees. The results of the current study showed miscarriage and fetal death at varying degrees in experimental groups dosed with the drinks used, as observed when aqueous solution of Arabic coffee was administered at a concentration of 4000 mg/kg body weight; the rate of abortion was 33 per cent and the death of some pregnant mice on the 15th day of pregnancy. The experimental group fed an aqueous solution of black tea at a concentration of 4000 mg/kg of body weight had a 29 percent miscarriage rate. On the 14th day of pregnancy, several pregnant mice had partial miscarriages and died.used, the current study found several abnormalities in white mouseembryos due to the various concentrations of the drinks use.

Figure a: embryo for control group

Figure b: The embryo of a deformed mouse is undefined. Dosed with an aqueous solution of Arabic coffee containing 4000 mg/kg body weight.

In terms of the findings of the study of abnormalities in embryos at various doses of the drinks used, the current study found several abnormalities in white mouse embryos due to the various concentrations of the drinks used. The embryos were elongated and enlarged, the fetus was elongated and deformed, and the embryos were mutated (Figures b, c).

Figure c: Completely mutated mouse embryo. Dosed with aqueous solution of Arabic coffee at a concentration of 4000 mg/kg of body weight.

Indicating that regular dosing of the aqueous solution of black tea caused deformities in pregnant mouse embryos and it curved in the shape of the letter C, and the emergence of mutated embryos that resemble the body of a fish (Figure d).

Figure d: A completely mutant mouse embryo with a fish-like body is shown from the front. Dosed with a 4000 mg/kg body weight concentration of black tea aqueous solution

Whereas the results showed that when dosed with Coca-Cola syrup, the percentage of mutilated embryos was different from what it is in coffee and tea, achieving (37-90 %) at concentrations of 3,4,6 ml/kg of body weight, and it was represented by the appearance of deformed embryos that looked like a water mermaid (Figure e) and distorted metamorphosed embryos (Figure f).

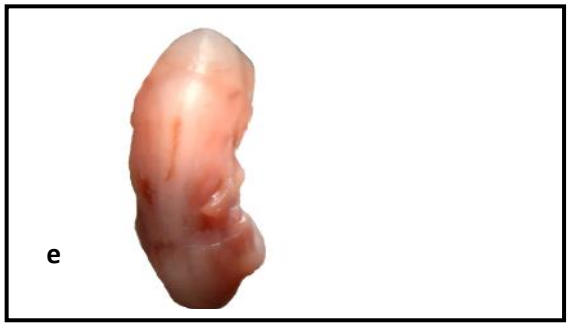

Figure e: A malformed water nymph-like mouse fetus missing front and hind limbs is seen from the back. Coca-Cola was given at a concentration of 4 mL per kilogram of body weight.

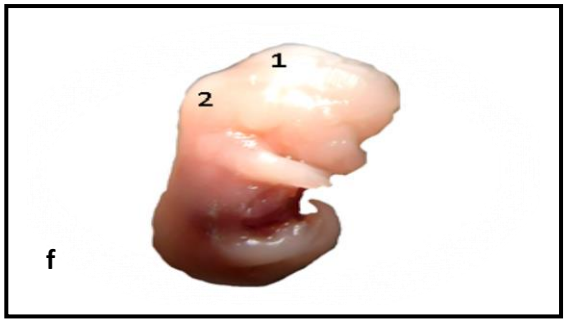

Figure f: A mutant mouse fetus with ischemic meningocele (1) and myelomeningocele in the dorsal anterior region is shown in a lateral view (2). Coca-Cola syrup was used as a dosing agent. 6ml per kilogram of body weight.

