



The Barberry Fruit Extract and Metformin as a Protective Effect on the Embryonic Development and Kidney Tissues Functioning in a Rat Model of Experimental Gestational Diabetes

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Citation: *Zainab Hasan Majeed. The Barberry Fruit Extract and Metformin as a protective effect on the embryonic development and kidney tissues functioning in a rat model of experimental gestational diabetes.*

Al-Kitab J. Pure Sci. [Internet]. 2026 Mar. 28 ;10(2):184-202 DOI.

<https://isnra.net/index.php/kjps/article/view/2026>.

<https://doi.org/10.32441/kjps.10.2.p14>

Keywords: Metformin, Gestational diabetes, Barberry, Fruit, kidney

Article History

Received 29 Jan. 2026

Accepted 28 Mar. 2026

Available online 01 Nov. 2026

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Abstract:

This study was carried out to assess the effects of *Berberis vulgaris* fruit extract and metformin on maternal metabolic parameters, fetal growth and organ development under the condition of gestational diabetes in albino rats. Gestational diabetes has been induced with a high-fat/ high-sugar diet (HFHS diet) that was followed by administration of streptozotocin. Pregnant rats were divided into 5 groups: control, HFHS diet, HFHS + metformin, HFHS + barberry extract, and HFHS + metformin + barberry extract. Maternal body weight, fasting blood glucose and insulin resistance were measured as well as fetal growth and placental efficiency. Histological study of fetal kidneys and skeletal staining was carried out in order to assess organ development and skeletal development. Results showed that the HFHS diet caused maternal hyperglycemia, insensitivity to insulin, deficiency in placental growth and organ abnormalities in fetuses. Both metformin and barberry extract alone improved maternal metabolic profiles and fetal outcomes, however, the combination of both had the greatest improvements including normalized glucose levels in maternal blood, improved fetal growth, and near complete normalization of kidney histology and skeletal ossification. These results

indicate the synergistic effect of barberry extract and metformin for reducing the negative impact of gestational diabetes on fetal development.

Keywords: Metformin, Gestational diabetes, Barberry, Fruit, kidney.

مستخلص ثمار البرباريس والميتفورمين كعامل وقائي على نمو الجنين ووظائف أنسجة الكلى في نموذج جرذ مصاب بسكري الحمل التجريبي

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الخلاصة:

أُجريت هذه الدراسة لتقييم تأثير مستخلص ثمار البرباريس الشائع (*Berberis vulgaris*) والميتفورمين على المؤشرات الأيضية للأمهات، ونمو الجنين، وتطور أعضائه في حالة سكري الحمل لدى فئران بيضاء. تم تحفيز سكري الحمل عن طريق اتباع نظام غذائي غني بالدهون والسكريات، تلاه إعطاء الستربتوزوتوسين. قُسمت الفئران الحوامل إلى خمس مجموعات: مجموعة ضابطة، ومجموعة نظام غذائي غني بالدهون والسكريات، ومجموعة نظام غذائي غني بالدهون والسكريات مع الميتفورمين، ومجموعة نظام غذائي غني بالدهون والسكريات مع مستخلص البرباريس، ومجموعة نظام غذائي غني بالدهون والسكريات مع الميتفورمين ومستخلص البرباريس. تم قياس وزن جسم الأم، ومستوى سكر الدم الصائم، ومقاومة الأنسولين، بالإضافة إلى نمو الجنين وكفاءة المشيمة. أُجريت دراسة نسيجية لكلى الجنين وتلوين الهيكل العظمي لتقييم تطور الأعضاء والهيكل العظمي. أظهرت النتائج أن النظام الغذائي الغني بالدهون والسكريات تسبب في ارتفاع سكر الدم لدى الأمهات، وانخفاض حساسية الأنسولين، ونقص نمو المشيمة، وتشوهات في أعضاء الأجنة. أدى كل من الميتفورمين ومستخلص البرباريس على حدة إلى تحسين المؤشرات الأيضية للأمهات ونتائج الحمل، إلا أن الجمع بينهما حقق أكبر قدر من التحسن، بما في ذلك استقرار مستويات الجلوكوز في دم الأم، وتحسين نمو الجنين، وعودة شبه كاملة لوظائف الكلى وتكوين العظام. تشير هذه النتائج إلى التأثير التآزري لمستخلص البرباريس والميتفورمين في الحد من التأثير السلبي لسكري الحمل على نمو الجنين.

الكلمات المفتاحية: ميتفورمين ، سكري الحمل ، برباريس ، فاكهة ، كلى.

