World Applied Sciences Journal 25 (12): 1690-1698, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.25.12.76142

Genetic Effects of Januvia and Galvus Alone or with Metformin on Pregnant Female Mice and Their Embryos

Hanaa M Roshdy and Salwa M Kassem

Department of Cell Biology, National Research Center, Dokki, Cairo, Egypt

Submitted: Sep 26, 2013; **Accepted:** Nov 1, 2013; **Published:** Nov 8, 2013

Abstract: Sitagliptin (Januvia) and vildagliptin (Galvus) are dipeptidyl peptidase-4 inhibitors newly approved to control blood sugar level in people with type 2 diabetes. They approved for use as mono therapy or in combination with metformin as (Janumet) and (Galvumet). Because recent information strongly suggest that abnormal blood glucose levels during pregnancy are associated with a higher incidence of abnormalities, anti diabetic drugs may be used during pregnancy to maintain blood glucose levels close to normal as possible. In fact there are no adequate and well controlled studies about the effects of Januvia and Galvus alone or in combination with metformin (Janumet and Galvumet) in pregnant women. To evaluate the cytogenetic effects of Januvia and Galvus alone or with metformin (Janumet and Galvumet) in pregnant female mice and their embryos. Pregnant female mice were divided into five groups. The females of the first group were administrated orally with a dose (0.04 mg/kg/day) of Januvia. The second group were administrated orally with (0.04 mg/kg/day) of Galvus. The third group were administrated orally with (0.04 mg Januvia, 0.2 mg metformin) of Janumet. The fourth group were administrated orally with a dose (0.04 mg Galvus, 0.2 mg metformin) of Galvumet and the fifth group were served as a control. All pregnant females were administrated orally from day (3) to day (18) of pregnancy at a doses equal to the recommended doses for human. Females were killed at day (19) of pregnancy and examined for the evidence of embryo toxic and cytogenetic effects. The results showed that the pregnant females and their embryos treated with Januvia and Galvus showed a significant increase in the embryo toxicity and in the total number of chromosomal aberrations compared with control, but Galvus treated group showed a significant decrease if compared with Januvia treated group. Also the females and their embryos treated with Janumet and Galvumet showed a slight increase in the embryo toxicity and total chromosomal aberrations but these increases were decreased than that of Januvia and Galvus alone without metformin and become close to the control group. These results indicate that januvia and Galvus had a mutagenic and toxic effects on the pregnant females and their embryos and should not be used during pregnancy, while, Janumet and Galvumet had a slight mutagenic and toxic effects on the pregnant females and their embryos but these effects decreased significantly than Januvia and Galvus treated groups and become close to the control group these results indicate that Janumet and Galvumet may be taken during pregnancy but under extreme medical control.

Key words: Galvus · Januvia · Janumet · Galvumet · Pregnant females · Embryos · Mice · Cytogenetic effects.

INTRODUCTION

Diabetes is a chronic disease that arises when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin produces.

Insulin is a hormone made by the pancreas that enables cells to take in glucose from the blood and use it for energy. Failure to produce insulin, or of insulin to act property, or both, leads to raised glucose (sugar) levels in the blood (hyperglycemia). This is associated with long-term damage to the body and failure of various organs and tissues.There are three main types of diabetes, type 1, type 2 and gestational diabetes [1]. Type 1 diabetes is sometimes called insulin-dependent or juvenile-onset diabetes. It is caused by an autoimmune reaction where the body's defense system attacks the insulin producing

Corresponding Author: Hanaa M. Roshdy, Department of Cell Biology, National Research Center, Dokki, Cairo, Egypt.

cells. People with type (I) diabetes produce very little or no Insulin this type usually occurs in children or young adults.

Type 2 diabetes is sometimes called non-Insulin dependent diabetes or adult onset diabetes and accounts for at least. 90% of all cases of diabetes. It is characterized by Insulin resistance and relative insulin deficiency this type usually occurs after the age of 40 but can occur earlier, especially in populations with high diabetes prevalence it is often, but not always, associated with obesity, which itself can cause insulin resistance and lead to elevated blood glucose levels.

