Effect Of Nifedipine on The Phenotypic Changes of Fetuses and on the Functions and Histological Formation of The Kidneys and the Protective Role of Cold Hibiscus Infusion Against It in Pregnant Albino Female Rats

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Abstract
The current study aimed to investigate the effect of nifedipine in causing morphological deformities in fetuses and changes in functions and histological formation in the kidneys of pregnant female rats and the protective role of aqueous infusion of hibiscus plant against them. Where this study showed the occurrence of negative changes when using the drug nifedipine at a concentration of 10 mg / kg in the phenotype of albino rat fetuses represented by abnormal elongation in the neck region with swelling in the abdomen region and enlargement in the head region with the disappearance of facial features and atrophy of the forelimbs, Changes in kidney function, as we notice a rise in the percentage of urea and creatine at levels higher than normal levels, as well as changes in the histological formation of the kidneys. Hemorrhage appears between the renal tubules and infiltration of inflammatory cells (IF) Infammatory cells with the presence of giant multinucleated phagocytic cells. As for the cold-water soak of the hibiscus plant 150 mg / kg, it had a positive effect, as the external appearance of the embryos was normal, and there were no changes in the kidney functions, as well as the tissue formation, which was normal.

1. Introduction

The term congenital malformations or defects is used to define abnormal physiological developments to describe the metabolic, functional and structural behavioral diseases that may exist at the birth of the fetus and that may increase the chance that the organism will be born with a congenital defect. The science that studies defects is known as Teratology. The term Teratogenesis stems from the Greek, meaning teratos. These abnormalities may be latent and obvious at birth, due to the combination of external or internal factors during prenatal developmental processes (Sadler, 2015). Some drugs have a major role in causing damage to fetal growth during pregnancy, although medical drugs are among the most important things that women need during that sensitive period, but they may cause many health problems for them in the long run and for the fetus as well. Many studies conducted on pregnant women confirmed the extent of the effect of medical drugs on them. Studies have shown that these drugs can cause a lot of problems for the fetus and can lead to deformities and miscarriages in many cases (Podymow & August 2008). Therefore, we conducted an experiment to find out the effect of the drug nifedipine, which is a drug considered a calcium blocker, as it prevents the flow of calcium ions through the membrane from the cell to the outside, as calcium ions do not reach in this way the smooth muscle cells in the heart muscle and blood vessels, which causes expansion in the coronary arteries and reduce muscle contraction. The heart, (Croom et al., 2006). Because of the mechanism of action of nifedipine in this way, it is appropriate to use it for the treatment of diseases of blood pressure, blood vessels, angina pectoris and hypertension (Varshosaz et al., 2002). The chemical characteristics are the common name of the drug is nifedipine and the chemical formula is C17H18N2O6 and its chemical name is dimethyl 6,2-dimethyl-4-(2-nitrophenyl)- 1,4-dihydropyridine-5,3-dicarboxylate and the molecular weight is 346.335 g / mol and the appearance of the drug is a white crystalline powder (Aderibigbe et al., 2012).It may be inaccurate use of drugs, and a number of these drugs sometimes work on the disappearance of the symptoms of the disease while the disease remains latent and can turn into a chronic condition, in addition to that some drugs have a negative effect on the body's immunity in its resistance to other diseases (Dar et al, 2017) Therefore, it was resorted to traditional medicine, where plants were used, because one of the most important advantages of treatment with plant extracts is the reduction of side effects that sometimes accompany industrial pharmaceutical compounds. 1992). For this reason, medicinal plants are characterized by a greater capacity than combined drugs in the treatment of many incurable diseases (Cacerves, 1991). The hibiscus plant (Rose rose) belongs to the Malvaceae family. It is known by various simultaneous and colloquial names, such as hibiscus, Indian Rumex vesicarius, Jamaican Rumex

doi.org/10.31838/hiv23.01.126 Received: 18.09.22, Revised: 19.10.22, Accepted: 20.12.22

vesicarius, Guinean Rumex vesicarius, red Rumex vesicarius, mesta (Sanou et al., 2022 .(It is also recognized as an important source of value-added compounds such as natural pigments and bioactive compounds from which it is isolated is of great importance in the food and pharmaceutical industries. In fact ,Many previous studies showed that H. sabdariffa is a treatment for many conditions, including high blood pressure, due to the presence of anthocyanin and proanthocyanidin compounds, as it can form biologically active compounds responsible for lowering blood pressure, as previous studies demonstrated the importance of the anthocyanin compound by inhibiting angiotensin-converting enzyme Hence the effect of vasodilation (Jonadet et al. 1990).