1. Introduction:

Diabetes in pregnancy is a major maternal and fetal health problem that impacts the organogenesis and general development of the fetus [1;2]. Gestational hyperglycemia is connected with the presence of oxidative stress and the disruption of the expression of genes and metabolic imbalance which can lead to the development of congenital malformation such as defects of the neural tube, heart, and skeletal system during early pregnancy [3]. Later on, uncontrolled maternal hyperglycemia may result in macrosomia, intrauterine growth retardation

and preterm births[4]. These complications highlight the significance of proper glycemic control during pregnancy in order to facilitate the best fetus outcome [5].

Metformin is a common pharmacological agent in the management of blood glucose levels among people who have type 2 diabetes and gestational diabetes [6]. It reduces glucose levels by a variety of processes, including the hepatic gluconeogenesis inhibition, increased peripheral glucose uptake and activation of AMP-activated protein kinase (AMPK), which is a marker of cellular energy homeostasis [7]. Also, metformin decreases intestinal glucose absorption which helps to regulate postprandial spikes in glucose levels. Although this treatment is effective, more interest is on natural-based complementary and alternative treatments that could have some other metabolic advantages with fewer side effects [8].

Among promising natural agents, there is barberry (*Berberis vulgaris*), a natural plant, which is a member of the family Berberidaceae and contains such pharmacologically active compounds as berberine, berbamine and jatrorrhizine [9]. The compounds have hypoglycemic, hypolipidemic, antioxidant, anti-inflammatory and antimicrobial properties. Particularly, berberine was also found to induce greater sensitivity to insulin, lower cholesterol, and protect the organs against oxidative stress [10]. Barberry extract is a possible therapy to be used alongside traditional antidiabetic agents in pregnancy due to its complex pharmacological characteristics [11]. The current study will examine the effects of barberry fruit extract as a mono and as a combination with metformin on maternal metabolic health, fetal growth, placental performance and organ development on a rat gestation diabetes model. Through discussion on biochemical, histological, and skeletal parameters, this study aims at giving an understanding of the possible synergistic effects, in terms of the combination of the conventional pharmacotherapy and plant-derived bioactive compounds in the future, in dose of providing safer and more effective management measures against gestational diabetes and its negative impact on offspring development.

2. Material and methods:

2.1. Experimental animals and housing conditions

The age-matched adult albino rats (10-week old) of both sexes and with similar body weights were acquired in the College of Veterinary Medicine of Tikrit University. The animals were kept in normal laboratory conditions and allowed to acclimatize after which the experiment started. During the study, rats were allowed to have access to food and water freely.

2.2. Experimental design

- both metformin and barberry extract.

Pregnant rats were randomly separated into 5 experimental groups (n= 6/group) as follows:

- Control group: fed a regular laboratory diet.
- HFHS group: Nourished on a high-fat, high-sugar diet.
- HFHS + Metformin group: fed the HFHS diet, metformin.
- HFHS + Barberry extract group: was administered HFHS diet and barberry extract.
- HFHS + Metformin + Barberry extract group: fed the HFHS diet and both Metformin and barberry extract were given

2.3. Dietary regimen and induction of gestational diabetes

Rats were placed on a high-fat, high-sugar (HFHS) diet for six weeks. The HFHS diet was composed of 66.5% normal diet, 10% animal fat, 2.5% cholesterol, 20% carbohydrate, Sucrose, and 1% chocolate. Following the dietary period mating was conducted and pregnancy was confirmed by the presence of a vaginal plug. The experiment did not include the non-pregnant females. A single intraperitoneal injection of streptozotocin (25mg/kg body weight) freshly dissolved in 0.1 M sodium citrate buffer [12] was used to induce gestational diabetes.

2.4. Barberry fruit extract and metformin preparation and administration.

The fresh berries of barberry (*Berberis spp.*) were picked, dried in the air, and pulverized into a fine powder. One hundred and fifty g of the powder were put in 500 mL of distilled water and allowed to soak in the water for three days after which they were filtered through regular filter paper. The filtrate was further concentrated in a water bath at 70 °C over two days and further evaporated through a rotary evaporator to eliminate the remaining solvent. The extract obtained was kept at 4 °C waiting to be used. The barberry extract was orally administered at a dose of 500 mg/kg body weight in normal saline (Ashraf et al., 2014). The metformin tablets were dissolved in normal saline and given to the subjects orally at a dosage of 15 mg/kg body weight [13].

2.5. Body weight measurement

The body weights of the mothers were obtained at the expiry of pregnancy. At gestational day 19, the fetuses were pooled and weighted at a sensitive and precise digital balance at an individual level.