Gestational diabetes (GDM) is a form of diabetes consisting of high blood glucose levels during pregnancy. It develops in one in 25 pregnancies world wide and is associated with complications in the period immediately before and after birth gestational diabetes usually disappear after pregnancy but women with GDM and their offspring are at an increased risk of developing type 2 diabetes later in life. Today, there is no cure for diabetes. Pharmacologic and non pharmacologic therapies are aimed at maintaining the quality of life of patients. Specifically, the treatment goals of diabetes therapy include maintenance of normal blood glucose levels, amelioration of signs and symptoms and reduction or prevention of the incidence of complications, morbidity and mortality.

Non pharmacologic therapy primarily consists of lifestyle modifications, which include exercise and diet. Pharmacologic therapy includes the use of oral hypoglycemic agents [2].

There are several drugs available to treat diabetes, such as sulfonylureas, biguanides and thiazolidinediones.

A relatively new class of drugs has been developed. The Dipeptidyl peptidase-4 (DPP) inhibitors, which include Januvia and Galvus [3].

Galvus (Vildagliptin) and Januvia (Sitagliptin) are the first in a new class of oral medications used to control blood sugar levels in people with type 2 they called DDP-4 inhibitors (dipeptidyl peplidase-4 inhibitors) (sometimes called gliptins) designed to enhance the body, natural system of lowering blood glucose. They do this by rising the levels of a naturally occurring hormone, called GLP-1 released in the stomach and intestines during eating this hormone causes the pancreas to produce more insulin while simultaneously discouraging the liver from producing sugar [4].

For some people, one medication alone is not enough to mange their sugar levels and several drugs from different classifications are necessary to maximize disease control. Recently, the two medications, januvia ad Galvus are combine with another anti diabetic drug metformin hydrochloride (Amophage) is also used in the management of type 2 diabetes [5].

Metaformin hydrochloride work by increasing glycogen synthesis and glucose uptake in the liver and also enhances insulin sensitivity in the liver and fat. The two combined medications are called janumet (Januvia/ metformin) combination and Galvumet (Galvus/metformin) combination [6].

Because recent information strongly suggest that abnormal blood glucose levels during pregnancy are associated with a higher incidence of congenital abnormalities, anti diabetic drugs may be used during pregnancy to maintain blood glucose level close to normal as possible.

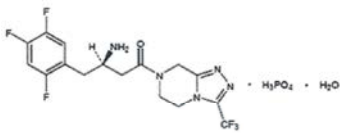
At present, there are no adequate and well controlled studies are available that illustrates the safety use of Galvus (vildagliptin), Galvumet (Vildagliptin/metformin), Januvia (sitagliptin) and Janumet (sitagliptin/ metformin) in the pregnant females and embryos.

Therefore, the present study was performed to evaluate the cytogenetic and developmental effects of Galvus and Januvia alone and in combination with metformin on pregnant females and their embryos if given orally from day 3 to 18 during pregnancy.

MATERIALS AND METHODS

Test Drugs:

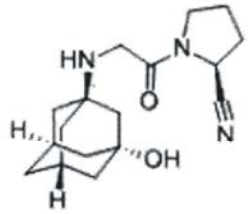
- Januvia tablets provided by (merck's) contain (sitagliptin phosphate monohydrate) is described chemically as 7-[(3R)-3-amino-1-Oxo-4-(2,4,strifluoropheyl)butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl)-1,2,4-triazolo[4,3-a]pyrazine phosphate (1:1) monohydrate.



The molecular formula is $C_{16}H_{15}F_6N_5O.H_3PO_4.H_2O$ and the molecular weight is 523.32. The structural formula is Sitagliptin phosphate monohydrate is a white to off white crystalline powder. It is soluble in water slightly soluble in methanol and insoluble in isopropanol and isopropyl acetate. Januvia tablets are supplied for oral administration as 50mg and 100mg of sitagliptin phosphate monohydrate. The recommended dose of Januvia is 100mg once daily.

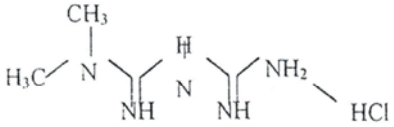
- Galvus tablets is provided by (Novaritis) contain (vildagliptin) is described chemically as [3-Hydroxy-adamantan-1-YI)amino]acetyl) pyrrolidine-2-Carbonitrile.