2. Materials and Methods

Preparing the watery infusion for hibiscus flowers

The process of preparing the infusion was conducted, where the dried hibiscus flowers are taken and converted into a fine powder using an electric grinder from AL-Araby 50 grams of fine vegetable powder was taken and placed in a glass beaker with a capacity of 1000 ml, and 100 ml of distilled water was added to it, after which it was soaked for six hours.Then the infusion is filtered through several layers of gauze. The process is repeated several times to obtain a sufficient amount of infusion, then it is kept in dark containers at a temperature of (-20) C until the experiment is conducted.

Animal preparation

In this study, (9) female white rats, aged between 10-12 weeks, with weights ranging from 150 to 200 grams, were used in this study. They were placed with males of the same breed in plastic cages at the onset of sunset and it was followed up until mating occurred. In addition, it was confirmed that mating occurred through the formation of the vaginal plug, which is considered evidence of the occurrence of fertilization, and in the next morning, the females are counted on the zero day of pregnancy. Then, it was distributed into three groups, with three females for each group. The animals were treated with the special treatments for the experiment from the fifth day of pregnancy to the tenth day, and the first group was the control group, which was treated with distilled water only. The second group was treated with nifedipine at a concentration of (10 mg/kg) once a day orally by oral tube feeding. The third group was treated with aqueous infusion of hibiscus plant at a concentration of (150 mg/kg) orally by oral tube feeding. All experimental animals were fed on the 19th day of pregnancy, the experimental animals were sacrificed for the purpose of collecting samples and drawing blood to conduct physiological and histological tests, to know the pathological and preventive effects that occurred in the studied groups.

Preparation of histological sections

Kidney tissue sections were prepared from pregnant females and fixed directly in formalin solution (10% formalin + 90% tap water) for 24 hours.The tissue pieces were prepared, which were excised, according to what was reported by (Al-Tarda and his group 2003), which were determined by the following operations:

The samples were washed with tap water for 45 minutes in order to remove the fixative from the excised tissues, then these washed samples were passed to different concentrations of ethyl alcohol in ascending order 70%, 80%, 90%, 95% and 100%. For half an hour for each concentration, except for absolute alcohol, twice every half hour, for the purpose of withdrawing water molecules or dehydration, and in order to make the samples more transparent and clearer, they are transferred to the Clearing stage. This is done by placing the samples in xylene for a period of (30-45) minutes, followed by the infiltration stage, where the tissue pieces were passed through a mixture of xylene and paraffin wax, melting point (58-60 °C), in a ratio of 1:1, and the mixture was placed in an electric oven at a temperature of 60 °C. Celsius for a period of (15) minutes, after which the samples were transferred to unused molten wax twice for a period of 45 minutes each time.

Followed by the embedding stage, where the molten paraffin wax was placed in iron molds special for this stage, in the form of the letter (L), and the information about the member was written on a sheet of paper and placed in the mold. To harden completely, these molds were placed in a rotary microtome and cut to a thickness of (5) µm. The strips of sections were placed with a dissecting needle and spread over water in a water bath device at a temperature of 37-40 °C, after which the sections were carried on the glass slide marked with a diamond pen, after wiping them with Meyer's salt and cholesterol (which is prepared from egg whites 50 ml, glycerol 50 ml, and thymol 1). Ml, after mixing the egg whites and glycerol, the mixture was well filtered through layers of medical gauze, after that thymol was added to it to prevent the growth of bacteria and fungi. After that, the slides were passed on xyol for 5-10 minutes in order to remove the paraffin wax, then left to dry. After that, these slides were passed on several concentrations of ethyl alcohol, but in a descending manner 100%, 95%, 70%, and 35%, not usually water, for (5) minutes in each concentration, then a drop or two of hematoxylin stain was added for a period of 7-8 minutes, after that the eosin stain with a duration of 30-60 seconds, Then it was washed with water to remove the excess dye, then the glass slides were re-passed in ethyl alcohol in ascending concentrations (70-100%) for two minutes.(D.PX) was used for docking purpose; As it dries faster, then it was covered with a cover slip, after which the slices were

dried on a hot plate at 40 degrees Celsius (Shaaban and Hamid, 2018).