2.6. Biochemical measurements related to diabetes

Blood samples were also collected by cardiac puncture after 12 hours of fasting in pregnant rats. Standard glucometer was used to assess the level of fasting blood glucose. Serum was centrifuged and assayed on insulin through ELISA assay. The insulin resistance was measured by determining the homeostasis model assessment of insulin resistance (HOMA-IR) by using the standard equation[14].

2.7. Histological examination

At gestational day 19, embryos were dissected and kidneys were excised and fixed in 10% neutral buffer formalin. Tissues were dried with the use of a graded ethanol, xylene cleared and embedded in paraffin wax [15]. Slices that are 5 mm thick were made with a microtome, placed on glass slides, and stained with hematoxylin and eosin (H&E). A light microscope was used to investigate and record the changes in the histology [16].

2.8. Embryonic skeletal staining

Embryos had been incubated in ethyl alcohol for a few days followed by transfer to acetone. Embryos were stained with Alizarin Red and Alcian Blue following the removal of the skin carefully, in order to visualize ossified bone and cartilage respectively. Specimens were then cleared using potassium hydroxide solution and then using different concentration of potassium hydroxide and glycerol. The skeletons were finally stained, kept in glycerol and photographed with a micro-camera [17].

2.9. Statistical Analysis

The SPSS was used to analyze the data and present them as mean plus SE. One-way ANOVA was the appropriate method of group comparison. A $p < 0.05$ was taken to be statistically significant.

2.10. Ethical approval

The experimental procedures were conducted as per the ethical principles of handling and use of laboratory animals. The protocol of the study was accepted by the Scientific Research Ethics Committee of the Department of Life Sciences, College of Education, University of Kirkuk.

3. Results:

3.1. Maternal body weight and metabolic values

The high-fat, high-sugar (HFHS) diet had a significant impact on maternal metabolic parameters as shown in Table 1. There was a significant maternal body weight increase in the HFHS group as opposed to the control group ($p < 0.05$). Conversely, metformin and barberry extract treatment, and especially in the combined treatment group (HFHS + M + B)[18] led to a massive decline in maternal body weight as compared to the HFHS group and the single treatment groups (Table 1, Figures 1,2).

The levels of fasting blood glucose (FBG) were significantly high in the HFHS group compared to the control group which validated the induction of gestational diabetes[19]. Metformin/barberry extract alone administration was significant in reducing the FBG levels but the highest reduction was observed within the combined treatment group that exhibited much lower levels of FBG than in the remaining treated groups[20]

In the same way, insulin resistance, as determined by the HOMA-IR index was also high in the HFHS compared to all other groups. The control group and the combined treatment group showed significantly lower HOMA-IR values than the control group and the metformin-only and barberry-only groups, which is an indication of a better insulin sensitivity after the combined treatment [21] (Table 1).

Table 1. Effect of metformin and barberry extract on maternal metabolic parameters

Group	Body weight at pregnancy (g)	Fasting blood glucose (mg/dL)	HOMA-IR
Control	270 ± 0.28 ^d	85 ± 0.016 ^d	1.4 ± 0.18 ^d
HFHS	334 ± 0.73 ^a	154 ± 0.41 ^a	5.6 ± 0.42 ^a
HFHS + Metformin	298 ± 0.54 ^b	110 ± 0.23 ^b	3.1 ± 0.29 ^b
HFHS + Barberry	321 ± 0.67 ^{ab}	136 ± 0.34 ^{ab}	4.7 ± 0.37 ^{ab}
HFHS + Metformin + Barberry	282 ± 0.41 ^c	98 ± 0.20 ^c	2.6 ± 0.23 ^c

Values are stated as mean ±SE.

The presence of different letters in the same column as a superscript suggests substantial differences below $p < 0.05$.