The molecular formula is $C_{17}H_{25}N_3O_2$ and the molecular weight is 303.4 the structural formula is Galvus is a white solid tablets it is soluble in water and supplied for oral administration as 50mg and 100mg of vildagliptin.



The usual recommended dose of Galvus is 50mg once daily and the maximum recommended dose is 100mg taken as 50mg twice a day.

- Janumet tablets provided by (mark) contain (sitagliptin/ metaformin). It is soluble in water and supplied for oral administration as 50mg/100mg sitagliptin and 1000mg metformin hydrochloride.
- Galvumet tablets provided by (Novaritis) contain (vildagliptin/ metaformin) It is soluble in water and supplied for oral administration as 50mg/100mg and 1000mg metformin hydrochloride.
- Metformin hydrochloride is an oral anti hyperglycemic drug used in the management of type 2 diabetes with a molecular formula of $C_4H_11N_5$.HCl and a molecular weight of 165.63. The structural formula is



The recommended dose of metformin is 500-1000mg/kg/day for human.

Experimental Design: Dilution of different concentrations was prepared by dissolving all tablets in distilled water. Females were housed in cages with adult males in a ratio of 3:1 respectively. One day after mating, the females which exhibiting vaginal plugs were considered as pregnant. The day of the appearance of the vaginal plug were considered the day I of pregnancy. The pregnant females were caged individually and divided into five groups.

The first group were administrated orally with a single dose of Januvia (0.04 mg/kg/day).

The second group were administrated orally with a single dose of Galvus (0.04mg/kg/day).

The third group were administrated orally with a single dose of Janumet (0.04mg Januvia 0.2mg metformin).

The fourth group were administrated orally with a single dose of Galvumet (0.04 mg Galvus 0.2mg metformin).

The fifth group served as control group were administrated orally with the same volume of distilled water. Januvia, Galvus, Janumet and Galvumet were tablets dissolved in distilled water and administrated orally from day (3) to day (18) of pregnancy with the dose equal to the recommended dose for human.

The doses of Januvia, Galvus, Janumet and Galvumet were modified to suit the small weight of albino mice according to pagat and Barnes [7].

The general behaviour of the pregnant females were observed every day.

On day 19 of pregnancy the pregnant females were sacrificed by cervical dislocation the uterus was opened and the number of live fetuses and dead fetuses were recorded.

The bone marrow of the mothers were collected and live embryos were randomly selected from each group to study the chromosomal aberrations in the mothers and their embryos.

MATERIALS AND METHODS

Developmental Toxicity: On day 19 of gestation, the females were sacrificed by cervical dislocation the uterus contents were evaluated for the number of implantation sites, resorption, dead and live embryos.

Methods for Preparing Chromosomes:

From Bone Marrow Cells of Pregnant Females: Chromosomes from bone marrow cells were prepared according to the methods of Hus and Patton [8] and Yosida *et al*. [9]. Bone marrow were collected in TCM 199. Culture medium and cholchicine was added to the tube (2ml of 0.05 cholchicine/5ml medium) then, the cells were incubated at 37°C for 90 minutes.

An amount of 5ml of hypotonic solution (0.56%) KCl was added and the cells were suspended and incubated at 37°C for 25 min then freshly prepared fixative (3:1 methyl alcohol/glacial acetic) was added drop by drop and one or three drops were added on a clean slide,

dried and stained with 10% Giemsa stain. About of 50 metaphase spreads were analyzed for each female and different types of chromosomal aberrations were recorded.

Methods for Preparing Chromosomes from Embryonic Cells: Chromosomal preparations from embryonic cells were prepared according to Evans *et al*.[10] and Romagnano *et al*. [11] as follows:

Embryos livers were collected from each group and placed in 5ml TCM 199 medium, 2ml of 0.05 colchicine was added for each tube and incubated at 37°C for 90 minutes, then the cells were centrifuged at 1000 rpm for 5 minutes.

After centrifugation the supernatant was discarded and 5ml of hypotonic solution of 0.56% KCl was added to the pellet. The cells were re suspended in the hypotonic solution and incubated at 37°C for 15 minutes.