Results also showed small, deformed, mutated and incomplete embryos when dosed at a concentration of 6 ml/kg of body weight of Coca-Cola syrup ,Results also showed that the head was deformed by 75 %-85.5 %

when dosed with an aqueous solution of Arabic coffee, and that the appearance of a cleft lip (Figure g) The appearance of the head in the form of a blurred triangle (Figure h)

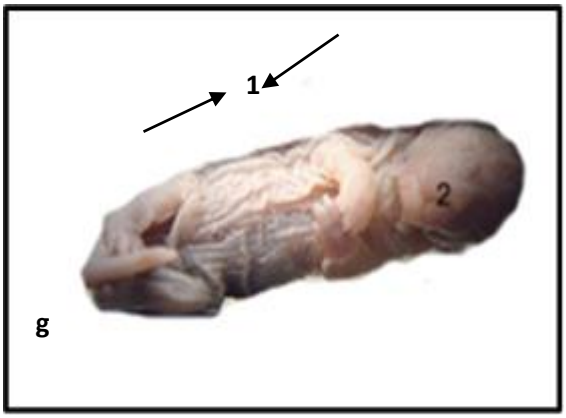

Figure g: Anterior view of a white mouse fetus reveals that the fetus appears elongated and enlarged (1), cleft lip (CL) (2). Dosed with an aqueous solution of Arabic coffee containing 2000 mg / kg body weight

Figure h: The head of a malformed mouse embryo stopped developing and took on an unclear triangular appearance. Dosed with 4000 mg of Arabic coffee per kg of body weight in an aqueous solution.

It reflects congestion and enlargement of the head in comparison to the rest of the body, distortion of the facial features of the nose, bulging eyes, and hemorrhage in the brain region when dosing with an aqueous solution of black tea and deformation of the head by (70%) (Figure i).

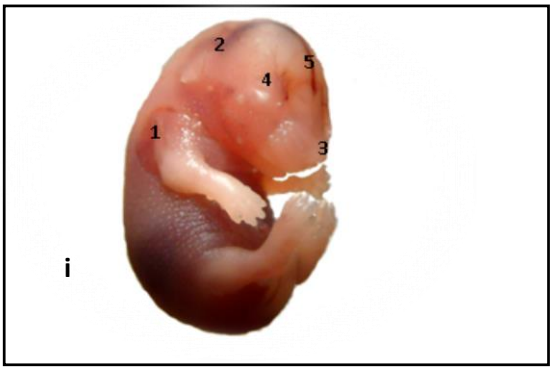

Figure i: Fetal curvature (1), congestion (CON) and enlargement of the head (2), deformation of nasal facial features (3), exophthalmos (4), and bleeding (BL) in the brain area (5) are all seen in this lateral view of a tiny white mouse fetus. Aqueous black tea solution at a concentration of 1000 mg / kg of body weight was given.

In addition to the formation of a head with a pointed end that resembles the beak of a bird, the brain is deformed by depression in the posterior cranial region of the skull (Figure k). Whereas, the severity of phenotypic head anomalies, when taken with Coca-Cola syrup, increased to a higher rate of 88% and was represented for the first time by the development of a new malformation, which is a cerebral meningocele in the form of a cystic tumor in the posterior brain region (Figure m), Compared to the control group (Figure a).

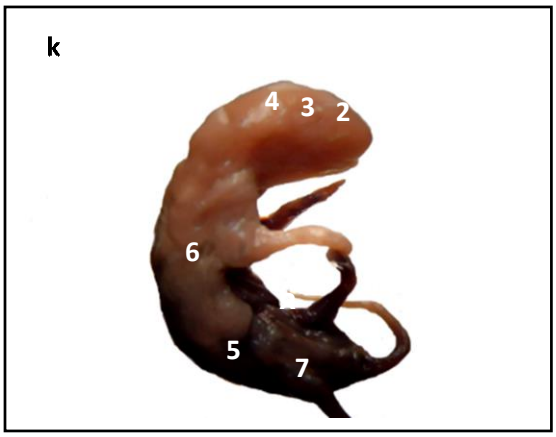

Figure k: Lateral figure of a white mouse embryo displaying round head with pointed end (1), occlusion of the eyes (2), loss of the auricle (3), posterior cranial depression (4), skin deformity and laxity (5), skeletal malformation, haemorrhage (7), abdominal atrophy and dissolution (8). At a concentration of 4000 mg / kg of body weight in an aqueous solution of blackte