3. Results and Discussion

kidney function

1-4-4 Concentration of urea in the blood serum:

The results of the current study, as shown in Figure (1-1), showed a significant increase (p≤0.05) in the urea concentration in the group treated with nifedipine 10 mg/kg (mg/dl (50 ± 2.516) when compared to the control group (44 ± 3.605) mg/ dl The study also showed that there was a significant decrease in urea concentration in the group treated with cold water infusion of hibiscus plant 150 mg/kg (43.677 ± 3.055) mg/dl when compared to the control group and the drug-treated group.

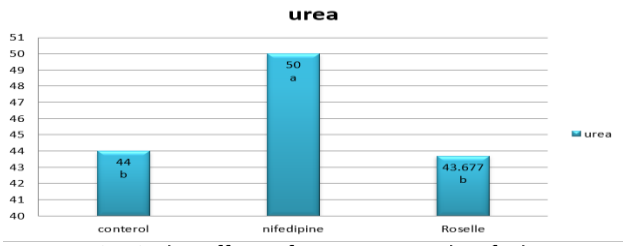


Figure (1-1) The effect of treatment with nifedipine 10 mg/kg and an aqueous decoction of hibiscus plant at a dose of 150 mg/kg on the effectiveness of urea concentration in the blood serum of pregnant female rats.

The different letters at the end of the columns indicate the presence or absence of significant differences at the level (p≤0.05).

2-4-4 Creatinine concentration in blood serum

The results of the current study, as shown in Figure (4-7), showed that there was no significant difference (p≤0.05) in the urea concentration in the group treated with nifedipine 10 mg/kg (mg/dl (0.627 ± 0.03) when compared to the control group (0.563 ± 0.04)mg. /dl, as the study showed that there was no significant difference in the concentration of urea in the group treated with cold water infusion of hibiscus plant 150 mg / kg (0.533 ± 0.02) mg / dl when compared to the control group and the group treated with nifedipine

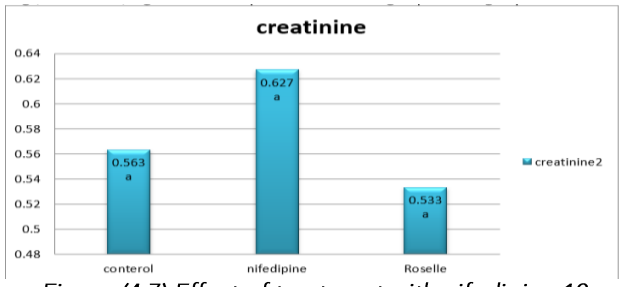


Figure (4-7) Effect of treatment with nifedipine 10 mg/kg and an aqueous decoction of hibiscus plant at a dose of 150 mg/kg on the activity of creatinine concentration in the blood serum of pregnant female rats.

The different letters at the end of the columns indicate the presence or absence of significant differences at the level (p≤0.05). This effect may be due to the renal dysfunction caused by nifedipine, possibly by blocking calcium entry into renal vascular smooth muscle cells, resulting in a modulated response to hormones such as norepinephrine and angiotensin II, Closely connected to the glomerulus, it modulates filtration and regulates renal blood flow. Alternatively, it may be mediated through the effect of nifedipine on the synthesis of prostaglandins, which leads to an increase in urea or a defect in renal function, as these results agree with (Del Rio et al., 1986) who used nifedipine to know its effect on the kidneys. While the watery infusion had a preventive role in preserving the kidney tissues from damage that may be caused by the treatment through a decrease in the level of urea and creatine, which indicates the integrity of the tissues. The hibiscus plant contains chlorogenic acid that can protect the kidneys through down-regulation of the p53 transcription factor, thus relieving oxidative stress, inflammation, and apoptosis in the renal tubules (Bhattacharyya et al., 2020).