5mL of freshly prepared fixative (3 methyl alchohol:1 glacial acetic acid) were added gently to the cells drop by drop. Two or three drops of cell suspension were dropped on the surface of a clean slide, dried and stained with 5% Giemsa stain for 14 min.

50 metaphase spreads were examined for each embryo and the different types of chromosomal abnormalities (structural and numerical) were recorded.

Statistical Analysis: The incidences of implantation, live and dead fetuses between experimental and control values were calculated non-parametrically using wilcoxons rank sum test [12].

The data for chromosomal aberrations in the females and embryos were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran [13]. Least significant differences were used to compare between means according to Waller and Duncan [14] at probability 5%.

RESULTS

Maternal Observations: Females administered with Januvia, Galvus, Janumet and Galvumet from day 3 to 18 of gestation showed no signs of illness or abnormal behaviour and appeared more or less normal on gross observation. The pregnant female mice exposed to januvia, Galvus, Janumet and Galvumet did not exhibit any toxic symptoms during pregnancy (Table 1).

Embryo Toxic Effect: The data are summarized in Table (1), the administration of Januvia and Galvus (diabetic drugs) to the pregnant females from day 3 to 18 of gestation resulted in an increase in the number of resorption and the number of dead embryos, while the number of implantations and number of live embryos decreased compared with the control group. On the other hand the administration of Janumet and Galvumet from day 3 to 18 of gestation resulted a slight increase in the number of resorption and the number of dead fetuses and a slight decrease in the number of implantation sites and live embryos compared with Januvia and Galvus groups and compared with the control.

Chromosomal Aberrations:

In the Bone Marrow Cells (In Pregnant Females): Mean value of the effect of oral administration of Galvus and Januvia on the bone marrow cells are given in Table (2).

In the group of pregnant females treated with Galvus, the frequencies of the total number of structural and numerical aberrations were increased significantly ($p > 0.05$) compared with the control.

The most frequent aberrations here are chromatid gaps, breaks, deletions, fragments. Endometosis, centromeric attenuation, Aneuploidy and polyploidy.

While, in the group of females treated with Januvia there were significant ($p > 0.05$) increase in the frequencies of structural and numerical aberrations than Galvus and control groups.

The frequencies of the total structural and numerical aberrations for Januvia group were (45.33 and 15) compared with (41.7 and 12.67) and (19.67 and 6.00) for Galvus and control groups respectively.

Mean values of the effect of oral administration of Janumet and Galvumet on the bone marrow cells of pregnant females are given in Table (4).

In the group of pregnant females treated with Galvumet (Galvus + metformin) from day 3 to 18 of pregnancy, the total number of structural and numerical aberrations were slightly increased significantly than the control group.

In the same time, in the group of pregnant females treated with Janumet (Januvia + metformin) the total number of structural aberrations was slightly increased significantly ($p > 0.05$) than the Galvumet and control groups while, the total number of numerical aberrations was slightly increased but in the same limit with Galvumet group.

The frequencies of the total number of structural and numerical aberrations for Janumet group were (26.67 and 9.33) compared with (23.00 and 7.67) and (19.67 and 6.00) for Galvumet and control groups respectively.

Table 1: The effect of diabetic drugs on the fertility and offspring development in mice at day 18 of pregnancy.

	Group treatments				
Parameters	Control	Januvia	Galvus	Janumet	Galvumet
No of females for study	10	10	10	10	10
No of pregnant females	10	10	10	10	10
Total no of implantation	75	70	72	73	74
Total no of Resorption	3	4	4	4	3
%	4%	5.7%	5.5%	5.4%	4.1%
Total no of dead fetuses	2	4	3	3	2
%	2.7%	5.7%	4.2%	4.1%	2.7%
Total no of life fetuses	70	62	65	66	69
%	93.3%	88.6%	90.3%	90.3%	93.2%

Table 2: The effects of Januvia and Galvus on bone marrow cells of pregnant females.