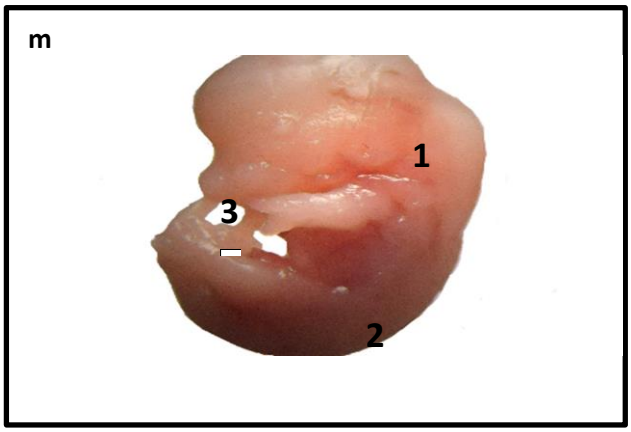

Figure m: Deformation and convexity of the trunk, differentiation of a congested cystic tumor (1), hyperemia of the dorsal area (2), and deformation and enlargement of the nasal characteristics are all visible in this lateral figure of a malformed mouse fetus (3). Coca-Cola was given at a concentration of 4 mL per kilogram of body weight.

4. Discussion

The study's findings revealed varied degrees of partial and entire abortion. The present study's findings revealed varying degrees of miscarriage and fetal mortality in experimental groups given the drinks. Excessive coffee intake and its caffeine content, which occurred at higher rates than other beverages, resulted in a doubling of the risk to the fetus within the uterus and a rise in the incidence of spontaneous and complete abortion during pregnancy (Hey ,2007), which could be due to the increase in contractions induced by stimuli containing caffeine. (Ullman *et al*., 1992).

The current study discovered several abnormalities in white mouse embryos due to various concentrations of the drinks used. The embryos were elongated and enlarged, the fetus was elongated and deformed, and the embryos were mutated, according to the findings of the study of abnormalities in embryos at various doses of the drinks used these findings backed up what was previously stated (Ormerod ,2001). Regular dosing of an aqueous solution of black tea produced abnormalities in pregnant mouse embryos, according to the findings. It curled in the shape of the letter C, as well. And the appearance of mutant embryos that resemble a fish's body and the reason may be attributed to the amount of caffeine in excessive doses of black tea, as indicated by the emergence of mutated embryos that resemble the body of (Grosso and Bracken,2005). When dosed with Coca-Cola syrup, the consequence was the emergence of malformed embryos that resembled a water mermaid, and distorted metamorphosed embryos. This may be due to the effect of Coca-Cola syrup, which contains caffeine in inhibiting growth during the stage of organogenesis, causing a lack of growth (Jana *et al.*,1994), or perhaps explaining the reason for increased fetal resorption and stunted growth of the fetus during the period of organogenesis on the sixth day of pregnancy (Al-Mamouri,2001), When Coca-Cola syrup was dosed at a concentration of 6 ml/kg of body weight, the results revealed tiny, malformed, mutated, and incomplete embryos and the cause may be attributed to damage to developing placenta that begins to function on the ninth day of pregnancy. Decreases in the exchange of nutrients between the fetus and the mother and reduces the process of protein building in the embryos, leading to delayed growth, low weights and the appearance of small embryos (Padmanabhan *et al*.,1981).

When given an aqueous solution of Arabic coffee, the head was deformed by 75 percent to 85.5 percent, and the appearance of a cleft lip was also seen this was similar to that indicated by (Matijasevich *et al*.,2005). The head appears to be in the shape of a blurring triangle and this combined with what the researcher pointed out (Hammoudi, 2005). When given an aqueous solution of black tea, it causes congestion and growth of the head in relation to the rest of the body, distortion of the facial characteristics of the nose, bulging eyes, and hemorrhage in the brain region, as well as deformation of the skull (70 %). The brain is deformed by depression in the posterior cranial region of the skull, in addition to the creation of a head with a pointed end that resembles a bird's beak, and that's in line with what reported in Fazal and Jalali, 2002.

