International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.363.373

Research Article
Protective Effect of Diosmin Against Streptozotocin-Induced Gestational Diabetes Mellitus via AGEs-RAGE Signalling Pathway

[1]Xifeng Zhang, [2]Shumin Zheng and [3]Haixia Li

[1]Department of Obstetrics, The Third Hospital of Jinan, Jinan, 250000, China
[2]Department of Obstetrics, Dezhou People's Hospital, Dezhou, Shandong Province, 253000, China
[3]Department of Obstetrics and Gynecology, Hanzhong People's Hospital, Hanzhong, 723000, China

Abstract

Background and Objective: Gestational Diabetes Mellitus (GDM) is the most common metabolic disease that affects 3-5% of total pregnancies. Diosmin (polyphenolic flavonoids) are commonly used in the treatment of various metabolic diseases. This experimental study, scrutinize the protective effect of Diosmin against the streptozotocin (STZ) induced GDM in rats. **Materials and Methods:** Sprague Dawley (SD) rats were used for the current protocol and STZ was used for the induction of GDM in female rats. The rats were divided into different groups and received the oral administration of diosmin (once a day) for up to 19 days. An Oral Glucose Tolerance Test (OGTT) was carried out. Body weight and litter size were estimated. Serum resistin and insulin were measured. Biochemical parameters were also determined at end of the experimental study. qRT-PCR was carried out for the estimation of different genes. **Results:** During the OGTT test, diosmin significantly ($p<0.001$) reduced the blood glucose level. Diosmin significantly ($p<0.001$) increased the body weight and litter size. Diosmin significantly ($p<0.001$) reduced the level of resistin and insulin in the serum. Diosmin also reduced the level of serum C peptide (CP), Free Fatty Acid (FFA), haemoglobin A1C (HbA1c) and reduced the level of hepatic glycogen. Diosmin significantly reduced the level of Triglyceride (TG), Low-Density Lipoprotein (LDL), Total Cholesterol (TG) and boosted the level of High-Density Lipoprotein (HDL). Diosmin also reduced the level of Thiobarbituric Acid Reactive Substance (TBARS) and increased the level of glutathione (GSH), catalase (CAT) and Superoxide Dismutase (SOD). Diosmin significantly ($p<0.001$) increased the mRNA expression of NF-κB and reduced the IκB. Diosmin also altered the mRNA expression of NOX-2, RAGE, EGFR, MCP-1, p65 and VCAM-1. **Conclusion:** Based on the result, Diosmin is a therapeutic and preventive effect against STZ induced GDM in rats.

Key words: Diosmin, gestational diabetes mellitus, inflammation, plasma insulin, NF-κB

Citation: Zhang, X., S. Zheng and H. Li, 2022. Protective effect of diosmin against streptozotocin-induced gestational diabetes mellitus via AGEs-RAGE signalling pathway. Int. J. Pharmacol., 17: 363-373.

Corresponding Author: Haixia Li, Department of Obstetrics and Gynecology, Hanzhong People's Hospital, Hanzhong, 723000, China

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Gestational Diabetes Mellitus (GDM) occurred due to develop glucose tolerance and insulin resistance during pregnancy; it affects 2-5% of normal pregnancies. GDM develop the diabetes risk factor, cardiovascular and obesity disease during pregnancy for the mother as well as the fetus[1]. GDM can induce maternal morbidities to include operative deliveries, preeclampsia and polyhydramnios. GDM also induces shortfalls in fetal expansion, with 15-45% of babies born via women with diabetes show macrosomia symptoms[2,3]. The GDM condition show the effect on offsprings such as birth trauma, prematurity, respiratory distress syndromes, macrosomia and more prominently, will demonstrate the relatively high incidence to develop the dysregulation the glucose tolerance (during short term). The serious side effects of GDM develop diabetes mellitus type II (T2DM) with high frequency[4]. GDM is considered the pre-diabetic condition that provides the opportunity to investigate the dysfunction that appears during the early stage of T2DM[4-6]. Moreover, the development and investigation of beneficial treatments against GDM are of critical clinical value.

At the end stage of gestation, the endogenous glucose synthesis increases around 30% to full the energy requirements throughout the pregnancy. However, the GDM suffer women and β cell dysfunction can boost insulin resistance that resultant develop hyperglycemia during pregnancy[5-7]. Due to enhancing insulin resistance, increasing the level of Free Fatty Acid (FFA), Low-Density Lipoprotein (LDL), Total Cholesterol (TC) and Triglycerides (TG), which further causes cardiovascular disease. The placenta is a vascularized structure that is rich in mitochondria and produces several Reactive Oxygen Species (ROS) and has a high metabolic rate. It is well documented that pregnancy is a pre-diabetic condition, which further increases oxidative stress[4,7,8].

It is well proved that high glucose level starts the production of hydroxyl and oxygen free radicals during diabetes condition[4,9]. It clearly shows that hyperglycemia directly boosts the oxidative stress in the embryo tissue and reduction of oxidative stress is the best treatment to reduce the manifestations related to diabetes. Recently, few investigations suggest that oxidative stress conditions, production of Reactive Nitrogen/Oxygen Species (RNS/ROS) via lipid peroxidation and mitochondria, RNA and DNA injury are the underlying mechanisms involved in the toxicity to immune-inflammatory reactions and oxidative stress[4,6]. Oxidative stress is a condition created during the imbalance between the endogenous antioxidant and production of ROS in the body, which is closely related to diabetes, ageing and several various diseases such as cardiovascular, atherosclerosis, arthritis and cancer[7,9].

It is well documented that advanced glycation endproducts (RAGE) and its ligands especially advanced glycation endproducts, (AGEs) interact with each other to form the AGEs-RAGE signalling pathway, which boosts the oxidative stress and inflammatory reaction that plays a crucial role in the pathophysiology of diabetes mellitus[10]. RAGE is a cell surface receptor for immunoglobulins. Decoy receptor (Carboxyl-terminally truncated or endogenous secretory RAGE) shows a V type immunoglobulin fragment that binds with AGE ligands[7,11]. AGEs are changed tissue proteins that are found in numerous tissues throughout the body as age, especially when the patients are under diabetic load or when the patients are exposed to more oxidative stress. The level of AGEs increased in pregnant women and it was also employed as a predictor of GDM-related unfavourable perinatal outcomes as well as a biomarker for GDM-related congenital abnormalities[7,11-13].

Flavonoids are polyphenolic tricyclic secondary metabolites commonly present in various species of plants[14]. Diosmin (flavone glycoside) is commonly found in the pericarp of various citrus fruits[15,16]. Diosmin having various pharmacological effects such as anti-inflammatory, antiapoptotic, antioxidant and anti-mutagenic[15-18]. Various reports suggest that the Diosmin have a protective effect on hypertension, myocardial infarction, liver injury, hepatocarcinogenesis and hepatocarcinogenesis[15-22]. The gestational protective effect of diosmin is still not explored. In this experimental study, the protective effect of Diosmin against the STZ induced GDM and explores the mechanism.