	Structural aberration								Numerical aberration			
Treatments	Chromatid gap	Chromoso mal gaps	Chromatid break	Deletion	Fragment	Endo-metosis	Centromic attenuation	Total structural aberration	<40	>40	Poly ploidy	Total numerical aberration
Control	4.00±0.00^{a}	2.67±0.58^{a}	1.67±0.57^{a}	3.00±1.00^{a}	2.67±0.57^{a}	3.33±0.57^{a}	2.33±0.57^{a}	19.67±1.155^{a}	4.00±0.00^{a}	2.00±0.00^{a}	0.0±0.00a	6.00±0.00^{a}
Galvus	7.33±0.57^{b}	5.00±0.00^{b}	6.00±1.00^{b}	5.33±0.57^{b}	6.33±0.57^{b}	6.67±0.57$^{(b)}$	5.00±0.00^{b}	41.7±1.53$^{(b)}$	5.67±0.57^{b}	4.33±0.57$^{(b)}$	2.67±0.57^{b}	12.67±1.53^{b}
Januvia	8.33±0.57^{r}	6.00±1.00^{b}	7.00±1.00^{b}	5.67±1.15^{b}	5.67±0.57^{b}	6.67±0.57$^{(b)}$	6.00±1.00^{b}	45.33±1.53$^{(c)}$	6.00±1.00^{b}	5.33±0.67$^{(c)}$	3.67±0.57^{c}	15.00±0.00^{c}

Means (± S.D.) of different letters (a,b,c,d) in the same column are significantly different.

•The column with the same letter is not significant

•50 metaphase were examined from each animals.

Table 3: The effects of Januvia and Galvus on embryos

	Structural aberration								Numerical aberration			
Treatments	Chromatid gap	Chromoso mal gaps	Chromatid break	Deletion	Fragment	Endo-metosis	Centromic attenuation	Total structural aberration	<40	>40	Poly ploidy	Total numerical aberration
Control	2.67±0.57a	1.33±0.57^{a}	1.67±0.57^{a}	1.33±0.57^{a}	1.33±0.57^{a}	2.33±0.057^{a}	2.00±1.00^{a}	12.67±1.528^{a}	2.33±0.57^{a}	1.67±0.57^{a}	0.00±0.00^{a}	4.00±1.00^{a}
Galvus	5.33±0.57^{b}	3.00±0.00^{b}	4.33±0.57^{b}	3.67±1.528^{b}	4.67±0.57^{b}	5.67±0.57^{b}	4.00±1.00^{b}	30.33±0.57^{b}	5.33±0.57^{b}	3.67±0.67^{b}	2.00±0.00^{b}	10.67±0.57^{b}
Januvia	5.00±5.00^{b}	5.00±0.00^{c}	5.00±1.00^{b}	5.00±1.00^{b}	5.00±1.00^{b}	4.67±0.57^{b}	5.00±1.00^{b}	34.67±0.57^{c}	5.67±0.57^{b}	5.67±0.57^{c}	3.33±0.57^{c}	14.67±0.57^{c}

Means (± S.D.) of different letters (a,b,c,d) in the same column are significantly different.

•The column with the same letter is not significant

•50 metaphase were examined from each animals.

Table 4: The effect of Janumet and Galvumet on bone marrow cells of pregnant females

	Structural aberration								Numerical aberration			
Treatments	Chromatid gap	Chromoso mal gaps	Chromatid break	Deletion	Fragment	Endo-metosis	Centromic attenuation	Total structural aberration	<40	>40	Poly ploidy	Total numerical aberration
Control	4.00±0.00^{a}	2.67±0.57^{a}	1.67±0.57^{a}	3.00±1.00^{a}	2.67±0.57^{a}	3.33±0.57^{a}	2.33±0.57^{a}	19.67±1.155^{a}	3.67±0.00^{a}	2.00±0.00^{a}	0.00±0.00^{a}	6.00±0.00^{a}
Galvus + metformin	4.00±1.00^{a}	3.33±0.57^{a}	3.00±1.00^{ab}	3.00±0.57^{ab}	3.33±0.57^{a}	3.67±0.57^{a}	3.33±0.58^{ab}	23.00±1.00^{a}	3.67±0.58^{a}	3.33±0.57^{b}	1.33±0.57^{b}	7.67±0.57^{ab}
Januvia + metformin	4.00±1.00^{a}	4.00±1.00^{a}	3.67±0.57^{b}	4.00±0.00^{b}	4.00±1.00^{a}	4.00±1.00^{a}	4.00±1.00^{b}	27.67±0.57^{c}	4.33±0.57^{a}	3.33±0.58^{b}	1.67±0.57^{b}	9.33±1.53^{b}

Means (± S.D.) of different letters (a,b,c,d) in the same column are significantly different.