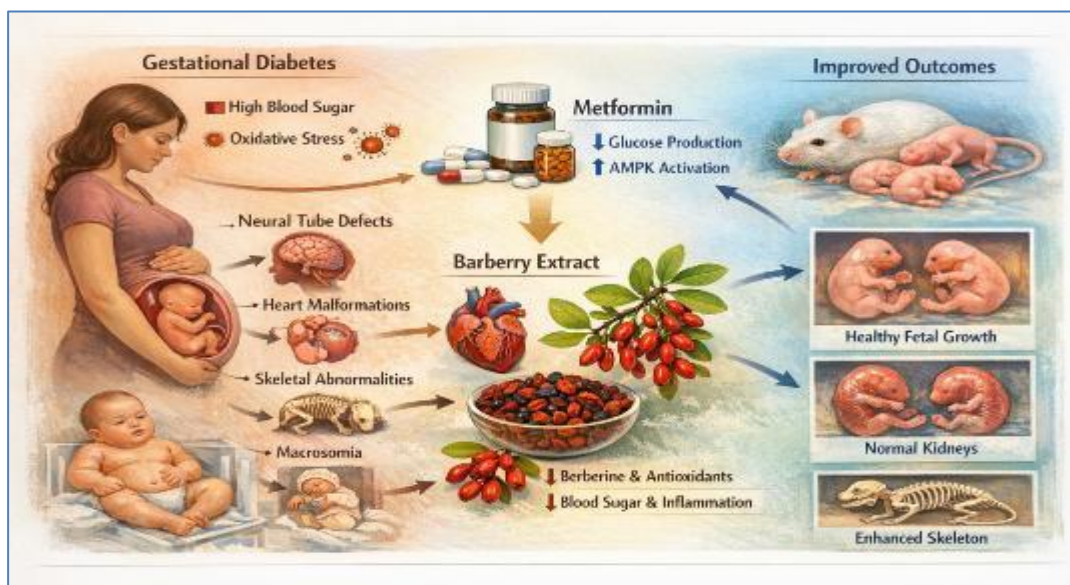


Figure 1. Figure illustrating effects of gestational diabetes on maternal and fetal should health and protective effects of Metformin and barberry extract

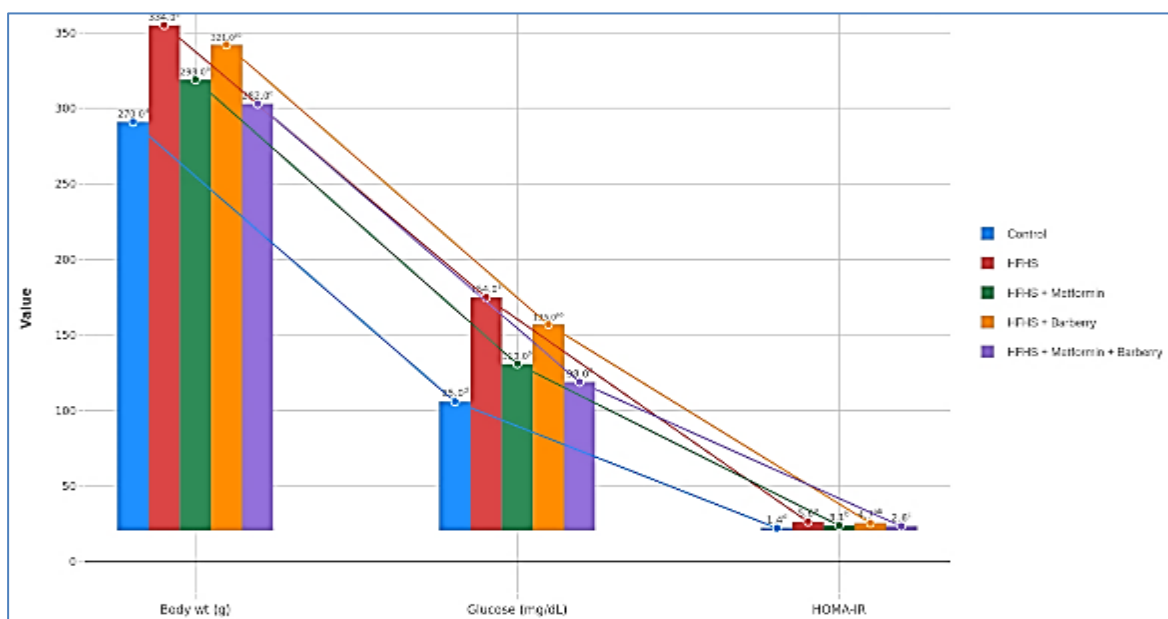


Figure 2. Effects of treatments on pregnancy body weight, fasting glucose and HOMA-IR

3.2. Parameters of fetal growth and placental index

The metabolic status of the mother had a significant impact on fetal growth parameters. The HFHS group showed a significant increase in fetal body weight relative to the control, meaning that the abnormal growth of fetuses has been related to maternal hyperglycemia (Figure 1). The

use of metformin or barberry extract partially normalized the fetal body weight and the combined treatment group (HFHS + M + B) had fetal weight that was not significantly different than those in the control group [22].

The placental index showed that there was a great reduction of the placental index in the HFHS group as compared to the control group indicating the maldevelopment of the placenta in diabetic conditions. It is important to note that the combined treatment group had a remarkable rise in placental index relative to both HFHS group and single-treatment groups indicating an enhancement of placental efficiency and fetal-maternal exchange [23] (Table 2).