4- The results of the study of kidney tissue in pregnant females. Histological changes on Kidneys in Pregnant females

1- Histological changes in the kidneys of the control group

Microscopic testes of the kidneys of a control group showed renal glomeruli (G), proximal convoluted tubules (PCT), distal convoluted tubules (DCT), space around the glomerulus (C) and the collecting tubule (picture 1-1).

2- Histological changes in the kidneys of the nifedipine group

Microscopic tests of the kidneys of the group treated with the drug 10 mg/kg showed a section of the kidney of the group treated with the drug nifedipine showing hemorrhage between the renal tubules and infiltration of inflammatory cells (IF) Infammatory Cell with the presence of giant multinucleated phagocytic cells (Picture (-21)

3- Histopathological changes in the kidneys of the group treated with hibiscus aqueous infusion

Microscopic examination of the kidneys of the group treated with aqueous decoction of hibiscus plants showed that there was no difference from the control group, where the renal glomeruli (G), the proximal convoluted tubules (PCT), the distal Convoluted tubules (DCT), the space around the glomerulus (C) space, and the collecting canal were visible. Tubule all appear normally as in the picture (3-1)

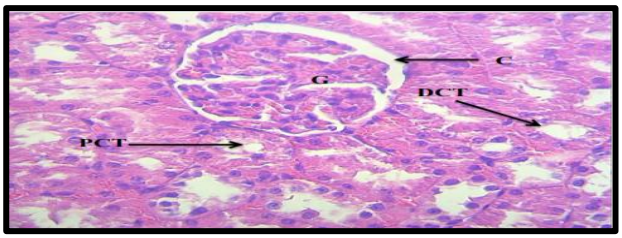
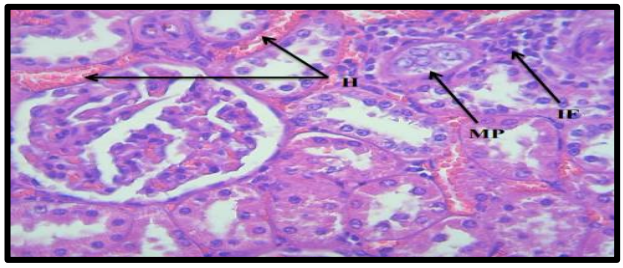
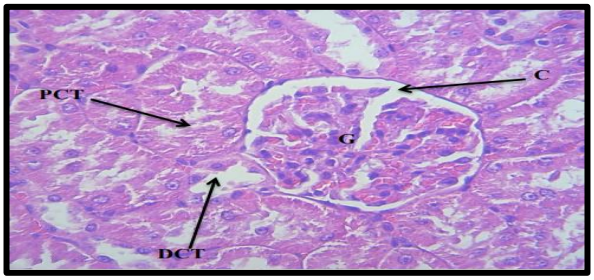


Figure 1-1. Section of a control group kidney showing the renal glomerulus (G), proximal and distal convoluted tubule (PCT) and distal convoluted tubule (DCT), and space around the glomerulus (C). H&E 400X,



Picture (1-2) a section of the kidney of the nifedipine treated group showing hemorrhage between the renal tubules (H) and infiltration of inflammatory cells (IF) with the presence of giant multinucleated macrophages (MP). H & E 400X



Picture (1-3) section of the kidney of the group treated with hibiscus plant infusion showing the renal glomerulus (G), the proximal and distal convoluted tubule (PCT) and the distal convoluted tubule (DCT), and the space around the glomerulus (C). H & E 400X

In the current study, it was found that the administration of nifedipine caused changes in the kidney tissue, where it led to the appearance of hemorrhage between the renal tubules and the infiltration of inflammatory cells (IF). Infammatory Cell with the presence of giant multinucleated phagocytic cells, and thus leads to disruption or weakness in kidney function, and thus leads to an increase in the proportion of urea and creatine (Hayashi et al, 2007). The current study also showed the protective role of the hibiscus plant, because it contains phenols and flavonoids, which work to protect the tissues of the kidneys and liver from damage that may result from the drug (Oršolić N et al., 2010).