When combined with Coca-Cola syrup, the severity of phenotypic head malformations grew to an 88 percent rate, which was represented for the first time by the development of a novel malformation, a cerebral meningocele in the shape of a cystic tumor in the posterior brain region this perhaps the explanation is due to the combination of maternal consumption of Caffeine-containing stimuli and the risk of developing brain myeloma (Rogwei *et al*., 2011).

5. Conclusion

The study showed that caffeinated drinks have congenital anomalies on the fetus and the pregnant mother should be careful when consuming.

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Conflict of interest: The authors declare that there are no conflicts of interest.

References

Al-Mamouri, Rafah, Hani Abdel-Latif. (2001). The effect of alcohol on implantation of embryos and fertility in the mouse. *Master Thesis*, College of Medicine, Tikrit University, Iraq.

AL-Rasheedi, A.A.(2009).study of effect of energy drinks on Biochemical and histological marks in Rats. Arab. J. Chem. 2 (1):113-126.

Ashmead ,T. M.(2003).Smoking and pregnancy .J. mater. Fet. and Neon. Medic.;14:297-304.

Bech, B. H., Nohr, E. A., Vaeth, M., Henriksen, T. B., & Olsen, J. (2005). Coffee and fetal death: a cohort study with prospective data. American journal of epidemiology, 162(10), 983–990. https://doi.org/10.1093/aje/kwi317 .

Bech, B. H., Obel, C., Henriksen, T. B., & Olsen, J. (2007). Effect of reducing caffeine intake on birth weight and length of gestation: randomised controlled trial. BMJ (Clinical research ed.), 334(7590), 409. https://doi.org/10.1136/bmj.39062.520648.BE.

Bierrnacki,B.; Wofodarezyk.; B. and Minta,M.(2000).Effect of sodium valporate on rat embryo development invitro. Bull .Vet. Inst. Pulawy .,44:201-205.

CARE Study Group (2008). Maternal caffeine intake during pregnancy and risk of fetal growth restriction: a large prospective observational study. BMJ (Clinical research ed.), 337, a2332. https://doi.org/10.1136/bmj.a23329.

Higdon, J.V.; and Frei, B.(2006) Coffee and health:A review of recent human research. Crit Rev Food Sci Nutr.;46(2):101-123.

Fazal ,A.; and Jalali ,M. (2002). Experimentally – induced exencephaly and spina bifida in mice. Archives of Iranian-medicine,5(3),179-183. https://www.sid.ir/en/journal/ViewPaper.aspx?id=13074.

Greenberg, J. A., Boozer, C. N., & Geliebter, A. (2006). Coffee, diabetes, and weight control. The American journal of clinical nutrition, 84(4), 682–693. https://doi.org/10.1093/ajcn/84.4.682 .

Grosso, L. M., & Bracken, M. B. (2005). Caffeine metabolism, genetics, and perinatal outcomes: a review of exposure assessment considerations during pregnancy. Annals of epidemiology, 15(6), 460–466. https://doi.org/10.1016/j.annepidem.2004.12.011.

Hammoudi, Hani Malallah. (2005). Study of the effect of paracetamol (acetaminophen) on the embryonic development of the Swiss white rat Mus musculus. Journal of Education and Science, Volume (7), Issue 1: 165-149.

Hey E. (2007). Coffee and pregnancy. BMJ (Clinical research ed.), 334(7590), 377. https://doi.org/10.1136/bmj.39122.395058.80.

Jana, N., Vasishta, K., Jindal, S. K., Khunnu, B., & Ghosh, K. (1994). Perinatal outcome in pregnancies complicated by pulmonary tuberculosis. Int J Gynaecol Obstet, 44(2), 119–124. https://doi.org/10.1016/0020-7292 (94)90064-7

Matijasevich, A., Santos, I. S., & Barros, F. C. (2005). Does caffeine consumption during pregnancy increase the risk of fetal mortality? A literature review. Cadernos de saude publica, 21(6), 1676–1684. https://doi.org/10.1590/s0102-311x2005000600014

Ormerod P. (2001). Tuberculosis in pregnancy and the puerperium. Thorax, 56(6), 494–499. https://doi.org/10.1136/thorax.56.6.494