Table 2. Effect of metformin and barberry extract on fetal growth and placental index

Group	Fetal body weight (g)	Placental index
Control	2.78 ± 0.51^d	8 ± 0.32
HFHS	4.14 ± 0.30^a	4 ± 0.09
HFHS + Metformin	3.70 ± 0.16^c	6 ± 0.19
HFHS + Barberry	3.92 ± 0.18^b	5 ± 0.13
HFHS + Metformin + Barberry	3.14 ± 0.23^{cd}	7 ± 0.25

Values are conveyed as mean $\pm$ SE

The significant differences at $p < 0.05$ are indicated by different superscript letters.

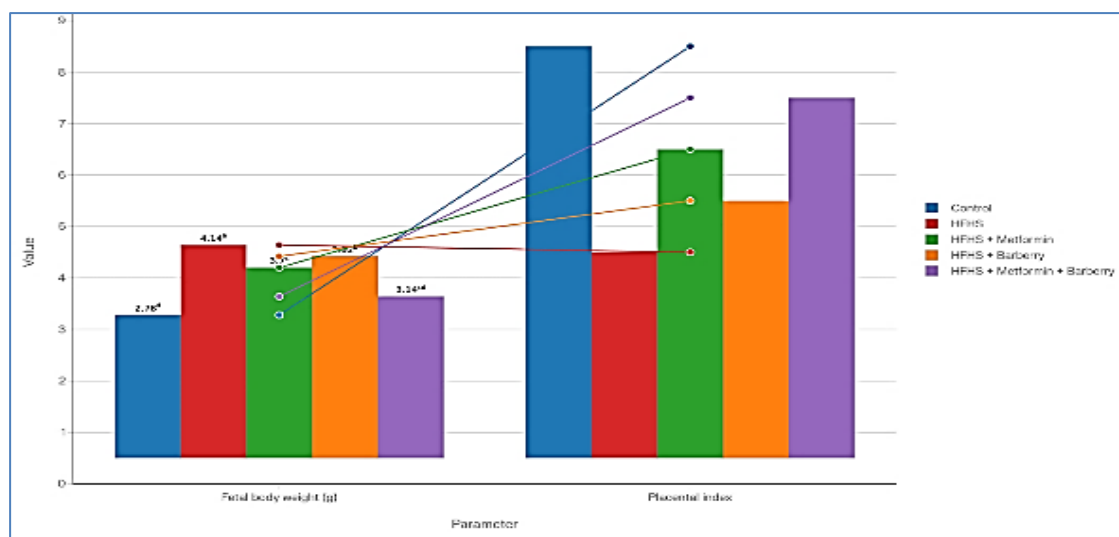


Figure 3. Effects of treatments on fetal body weight and placental index

3.3. Histological assessment of fetal kidney development

The histological analysis of fetal kidneys showed that there are significant differences within the experimental groups as depicted in Figure 4 (A–E). Kidney sections in the control group exhibited normal renal architecture with well-developed glomeruli, clearly identified Bowman space and normal distribution of erythrocytes in glomerular capillaries. The proximal and distal renal tubules were easily identifiable and intact (Figure 4A).

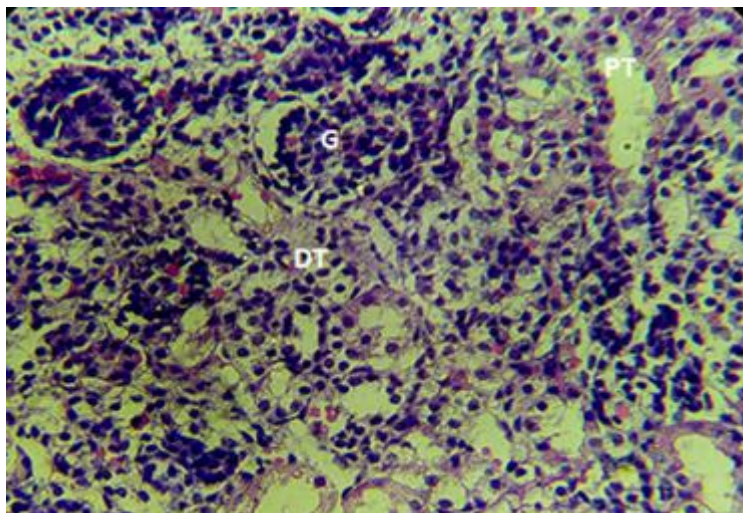


Figure 4 A : Control fetal kidney (normal histology)

Conversely, severe histopathological changes which were cellular degeneration and necrosis were observed in the HFHS group. Glomeruli were not well differentiated, Bowman space was not found, and renal tubules looked chaotic and indistinct which signified severe damage to renal development in diabetic cases (Figure 4 B) .