MATERIALS AND METHODS

Study area: The study was carried out in the Hanzhong people's Hospital, China from November, to December, 2020.

Chemical: Diosmin (98%) and streptozotocin were purchased from Sigma Aldrich, USA. Blood glucose levels were estimated using the ACCU-CHEK Glucometer and also using the blood sugar test paper (Roche Company, Sweden). Insulin was estimated using the ELISA kits (Mercodia Company, Sweden). All the chemicals and reagents used in the experimental study were analytical grade.

Rodent: Sprague Dawley (SD) (weight 220-250 g, sex male 6 rats and 30 female rats) were procured from the animal house and maintained at 22±5°C temperature; 50-75%

Table 1: List of primers

	Primers (5'-3')	
Genes	Forwarded	Reverse
NF-κB	AGTTGGAGCAGCTGCAGCGC	GGAGGGCTCCGCTGGTT
IκB	GCAACCCAGTGGTGGCCCAG	CTCCCGCTGGCTGACCTGGA
VCAM-1	GAAGCCGGTCATGGTCAAGT	GACGGTCACCCTTGAACAGTTC
P65	AGCACCATCAACTATGATGAGTTTC	GAGTTATAGCCTCAGGGTACTCCAT
Nox2	CCCTTTGGTACAGCCAGTGAAGAT	CAATCCCAGCTCCCACTAACATCA
MCP-1	CCCCAGTCACCTGCTGTTAT	TGGAATCCTGAACCCACTTC
VEGF	GAGAATTCGGCCCCAACCATGAACTTTCTGCT	G AGCATGCCCTCCTGCCCGGCTCACCGC
RAGE	CCTGAGACGGGACTCTTCACGCTTCGG	CTCCTCGTCCTCCTGGCTTTCTGGGGC
β-actin	AGAACATCATCCCTGCATCC	TGGATACATTGGGGGTAGGA

relative humidity and finally maintain the 12 hrs light and 12 hrs dark cycle. The current experimental study was carried our accordance with the animal guidelines of the institute. In the incubation periods, the rats received oral administration of saline (20 mL kg^{-1}) using oral gavage.

Experimental grouping: The previously reported method was used for established the GDM models with minor modifications[23]. Female rats were fed according to the standard experimental protocol. The rats were divided into different groups such as normal rats who received diosmin and GDM control (no treatment). The GDM group rats received a high-fat diet presented in Table 1. The rats received HFD for 12 weeks and STZ (20 mg/kg/day) treatment for 12 weeks. The normal control group rats received the normal diet for 12 weeks. After 12 weeks, all three groups of female rats were mated with male rats and incidence of vaginal plug was noted as day 0 of pregnancy. The rats in the GDM group received HFD in the whole experimental study, while the GDM group rats received the HFD treated with diosmin (10 mg kg^{-1}, daily).

Oral glucose tolerance test (OGTT): An oral glucose tolerance test was performed using the previously reported method with minor modification for estimating glucose from the rats[1]. Before the experimental study (24 hrs), the rats in each group were fasted overnight (12 hrs) and estimated the BGL. The rats were received oral administration of glucose (2 g kg^{-1}) via oral gavage. The solution of glucose was prepared in water. After being given the glucose, the BGL was estimated at regular time intervals (15, 30, 60 and 120 min) by collecting the blood from the tail.

Insulin: On the 18th day of pregnancy, the rats were fasted overnight (12 hrs). The rats were anaesthetized using isoflurane and blood was collected from the aortic. The blood was centrifuged at 5000 rpm for 5 min to separate the supernatant. The supernatant was stored at -20°C. Resistin and insulin level was estimated using the ELISA kits following the manufacture instruction.

AGEs and RAGE level: The level of AGE and RAGE was estimated in pregnant rats using the available commercial kits following the manufacturing method (Bio-Engineering Co., Ltd. Wuhan gorgeous).

Determination of mRNA expression of NF-κB and IκB in pancreatic tissues: On the induction of gestation (20th day), the rats were anaesthetized using isoflurane and sacrificed via cervical dislocation and successfully isolated the islet tissue. For isolation, the total RNA and Trizol lysate reagent was used. The cDNA and RNA extraction was done using the appropriate kits following the manufacture protocol. The primers are given in Table 1.

Statistical analysis: GraphPad Prism version 7 (St. Louis, USA) was utilized for statistical analysis. The estimated data are presented as x±SEM. Two sample comparison was carried out with t-test groups. Where $p<0.05$ was expected as statistical significance.

RESULTS

Oral glucose tolerance test: Figure 1 showed the effect of diosmin on glucose tolerance. GDM group rats exhibited increased glucose levels after the administration of glucose (first 15 min) and remain higher time end of the protocol (120 min). Diosmin significantly suppressed the BGL level compared to GDM group rats in Fig. 1a.

Body weight and glucose level: The body weight of all group rats augmented as time increases except for GDM control. Normal rats showed an enhancement in the body weight of rats (increases the body weight with time). GDM rats demonstrated reduced body weight compared with the other group and initial body weight in Fig. 1b. GDM rats treated with Diosmin significantly ($p<0.001$) increased the body weight.

Table 2 exhibited the effect of diosmin on the blood glucose level of GDM rats. GDM group rats demonstrated an enhanced blood glucose level (22.32±4.34 and 23.43±5.23)

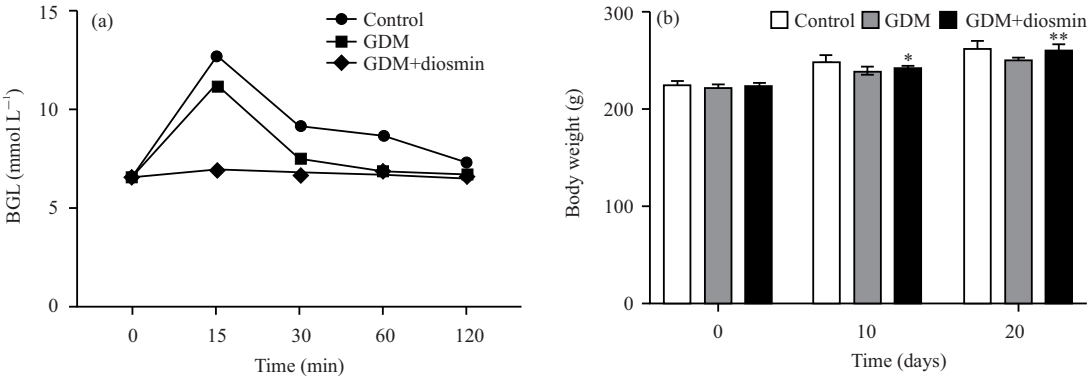

Fig. 1(a-b): Effect of diosmin on the level of blood glucose (oral glucose tolerance test) and body weight of the GDM group of rats. (a) Blood glucose level (oral glucose tolerance test) and (b) Body weight
* vs. control group, *p<0.05, **p<0.01 and ***p<0001