Embryos Variations

4-8-1 Embryos Phenotypic Variation

1 The control group

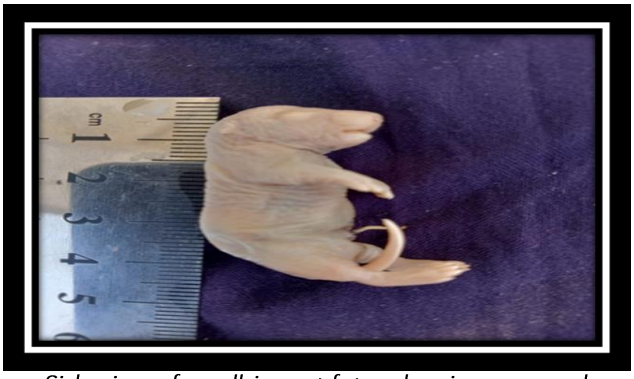
The fetuses appeared in the control group in normal numbers and were distributed on the two horns of the uterus almost equally.

2- The group treated with nifedipine

The group treated with nifedipine showed a set of deformities, the most prominent of which was abnormal elongation in the neck area with swelling in the abdomen area and enlargement in the head area with the disappearance of facial features and atrophy of the forelimbs as in the picture

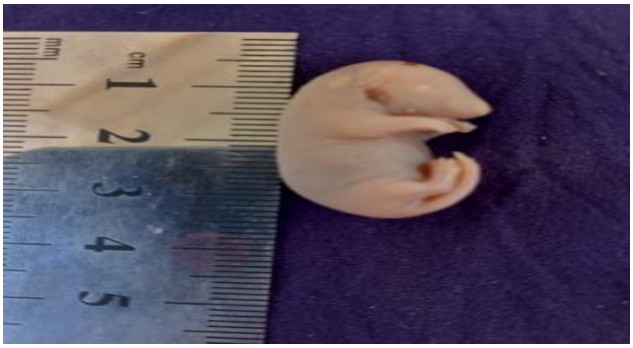
3- The group treated with the hibiscus plant infusion

The embryos appeared in the group treated with water soaked hibiscus plant. There was no difference from the control group. The size of the embryo was about 3 cm, with clear features in terms of the head, snout, trunk, front and back limbs, moderate spine and tail, as in the picture.



Side view of an albino rat fetus showing a normal control *fetus*

The fetus treated with nifedipine has a swollen tail, abnormal elongation in the neck, and swelling in the abdomen.



It shows the embryo treated with the aqueous infusion of the hibiscus plant

Occasionally nifedipine may cause reactive oxygen species (ROS) in the trunk region that showed marked growth arrest, indicating that tissues under stress are likely to produce pathological ROS as in the study by Robinson et al,.2020. This study also agrees with Chihara et al., 1998, who administered oral nifedipine to pregnant rats from the seventh to the seventeenth day of pregnancy and dissected the pregnant rats on the 19th day of pregnancy and noted the phenotypic changes on the fetuses that appeared, which are deformities in the forelimbs .As for the watery soak of the hibiscus plant, it did not affect the visual appearance of the fetus, because it contains antioxidants and effective compounds such as flavonoids and phenols that have a role in increasing the activity of insulin-like growth factor IGF-1, which plays an important role in the growth

and development of tissues (Sair et al, 2022;Xu et al 2021), Thus, it increases the AKt phosphorylation pathway, which works to increase the metabolism of fats and glucose, which leads to the occurrence of normal growth of the fetus. Thus, the decoction inhibits the teratogenic effect of nifedipine, as the fetuses are fully developed and of normal size.

4. Conclusions

1- The use of nifedipine during pregnancy has negative effects in causing fetal deformities, an increase in kidney function, and changes in the kidney tissue.
2- Using the hibiscus plant during pregnancy leads to positive results, as all results are positive

5. References

Al-Tarda, Mahmoud Muhammad, Al-Ratrout, Osama Khaled, Othman, Jamal Muhammad Abudayyah, Muhammad. (2009), Basics of Histological Preparation, Dar Al-Thaqafa, Amman. Jordan

• Shaaban, Rashed Khamis Hamid, Aziz Khaled. (2018) Study of the effect of obesity on the livers and kidneys of white rat fetuses and the possibility of treating these effects using grape seed oil (phenotypic and histological study). Tikrit Journal of Pure Sciences, Volume 22, Issue 4, p. 1

Sadler TW (2015) Birth defects and prenatal diagnosis, Chapter 9. In: Sadler TW (ed) Langman's medical embryology, 13th edn. Lippincott Williams & Wilkins, Philadelphia

Croom, Katherine F., and Keri Wellington. "Modified-release nifedipine." *Drugs* 66.4 (2006): 497-528.