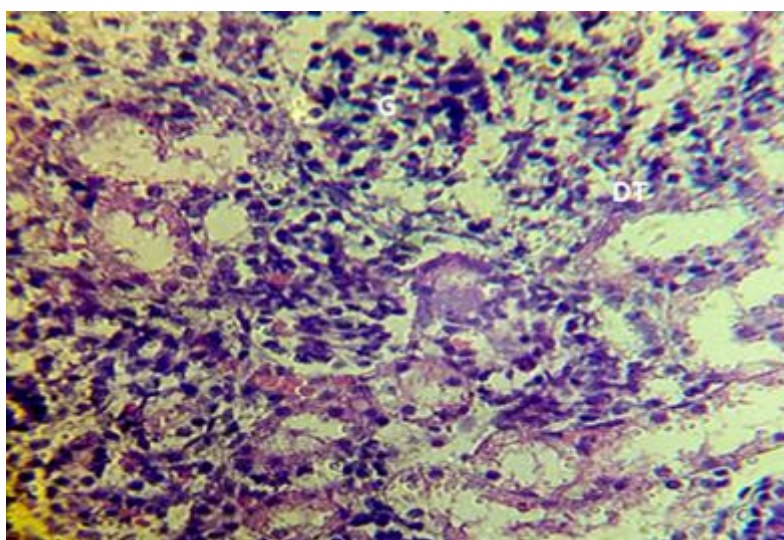


Figure 4 B. HFHS fetal kidney (severe histopathological alterations)

Partial histological improvement was found in the HFHS + Metformin group. It was possible to identify but shrunk glomeruli with partial recovery of Bowman space and erythrocyte distribution. Renal tubules were also still impacted but more noticeable as compared to the HFHS group (Figure 4C) .

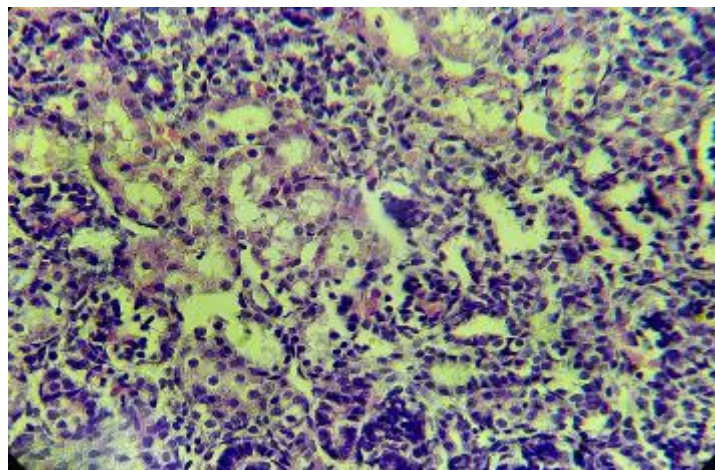


Figure 4C. HFHS + Metformin fetal kidney (partial histological improvement)

Kidney slices of HFHS + Barberry extract group were even better than HFHS group. The distinctions between glomeruli were also better defined, and the space between Bowman had not been completely regained. Proximal and distal tubules could be observed and had narrowed or partially blocked lumen (Figure 4D).

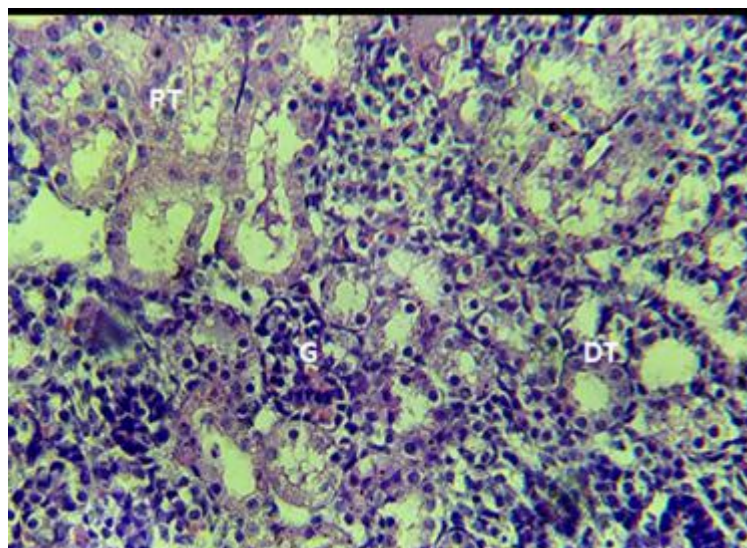


Figure 4D. HFHS + Barberry extract fetal kidney (moderate histological recovery)

HFHS + Metformin + Barberry extract group showed the greatest histological recovery following the control group. Gomeruli were almost of normal size and structure and Bowman

space was evident and renal tubules with well-defined lumens with minimum pathological alterations (Figure 4E) could be observed.