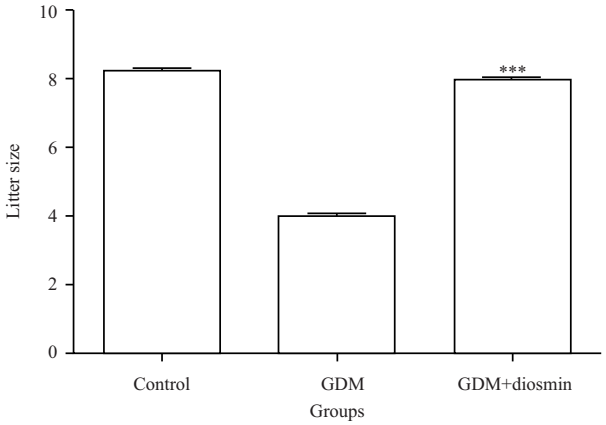

Fig. 2: Effect of diosmin on litter size of the GDM group of rats
* vs. control group, *p<0.05, **p<0.01 and ***p<0001

Table 2: Effect on the blood glucose level

	Time (days)		
Groups	GD 0	GD 10	GD 20
NC	6.03±0.84	6.3±0.93	6.2±0.98
GDM	5.98±0.73	22.32±4.34	23.43±5.23
GDM+diosmin	6.02±0.69^{ns}	18.23±3.45*	9.08±3.28***

* vs. control group, *p<0.05, **p<0.01 and ***p<0.001

on GD 10 and GD 20. Diosmin treatment significantly suppressed the blood glucose level (18.23±3.45 and 9.08±3.28) on GD 10 and GD 20.

Litter size: The without treated rats exhibited normal litter size (no changes observed). GDM control rats showed reduced litter size compared to normal and diosmin-treated group rats. GDM rats treated with diosmin significantly (p<0.001) increased the litter size and showed almost near the normal group in Fig. 2.

Insulin and resistin: During the GDM, the level of insulin and resistin considerably boosted. The levels of resistin and insulin were normally observed in the without the treatment group. GDM group rats exhibited the increased resistin and insulin level. GDM group rats treated with diosmin significantly (p<0.001) decreased the level of insulin in Fig. 3a and resistin in Fig. 3b.

Serum C-peptide, hepatic glycogen, free fatty acid and HbA1c: GDM rats showed increased levels of C-peptide in Fig. 4a, FFA in Fig. 4b, HbA1c in Fig. 4c and declined the level of hepatic glycogen in Fig. 4d compared to normal rats. GDM rats treated with diosmin significantly (p<0.001) reduced the C-peptide, FFA, HbA1c levels and increased hepatic glycogen level.

Lipid parameters: Lipid parameters viz., TG in Fig. 5a, TC in Fig. 5b, LDL in Fig. 5c and HDL in Fig. 5d were estimated in the different groups of rats. GDM rats exhibited an amplified level of TG, TC, LDL and declined levels of HDL compared to normal rats. Diosmin treated rats significantly (p<0.001) reduced the level of TG, TC, LDL and increased the level of HDL.

Antioxidant parameters: GDM rats showed boosted levels of TBARS in Fig. 6a and reduced levels of SOD in Fig. 6b, GSH in Fig. 6c and CAT in Fig. 6d compared to normal rats. Diosmin treated rats demonstrated suppressed levels of TBARS (Fig. 6a) and boosted levels of SOD (Fig. 6b), GSH (Fig. 6c) and CAT (Fig. 6d).

Inflammatory cytokines: The level of TNF-α in Fig. 7a, IL-1β in Fig. 7b and IL-6 Fig. 7c, boosted in the GDM group rats and diosmin significantly (p<0.001) reduced the level of

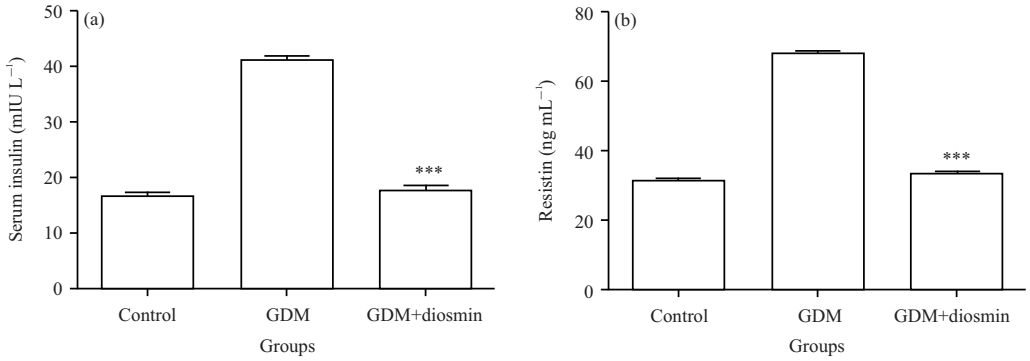

Fig. 3(a-b): Effect of diosmin on the level of insulin and resistin of the GDM group of rats. Effect of diosmin on the level of (a) Insulin and (b) Resistin