Padmanabhan, R., Singh, G., & Singh, S. (1981). Malformations of the eye resulting from maternal hypervitaminosis A during gestation in the rat. Acta anatomica, 110(4), 291–298. https://doi.org/10.1159/000145439

Rogwei, Y.E.; Aiguo, R.; Zhang, Le.; Zhiwen, Li.; Jianmeng, Liu.; Lijun Pei.; and Xiaoying ,Z.(2011).Tea Drinking as a Risk Factor for Neuraltube Defects in Northern China. Epidemiology. 22. (4);22:491-496.

Ullman, A., Beutsch, J. Wilber T.D. (1992). The role of drug R4486 in early miscarriage. Journal of Science, 8(7): 19-12.

Weng X, Odouli R, Li DK. (2008).Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. Am J Obstet Gynecol. 198(3):279.e1-8. doi: 10.1016/j.ajog.2007.10.803. Epub 25. PMID: 18221932.

Yen WJ, Wang BS, Chang LW, Duh PD. (2005).Antioxidant properties of roasted coffee residues. J Agric Food Chem. 6;53(7):2658-63. doi: 10.1021/jf0402429.

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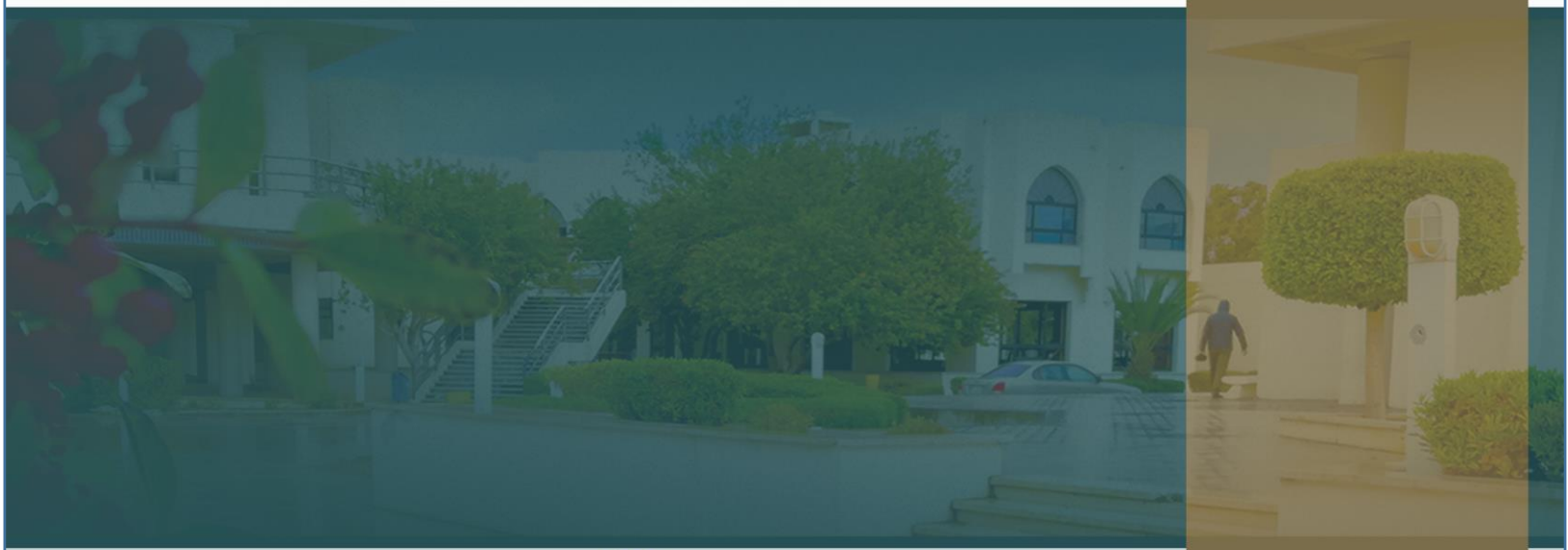

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