Podymow, T., & August, P. (2008). Update on the use of antihypertensive drugs in pregnancy. *Hypertension*, *51*(4 PART 2 SUPPL.), 960–969. https://doi.org/10.1161/HYPERTENSIONAHA.106.075895

Dar. R.A, Shahnawaz. M, Qazi. P.H General overview of medicinal plants: A review. The Journal of Phytopharmacology. 6 (6), 2017, 349-351.

Unesco, (1992). Seventh Asian Symposium on Medicinal Plants spices and other natural products, In Unesco Sources, (35), March

Cacerves, A., Lopez, B Giron, M., and logemann, H. (1991). Plants used in Gnatemale for the Treatment of Dermatophytic Infection: Secreening for Antimycotic activity of 44 plant extracts, Journal of Ethnopharmacology, 31, p.p. 263-276

Aderibigbe, S. A., Adegoke, O. A., & Idowu, O. S. (2012). A new colorimetric method for the determination of nifedipine tablets by derivatization using 4-carboxyl-2, 6-dinitrobenzenek8 diazonium ion. *International Journal of Industrial Chemistry*, *3*(1), 1-8.

Sioud, F., Ben Toumia, I., Lahmer, A., Khlifi, R., Dhaouefi, Z., Maatouk, M., ... & Chekir-Ghedira, L. (2020). Methanolic extract of Ephedra alata ameliorates cisplatin-induced nephrotoxicity and hepatotoxicity through reducing oxidative stress and genotoxicity. *Environmental Science and Pollution Research*, *27*(11), 12792-12801.

Jonadet, M., Bastide, J., Bastide, P., Boyer, B., Carnat, A.P., Lamaison, J.L., 1990. In vitro enzyme inhibitory and in vivo cardioprotective activities of Hibiscus (Hibiscus sabdariffa L). Pharm. Belg. 45, 120–124.

Del Rio, A., Romero, R., Novoa, D., & Arcocha, V. (1986). Nifedipine and renal function. *Nephron*, *44*(1), 78-79.

Bhattacharyya, S., Kumar, R., Sengupta, G., Hazra, A. K., & Sur, T. K. (2020). Chlorogenic acid enriched green coffee ameliorated renal injury in rats. *Mymensingh Med J*, *29*(4), 991-1000.

Hayashi, K., Wakino, S., Sugano, N., Ozawa, Y., Homma, K., & Saruta, T. (2007). Ca2+ channel subtypes and pharmacology in the kidney. *Circulation research*, *100*(3), 342-353.

Oršolić, N., Benković, V., Lisičić, D., Đikić, D., Erhardt, J., & Horvat Knežević, A. (2010). Protective effects of propolis and related polyphenolic/flavonoid compounds against toxicity induced by irinotecan. *Medical oncology*, *27*(4), 1346-1358.

Robinson, B. L., Gu, Q., Tryndyak, V., Ali, S. F., Dumas, M., & Kanungo, J. (2020). Nifedipine toxicity is exacerbated by acetyl l-carnitine but alleviated by low-dose ketamine in zebrafish in vivo. *Journal of Applied Toxicology*, *40*(2), 257-269.

Chihara, K., Funabashi, H., Mukumoto, K., Imura, Y., & Matsuoka, N. (1998). Effects of calcium channel blockers on embryo-fetal development in rats. *Toxicology Letters*, *95*(1001), 209-210.

Sair, A. T., & Liu, R. (2022). Molecular regulation of phenolic compounds on IGF-1 signaling cascade in breast cancer. Food & Function

Xu, Z., Yang, H., Poolsawat, L., Rahman, M. M., Xu, X., Jiang, X., ... & Leng, X. (2021). Flavonoid-enriched diets improved the growth and flesh quality of grass carp (Ctenopharyngodon idellus) based on metabolomics. Aquaculture Nutrition, 27(6), 2514-2528