* vs. control group, *p<0.05, **p<0.01 and ***p<0001

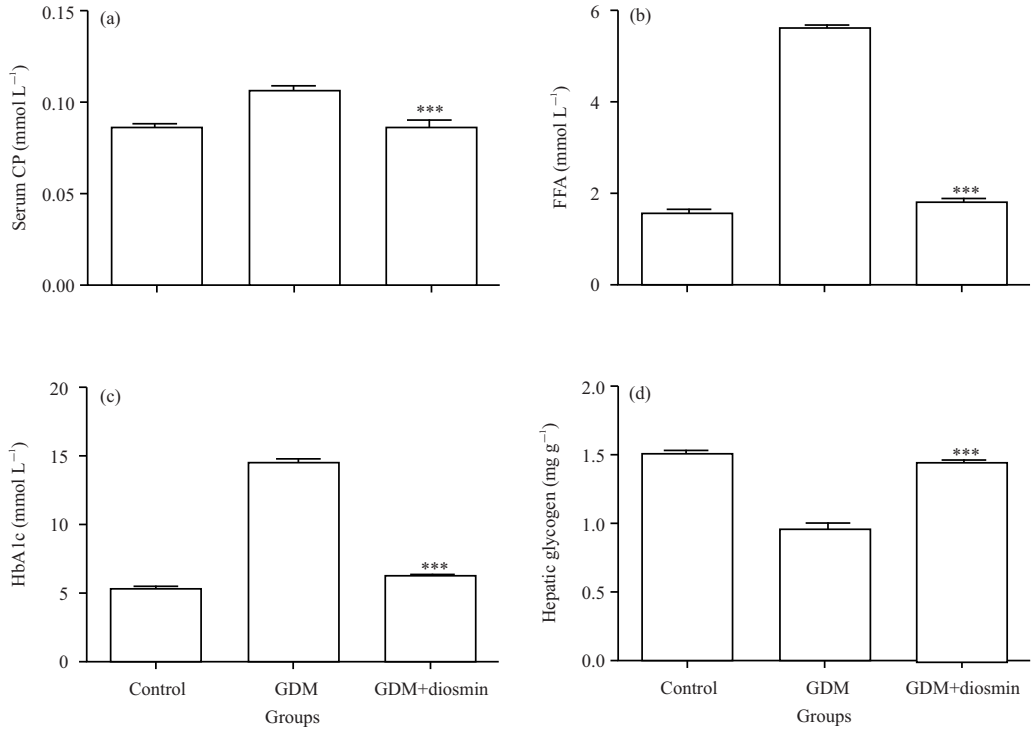

Fig. 4(a-d): Effect of diosmin on the level of serum C-peptide (CP), hepatic glycogen, free fatty acid (FFA) and HbA1c of GDM group of rats. Effect of diosmin on the level of (a) CP, (b) FFA, (c) HbA1c and (d) Hepatic glycogen

* vs. control group, *p<0.05, **p<0.01 and ***p<0001

inflammatory cytokines, the level of pro-inflammatory cytokines reached almost near the control group rats.

mRNA expression: The higher mRNA expression of NF-κB Fig. 8a and reduced mRNA expression of IκB Fig. 8b were observed in the pancreatic tissue of GDM group rats. Diosmin treatment significantly (p<0.001) enhanced the expression of NF-κB and suppressed the expression of IκB.

Figure 9a showed the mRNA expression of RAGE in the placenta, offspring and heart tissue. GDM group rats showed the increased mRNA expression of RAGE in the placenta, offspring and reduced in the heart tissue and diosmin treated rats exhibited the reduced expression of RAGE in the placenta, offspring and increased in heart tissue.

Figure 9b demonstrated the enhanced mRNA expression of NOX2 in the placenta, offspring and reduced the mRNA

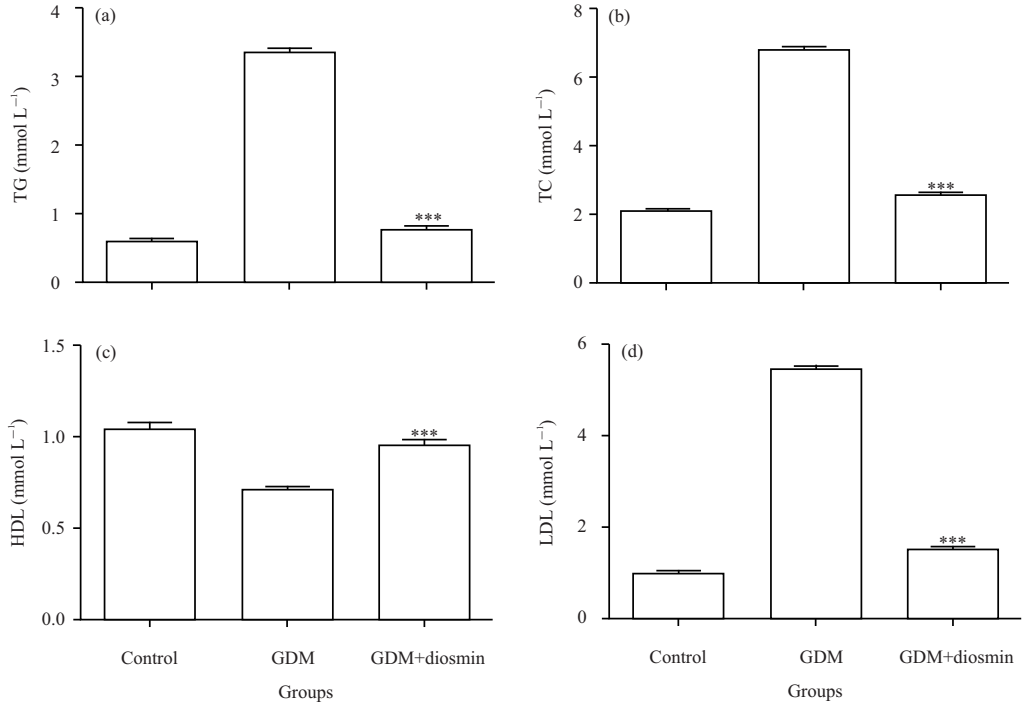

Fig. 5(a-d): Effect of diosmin on the level of lipid parameters of GDM group of rats. Effect of diosmin on the level of (a) TG, (b) TC, (c) HDL and (d) LDL

* vs. control group, *p<0.05, **p<0.01 and ***p<0001

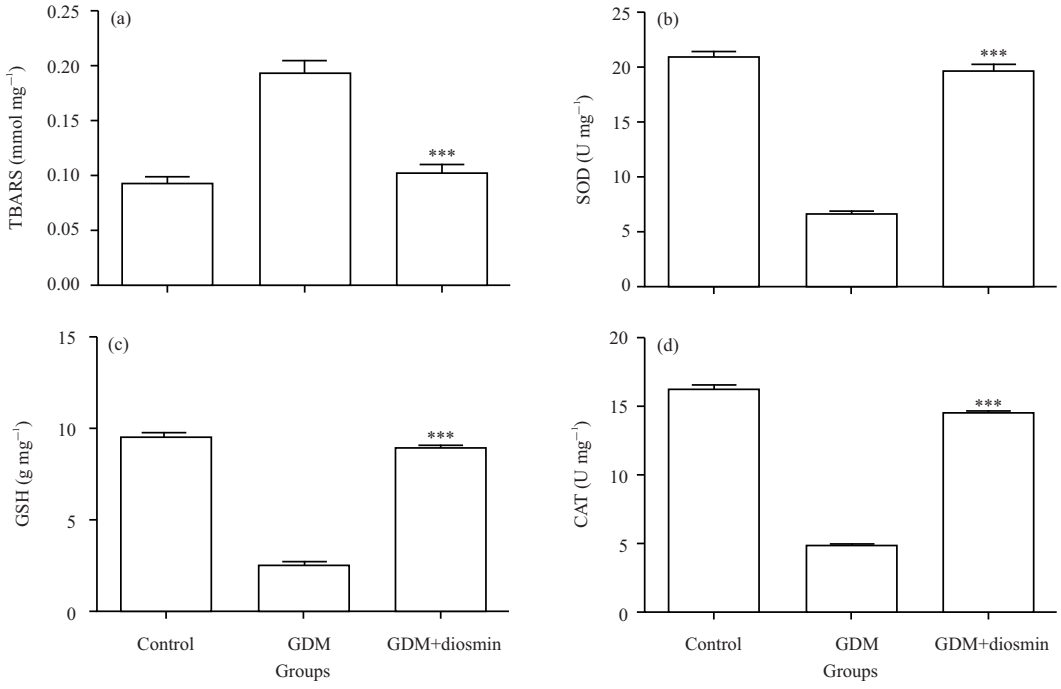

Fig. 6(a-d): Effect of diosmin on the level of antioxidant parameters of GDM group of rats. Effect of diosmin on the level of (a) TBARS, (b) SOD, (c) GSH and (d) CAT