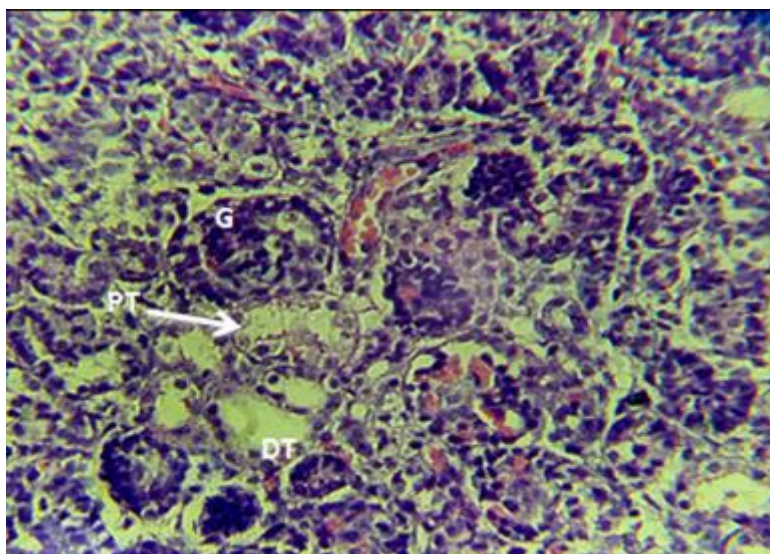


Figure 4E. HFHS + Metformin + Barberry extract fetal kidney (near-normal histology)

3.4. Embryonic skeletal development

The bone staining in Figure 5 showed that there were clear changes in the ossification patterns between the experiment groups. The embryos in the control group depicted normal formation of the bones in which the ossified structures showed strong dark red staining and certain bone and cartilage differentiation.

Contrary to this, the embryos in the HFHS group were significantly less stained, which means that the ossification was delayed and impaired. This was especially noticeable in the skull bones, the forelimbs, hindlimbs and the area of the tail which is an adverse outcome of maternal diabetes on skeletal formation.

Embryos of the metformin- and barberry-treated embryos showed some improvement in the skeletal ossification, and the bone staining was more intense in these groups than in the HFHS group. It is worth noting that the skeletal development pattern of the embryos in the combined treatment group was nearly similar to that of the control group with superior ossification and better bone-cartilage differentiation.

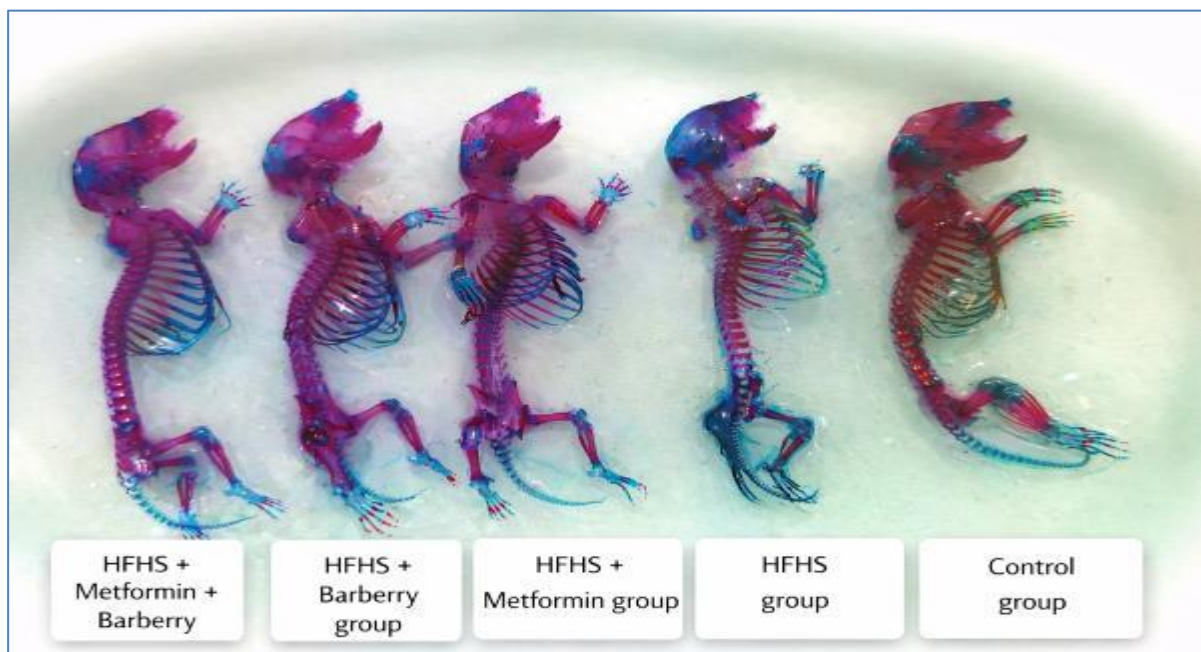


Figure 5. Ossification of skeletal structural organs in various experimental groups after staining with Alizarin red and Alcian blue.