* vs. control group, *p<0.05, **p<0.01 and ***p<0001

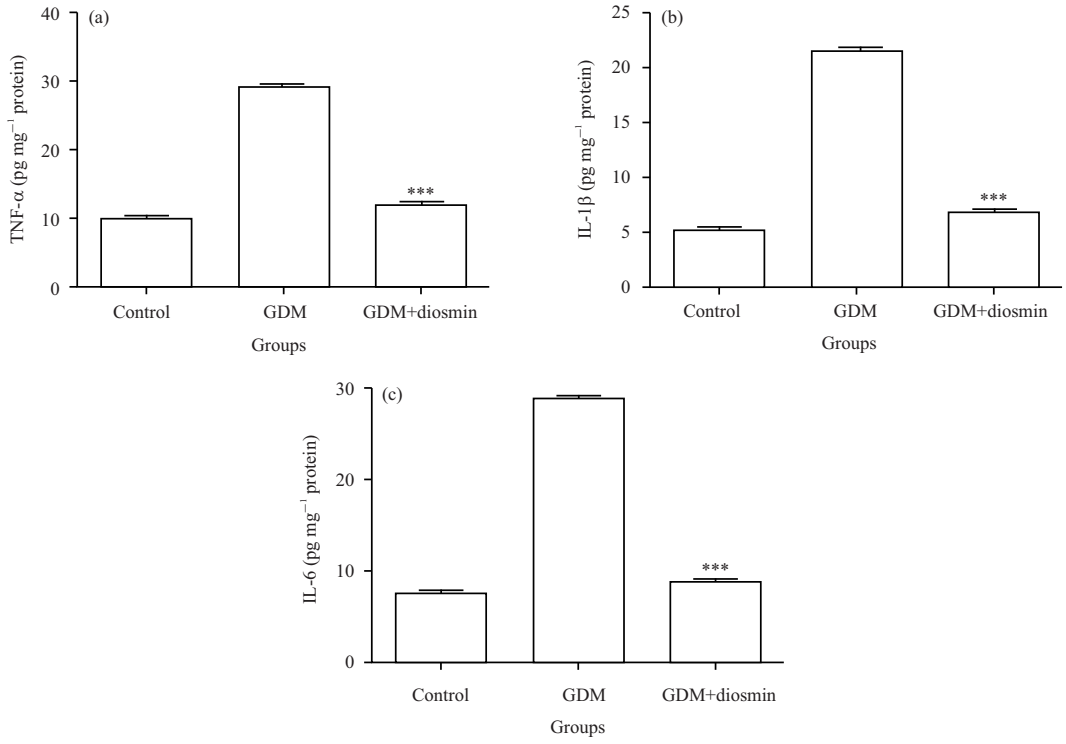

Fig. 7(a-c): Effect of diosmin on the level of pro-inflammatory cytokines of GDM group of rats. Effect of diosmin on the level of (a) TNF-α, (b) IL-1β and (c) IL-6

* vs. control group, *p<0.05, **p<0.01 and ***p<0001

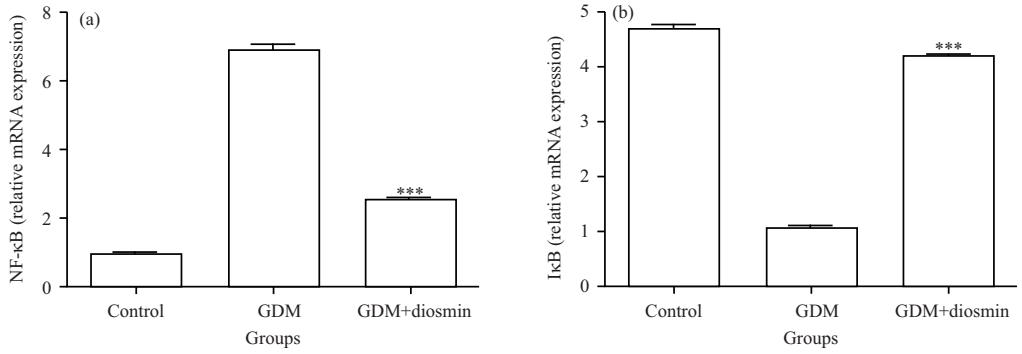

Fig. 8(a,b): Effect of diosmin on the mRNA expression of NF-κB and IκB in the pancreatic tissue of GDM group of rats. Effect of diosmin on the mRNA expression of (a) NF-κB and (b) IκB

* vs. control group, *p<0.05, **p<0.01 and ***p<0001

expression in heart tissue. Diosmin significantly (p<0.001) suppressed the NOX2 mRNA expression in the placenta, offspring and enhanced in heart tissue.

Figure 9c exhibited the increased mRNA expression of MCP-1 in the placenta and decreased mRNA expression in offspring and heart tissue. Diosmin significantly (p<0.001) suppressed the mRNA expression of MCP-1 in the placenta and enhanced the mRNA expression in offspring and heart tissue.

Figure 9d showed the increased mRNA expression of EGFR in the placenta, heart tissue and reduced in the offspring. Diosmin significantly (p<0.001) suppressed the mRNA expression of EGFR in the placenta, heart tissue and increased the mRNA expression of EGFR in the offspring.

Figure 9e demonstrated the enhanced mRNA expression of VCAM-1 in the placenta and suppressed in the offspring and heart tissue. Diosmin significantly (p<0.001) down-regulated

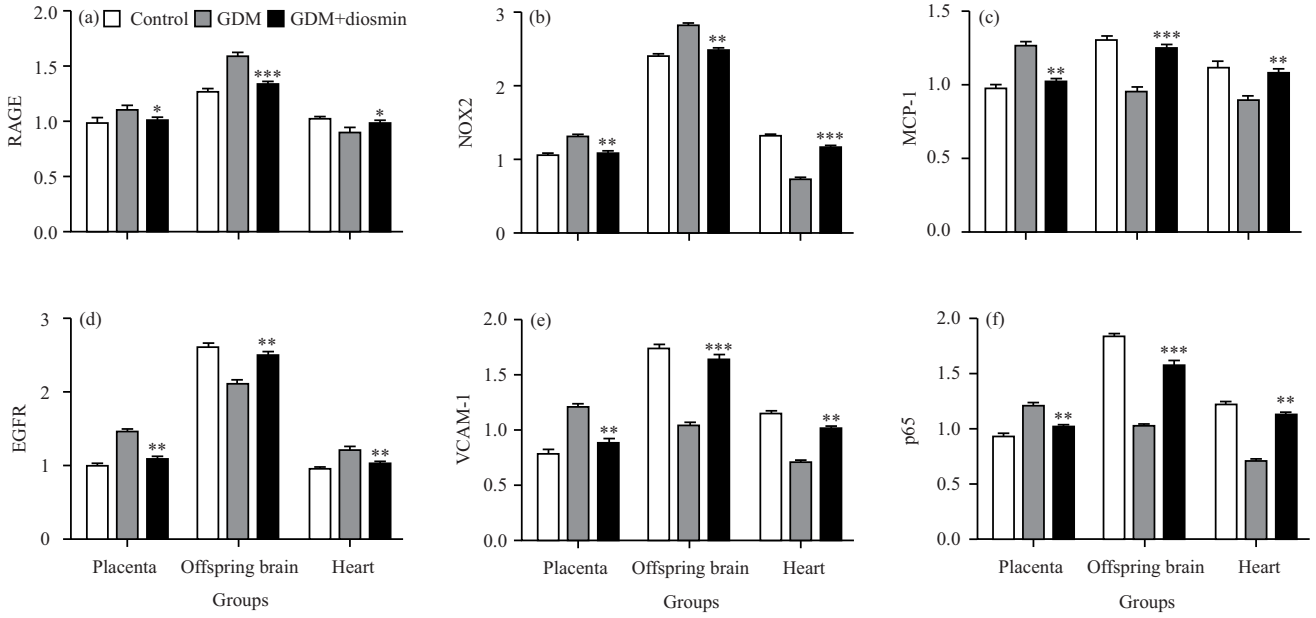

Fig. 9(a-f): Effect of diosmin on the mRNA expression of GDM group of rats. Effect of diosmin on the mRNA expression of (a) RAGE, (b) NOX2, (c) MCP-1, (d) EGFR, (e) VCAM-1 and (f) p65

* vs. control group, *p<0.05, **p<0.01 and ***p<0001

the mRNA expression of VCAM-1 in the placenta and up-regulated the mRNA expression in offspring and heart tissue.

Figure 9f demonstrated the increased mRNA expression of p65 in the placenta and reduced in offspring and heart tissue. Diosmin significantly (p<0.001) reduced the mRNA expression of p65 in the placenta and increased in offspring and heart tissue.

DISCUSSION

It is well known that GDM is a heterogeneous dysfunction, which involves numerous factors that leading the hyperglycemia condition and inducing the vascular complication in the mother and its offspring[23,24]. Various factors, such as impaired insulin secretion, suppress insulin sensitivity, reduction of pancreatic beta cells and enhanced inflammatory reaction and many more complications[23,25,26]. Oxidative stress is determined to be the primary cause of maternal hyperglycemia, which causes alterations in the intrauterine environment. Therefore, no direct evidence shows the involvement of the ROS signalling pathway in the pathophysiology of GDM and few studies demonstrate that AGEs play a significant role in the production of oxidative stress in diabetics[24-26]. In this experimental study, the authors demonstrate the increased AGE having a strong correlation with the LPO. Recent investigation suggests that the expansion of AGE is inducing the vascular inflammation during the GDM via activation of early growth response-1[8,12]. In this experimental investigation, the authors have found enhanced AGEs expression and oxidative stress and inflammatory reactions. This is enough data to suggest that AGEs play a role in GDM pathophysiology and have an effect on the fetoplacental vasculature, which may have long-term consequences for the mother and offspring.

It's worth noting that the change in GD offspring body weight at birth is greater in mice than in rats, which could be due to differences in species or facilities[8,14]. In this current investigation, the authors observed decreased litter size and increased body weight of offspring in GDM rats. Increased body weight and decreased litter size are the main phenotypes characteristic of GDM. Diosmin treated rats exhibited the enhanced fetus growth and alteration of fetal growth affected by GDM.

Insulin therapy is most recommended to treat hyperglycemia during diabetes and pregnancy[27,28]. Furthermore, due to uncontrolled administration and potential increase body weight and induce hypoglycemia and clinical practice is insufficient to cure. The researcher suggests that some time the GDM patient develop insulin resistance[27-29]. In this experimental study, diosmin treatment exhibited a

protective effect against hyperglycemia and insulin resistance in GDM rats. Diosmin significantly enhance fetal development and reproductive outcome. Accordingly, the authors can say that diosmin might be considered the alternative therapy for treating GDM.

A previous study suggests that AGEs induce RNS and ROS production starts after the activation of the NADP(H) oxidase pathway [8,12]. The current study showed the boosted level of MDA in GDM rats compared to normal pregnant rats, which suggests the induction of oxidative stress[30]. The reduction of GDH, SOD and CAT level provide the strength of the current statement. The report suggests that AGEs inactivate the GDH related enzymes and also affect the GSH level in circulation[30]. Therefore, boosted AGEs may be responsible for a decline in GSH activity and in turn also take part to reduce the antioxidant levels in GDM rats.

Previous investigation suggests that the AGEs-RAGE induce intracellular pathways involved in the expansion of inflammatory cytokines, pro-atherogenic mediators and NF-κB dependent mediators[7,11-13]. GDM rats exhibited the up-regulation in the expression of p65, EGFR, MCP-1, RAGE, VCAM-1 and Nox2. AGE-RAGE signalling pathway activation involves enhancing the transcription factor NF-κB, which further up-regulate the production/secretion of inflammatory cytokines including IL-6, ICAM-1, TNF-α and IL-1α. The AGE-RAGE signalling pathway is also implicated in the generation of oxidative stress, which is linked to the development of diabetes complications like foetal abnormalities, diabetic retinopathy, diabetic nephropathy and diabetes-related cardiovascular disease[10]. Previous studies showed a strong relation between AGEs and blood sugar levels. During the GDM, increases the AGE levels exhibited the effect on the offspring (fetal complication)[31,32]. Diosmin treatment significantly suppressed the BGL and also diminution the AGE production in the serum of GDM rats. GDM rats received the administration of diosmin showed the fewer defects in pups in GDM pregnant rats.

Few published reports suggest that the GDM is the early stage of T2DM and IR plays a significant role in the T2DM and GDM pathogenesis[33]. In this experimental study, the authors used the diosmin to treat the GDM via reduction of BGL, lipid profile as well as enhanced IR. According to Kumar *et al*., hyperglycemia often induces the generation of free radicals in the body, enhance the glucose oxidation and glycation of proteins by degrading the glycated proteins and is also responsible for the production of oxygen free radicals in patients with diabetes[34,35]. Antioxidant parameters (enzymatic and non-enzymatic) having the capability to scavenge free radicals and remove toxic effects. Diosmin (flavone glycoside) is commonly found in citrus fruits and has numerous medicinal important against various diseases[36,37]. Diosmin possesses an antidiabetic and antihyperlipidemic effect on diabetic rats. In this experimental study, the authors compared the antioxidant levels of the diosmin group with the GMD group and the result showed the reduction in antioxidant levels and suggesting the antioxidant effect. Diosmin significantly reduced glucose oxidation due to suppression of the formation of superoxide anion and restore the level of endogenous antioxidants.