4. Discussion

Gestational diabetes is a complex metabolic syndrome, which is characterized by hyperglycemia, insulin resistance, and oxidative stress in the mother [24]. This kind of metabolic perturbation can disrupt normal fetal development and organogenesis in multiple ways including excessive production of reactive oxygen species (ROS), mitochondrial dysfunction and altered expression of genes in developing tissues [25]. The increased oxidative conditions in the maternal blood can impair the functioning of the placenta causing the placental failure to transport nutrients and oxygen and hence the development of fetal growth and organ maturation [26]. Furthermore, the endoplasmic reticulum stress and inflammatory signaling cascaded by hyperglycemia may disrupt appropriate differentiation of renal and skeletal tissue in developing organs, which shows the susceptibility of the developing organs during the critical gestation phases [27].

The pharmacological treatment of gestational diabetes with metformin is associated with a significant level of benefits because of its multimodal effects on glucose homeostasis[28]. The main mechanism of action of metformin is prevention of hepatic gluconeogenesis, stimulation of peripheral glucose uptake, and activation of AMP-activated protein kinase (AMPK) which is a key controller of cellular energy homeostasis [29]. AMPK activation has shown not only

to cut down hepatic glucose levels, but also to increase mitochondrial lipid metabolism, reduce oxidative stress and stimulate mitochondrial biogenesis [30;31]. These processes combine to create a better intrauterine environment that is useful in the development of the fetal organs and prevents developmental abnormalities [32]. Also, the influence of metformin in lowering the intestinal glucose uptake modulates postprandial glycemic fluctuations, which further safeguard the fetus against the changes in glucose levels that may cause metabolic stress in the fetus [33].

Complementary natural therapies are becoming more and more popular as having the potential to reduce diabetic complications by their antioxidant, anti-inflammatory, and metabolic modulatory activities. The promising candidate is barberry (*Berberis vulgaris*) because of the presence of bioactive alkaloids, such as berberine, berbamine, and jatrorrhizine [34]. Berberine has been demonstrated as an improved insulin sensitivity and glucose transporter activity and lipid metabolism through AMPK-dependent and independent mechanisms [35]. Moreover, its antioxidant effect prevents ROS-induced injury of mother and fetal tissues, and prevents structural and functional defects in organs like the kidney [36]. It is theoretically beneficial that metformin and barberry extract be used in combination because they may work synergistically [37]. Although the metformin drug is mainly aimed at hepatic glucose production and systemic metabolic control, barberry bioactive compounds are considered to give extra antioxidant and anti-inflammatory effects [38]. This twofold solution can improve maternal insulin response, stabilize the glycemic conditions, and reduce the level of oxidative stress to build optimal conditions to support fetal organogenesis and skeletal development [39]. In addition, the efficiency of the placenta may also be improved due to the alleviation of disturbances in maternal metabolism and reduction in the levels of inflammatory mediators, which would allow the placenta to transfer nutrients more effectively and would support the growth and development of the fetus [40].

The fetuses under development are incredibly vulnerable to the metabolic conditions of the mother in regard to histological and skeletal integrity [41]. Interference in the differentiation and mineralization process of renal and skeletal tissues can be caused by hyperglycemia and oxidative stress [42]. Protective replacement of the lost redox equilibrium, glucose control and inflammatory state can therefore be used to preserve normal tissue structure and function [43]. These trends have implied that the effectiveness of the combination of the traditional pharmacological therapy with the bioactive components of the vegetable origin may be employed to enhance the fetal tolerance of the adverse intrauterine conditions of the gestational diabetic complication [44;45].

5. Conclusions

Gestational diabetes has an adverse impact on maternal metabolism and unborn child development by impacting hyperglycemia, insulin resistance, and oxidative stress. Metformin and barberry fruit extract combination is complementary to bring about better metabolic balance, placental functions, and preserve the development of fetal organs and skeletal structures. This integrated intervention is possibly a promising solution to the problem of decreasing complications associated with gestational diabetes and positively influencing the outcomes of pregnancy.

6. Acknowledgments

The authors would like to acknowledge that they are truly indebted to the Department of biology, College of Education, Pure Sciences University of Kirkuk, to offer the required laboratory facilities and technical support which played a great part in ensuring the success of this study.

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