Previous investigation suggests that oxidative stress increases DM and related complications. It also boosts the late stage of DM and increases LDL oxidation and oxidized substances[38,39]. The antioxidant parameter such as SOD directly or indirectly remove oxygen free radicals, during the GDM. GDM rats showed excessive absorption of SOD and other endogenous antioxidant enzymes, which is beneficially to remove the ROS in the body[40]. ROS is a signal molecule that activates redox-sensitive signal pathways, triggering phosphorylation of insulin receptors and insulin receptor substrate in insulin signal pathways, resulting in the lower activity of downstream signal molecules, diminished insulin-sensitizing action and finally IR[40-42]. In this experimental study, diosmin treatment significantly removing the ROS and improved the level of endogenous antioxidant enzymes, which weakened the IR and increased insulin-sensitizing effect. This occurs due to an increase in the BGL level in the GDM rats.

CONCLUSION

The result suggests that diosmin significantly increased the body weight and litter size along with a reduction of the blood glucose level. Diosmin significantly suppressed the level of CP, FFA, HbA1c and reduced the level of hepatic glycogen. It also suppressed the level of LDL, TC, TG and increased the level of HDL. Diosmin significantly downregulated the level of TBARS and boosted the level of SOD, GSH and CAT. Diosmin significantly reduced the level of cytokines. Diosmin also reduced the mRNA expression of NF-κB and increased the mRNA expression of IκB in the pancreatic tissue. Diosmin significantly reduced the inflammatory reaction, oxidative stress and RAGE related genes in the STZ induced GDM rats. In future, more experimental studies require scrutinizing the possible mechanism of action.

SIGNIFICANCE STATEMENT

This study is novel and explores the protective effect of Diosmin (polyphenolic flavonoids) against the STZ induced gestational diabetes mellitus in the rats via improved level of

endogenous antioxidants and reduced inflammatory reaction. This study helps the researcher to uncover the critical complication associated with the pregnancy. Thus a new beneficial therapy on gestational diabetes mellitus during the pregnancy may have arrived.

REFERENCES

1. Yang, J., J. Jia, Y. Yang, Y. Zhao and Q. Li, 2020. Protective effect of specnuezhenide on islet β cell of rats with gestational diabetes mellitus. Cell. Mol. Biol., 66: 60-64.
2. Ziegler, A.G., M. Wallner, I. Kaiser, M. Rossbauer and M.H. Harsunen *et al*., 2012. Long-term protective effect of lactation on the development of type 2 diabetes in women with recent gestational diabetes mellitus. Diabetes, 61: 3167-3171.
3. Meng, Z., J. Wang, R. Li, Q. Tian, H. Mu, W. LI and X. Yang, 2020. An *in vivo* experimental study on protective effect of mangiferin in gestational diabetes mellitus rats. Latin Am. J. Pharm., 39: 1415-1420.
4. Tian, Z.H., F.T. Miao, X. Zhang, Q.H. Wang, N. Lei and L.C. Guo, 2015. Therapeutic effect of okra extract on gestational diabetes mellitus rats induced by streptozotocin. Asian Pac. J. Trop. Med., 8: 1038-1042.
5. Du, S., Y. Lv, N. Li, X. Huang and X. Liu *et al*., 2020. Biological investigations on therapeutic effect of chitosan encapsulated nano resveratrol against gestational diabetes mellitus rats induced by streptozotocin. Drug Delivery, 27: 953-963.
6. Xu, W., M. Tang, J. Wang and L. Wang, 2020. Anti-inflammatory activities of puerarin in high-fat diet-fed rats with streptozotocin-induced gestational diabetes mellitus. Mol. Biol. Rep., 47: 7537-7546.
7. Wang, Y., L. Wang, G. Xu and D. Wei, 2019. Hesperidin exerts the gestational diabetes mellitus via ages-rage signalling pathway. Int. J. Pharmacol., 15: 604-615.
8. Kassab, B.M., H.H. Hussein, O.M. Mahmoud and G. Abdel-Alrahman, 2019. Effects of insulin and metformin on fetal kidney development of streptozotocin-induced gestational diabetic albino rats. Anat. Cell Biol., 52: 161-175.
9. Osinubi, A.A.A., L.J. Medubi, E.N. Akang, L.K. Sodiq and T.A. Samuel *et al*., 2018. A comparison of the anti-diabetic potential of D-ribose-L-cysteine with insulin, and oral hypoglycaemic agents on pregnant rats. Toxicol. Rep., 5: 832-838.
10. Tang, X., Q. Qin, X. Xie and P. He, 2015. Protective effect of sRAGE on fetal development in pregnant rats with gestational diabetes mellitus. Cell Biochem. Biophys., 71: 549-556.
11. Dai, S., X. Meng, X. Cai, C. Yuan and Z. Zhao *et al*., 2021. Therapeutic effect of ursolic acid on fetal development in pregnant rats with gestational diabetes mellitus via AGEs RAGE signaling pathway. J. Food Biochem., Vol. 45. 10.1111/jfbc.1365.
12. Kawaharada, R., H. Masuda, Z. Chen, E. Blough, T. Kohama and A. Nakamura, 2017. Intrauterine hyperglycemia-induced inflammatory signalling via the receptor for advanced glycation end products in the cardiac muscle of the infants of diabetic mother rats. Eur. J. Nutr., 57: 2701-2712.
13. Rajaraman, B., N. Ramadas, S. Krishnasamy, V. Ravi and A. Pathak *et al*., 2019. Hyperglycaemia cause vascular inflammation through advanced glycation end products/early growth response-1 axis in gestational diabetes mellitus. Mol. Cell. Biochem., 456: 179-190.
14. Bednarska, K., P. Kuś and I. Fecka, 2020. Investigation of the phytochemical composition, antioxidant activity, and methylglyoxal trapping effect of *Galega officinalis* L. herb *in vitro*. Molecules, Vol. 25. 10.3390/molecules25245810.
15. Ali, T.M., O.M. Abo-Salem, B.H. El Esawy and A. El Askary, 2020. The potential protective effects of diosmin on streptozotocin-induced diabetic cardiomyopathy in rats. Am. J. Med. Sci., 359: 32-41.
16. Tanrikulu, Y., M. Sahin, K. Kismet, S.S. Kilicoglu and E. Devrim *et al*., 2013. The protective effect of diosmin on hepatic ischemia reperfusion injury: An experimental study. Bosn J. Basic Med. Sci., 13: 218-224.
17. Ali, N., A.F. AlAsmari, F. Imam, M.Z. Ahmed and F. Alqahtani *et al*., 2021. Protective effect of diosmin against doxorubicin-induced nephrotoxicity. Saudi J. Biol. Sci., Vol. 2021. 10.1016/j.sjbs.2021.04.030.
18. Prabhu, V.V., D. Sathyamurthy, A. Ramasamy, S. Das, M. Anuradha and S. Pachiappan, 2016. Evaluation of protective effects of diosmin (a citrus flavonoid) in chemical-induced urolithiasis in experimental rats. Pharm. Biol., 54: 1513-1521.
19. Jain, D., M.K. Bansal, R. Dalvi, A. Upaganlawar and R. Somani, 2014. Protective effect of diosmin against diabetic neuropathy in experimental rats. J. Integ. Med., 12: 35-41.
20. Islam, J., A. Shree, S.M. Afzal, A. Vafa and S. Sultana, 2020. Protective effect of diosmin against benzo(a)pyrene induced lung injury in swiss albino mice. Environ. Toxicol., 35: 747-757.
21. Mirzaee, S., E. Mansouri, M. Shirani, M. Zeinvand-Lorestani and M.J. Khodayar, 2019. Diosmin ameliorative effects on oxidative stress and fibrosis in paraquat-induced lung injury in mice. Environ. Sci. Pollut. Res., 26: 36468-36477.
22. Queenthy, S.S. and B. John, 2013. Diosmin exhibits anti-hyperlipidemic effects in isoproterenol induced myocardial infarcted rats. Eur. J. Pharmacol., 718: 213-218.
23. Lu, X., F. Wu, M. Jiang, X. Sun and G. Tian, 2019. Curcumin ameliorates gestational diabetes in mice partly through activating AMPK. Pharm. Biol., 57: 250-254.
24. Sun, C.C., Y.N. Lai, W.H. Wang, X.M. Xu and X.Q. Li *et al*., 2020. Metformin ameliorates gestational diabetes mellitus-induced endothelial dysfunction via downregulation of p65 and upregulation of NRF2. Front. Pharmacol., Vol. 11. 10.3389/fphar.2020.575390.

25. Sha, H., H. Zeng, J. Zhao and H. Jin, 2019. Mangiferin ameliorates gestational diabetes mellitus-induced placental oxidative stress, inflammation and endoplasmic reticulum stress and improves fetal outcomes in mice. Eur. J. Pharmacol., Vol. 859. 10.1016/j.ejphar.2019.172522.
26. Hua, S., Y. Li, L. Su and X. Liu, 2016. Diosgenin ameliorates gestational diabetes through inhibition of sterol regulatory element-binding protein-1. Biomed. Pharmacother., 84: 1460-1465.
27. Szmuilowicz, E.D., J.L. Josefson and B.E. Metzger, 2019. Gestational diabetes mellitus. Endocrinol. Metab. Clin. North Am., 48: 479-493.
28. Chiefari, E., B. Arcidiacono, D. Foti and A. Brunetti, 2017. Gestational diabetes mellitus: An updated overview. J. Endocrinological Invest., 40: 899-909.
29. Johns, E.C., F.C. Denison, J.E. Norman and R.M. Reynolds, 2018. Gestational diabetes mellitus: Mechanisms, treatment, and complications. Trends Endocrinol. Metab., 29: 743-754.
30. Shivananjappa, M.M. and Muralidhara, 2012. Taurine attenuates maternal and embryonic oxidative stress in a streptozotocin-diabetic rat model. Reprod. BioMed. Online, 24: 558-566.
31. Panee, J., 2012. Monocyte chemoattractant protein 1 (MCP-1) in obesity and diabetes. Cytokine, 60: 1-12.
32. Sartipy, P. and D.J. Loskutoff, 2003. Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc. Natl. Acad. Sci. USA., 100: 7265-7270.
33. Thomann, R., N. Rossinelli, U. Keller, B.F. Tirri and C.D. Geyter *et al*., 2008. Differences in low-grade chronic inflammation and insulin resistance in women with previous gestational diabetes Mellitus and women with polycystic ovary syndrome. Gynecological Endocrinol., 24: 199-206.
34. Kumar, V., K. Sharma, B. Ahmed, F.A. Al-Abbasi, F. Anwar and A. Verma, 2018. Deconvoluting the dual hypoglycemic effect of wedelolactone isolated from *Wedelia calendulacea*: Investigation via experimental validation and molecular docking. RSC Adv., 8: 18180-18196.
35. Kumar, V., R. Sachan, M. Rahman, K. Sharma, F.A. Al-Abbasi and F. Anwar, 2020. *Prunus amygdalus* extract exert antidiabetic effect via inhibition of DPP-IV: *In-silico* and *in-vivo* approaches. J. Biomol. Struct. Dyn., 39: 4160-4174.
36. Eneni, A.E.O., B. Ben-Azu, A.M. Ajayi and A.O. Aderibigbe, 2020. Diosmin attenuates schizophrenia-like behavior, oxidative stress, and acetylcholinesterase activity in mice. Drug Metab. Personalized Ther., Vol. 2020. 10.1515/dmdi-2020-0119.
37. Anwer, M.K., M.M. Ahmed, A. Alshetaili, B.K. Almutairy and A. Alalaiwe *et al*., 2020. Preparation of spray dried amorphous solid dispersion of diosmin in soluplus with improved hepato-renoprotective activity: *In vitro* anti-oxidant and *In-vivo* safety studies. J. Drug Delivery Sci. Technol., Vol. 60. 10.1016/j.jddst.2020.102101.
38. Gupta, R., M. Mathur, V.K. Bajaj, P. Katariya, S. Yadav, R. Kamal and R.S. Gupta, 2012. Evaluation of antidiabetic and antioxidant activity of *Moringa oleifera* in experimental diabetes. J. Diabetes, 4: 164-171.
39. Yu, L., X. Li, Z. Zhang, P. Du and J.L. Liu *et al*., 2019. Dimorphic autoantigenic and protective effects of Reg2 peptide in the treatment of diabetic β cell loss. Diabetes Obesity Metab., 21: 1209-1222.
40. Yao, L., J. Wan, H. Li, J. Ding, Y. Wang, X. Wang and M. Li, 2015. Resveratrol relieves gestational diabetes mellitus in mice through activating AMPK. Reprod. Biol. Endocrinol., Vol. 13. 10.1186/s12958-015-0114-0.
41. Li, S., Y. Zhang, Y. Sun, G. Zhang and J. Bai *et al*., 2019. Naringenin improves insulin sensitivity in gestational diabetes mellitus mice through AMPK. Nutr. Diabetes, Vol. 9. 10.1038/s41387-019-0095-8.
42. Chen, Y., J. Tang, Y. Zhang, J. Du, Y. Wang, H. Yu and Y. He, 2020. Astaxanthin alleviates gestational diabetes mellitus in mice through suppression of oxidative stress. Naunyn-Schmiedeberg's Arch. Pharmacol., 393: 2517-2527.