•The column with the same letter is not significant

•50 metaphase were examined from each animal.

Table 5: The effect of Janumet and Galvumet on embyos

	Structural aberration								Numerical aberration			
Treatments	Chromatid gap	Chromoso mal gaps	Chromatid break	Deletion	Fragment	Endo-metosis	Centromic attenuation	Total structural aberration	<40	>40	Poly ploidy	Total numerical aberration
Control	2.67±0.57^{a}	1.33±0.57^{a}	1.67±0.57^{a}	1.33±0.57^{a}	1.33±0.57^{a}	2.33±0.57^{a}	2.00±1.00^{a}	12.67±1.53^{a}	2.33±0.57^{a}	1.67±0.57^{a}	0.00±0.00^{a}	4.00±1.00^{a}
Galvus + metformin	2.67±0.57^{a}	1.67±0.57^{a}	2.67±1.00^{ab}	2.33±0.58^{a}	3.00±0.57^{b}	2.33±1.00^{a}	2.67±0.57^{a}	18.00±4.00^{b}	3.67±0.57^{ab}	2.00±1.00^{a}	0.67±0.57^{a}	6.33±1.15^{b}
Januvia + metformin	3.00±1.00^{a}	3.33±0.57^{b}	3.67±0.57^{a}	2.33±0.58^{a}	3.23±1.00^{b}	3.33±0.57^{a}	2.67±1.15^{a}	20.33±0.57^{b}	4.00±1.00^{b}	3.00±0.00^{a}	1.67±0.57^{b}	8.67±0.57^{c}

Means (± S.D.) of different letters (a,b,c,d) in the same column are significantly different.

•The column with the same letter is not significant

•50 metaphase were examined from each animal.

In the Embryonic Cells (Embryos): Mean values of the effect of oral administration of Galvus and Januvia on the chromosomes of embryos are given in Table (3).

Cytogenetic examination showed that the total number of structural and numerical aberrations in the embryos treated with Galvus were increased significantly than that of the control group.

Also the frequencies of the total number of structural and numerical aberrations in the group of embryos treated with Januvia were increased significantly than those of the Galvus and control groups.

The total number of structural and numerical aberrations in the Januvia group were (34.67 and 14.67) compared with (30.33 and 10.67) and (12.67 and 4.00) for Galvus and control groups respectively.

Mean values of the effect of oral administration of Janumet and Galvumet on the chromosomes of embryos are given in Table (5).

The frequencies of the total number of structural and numerical aberrations in the group treated with Galvumet were slightly increased than the control group. The total number of structural and numerical in Galvus group were (18.00 and 6.33) compared with control (12.67 and 4.00).

While, the frequencies of the total number of structural and numerical aberrations in the embryos treated with Janumet were increased significantly than Galvumet and control groups. The total structural and numerical aberrations of Janumet embryo group were (20.33 and 8.67) compared with (18.00 and 6.33) and (12.67 and 4.00) for Galvumet and control groups respectively.

Comparison Between the Effect of (Galvus and Galvumet) and (Januvia and Janumet) in Pregnant Females: Cytogenetic data between the mothers treated with Galvus and the mothers treated with Galvumet showed that the mothers treated with Galvus had increases in the frequencies of total chromosomal aberrations (Structural and numerical) than mothers treated with Galvumet.

The total structural and numerical aberrations of Galvus were (41.7 and 12.67) compared with (23 and 7.67) for Galvumet group.

Also, cytogenetic examinations in mothers treated with Januvia had increases in the frequencies of total chromosomal aberrations than mothers treated with Janumet.

The total number of structural and numerical aberrations of Januvia (43.33 and 15.00) compared with (27.67 and 9.33) for Janumet group.

Comparison Between the Effect of (Galvus and Galvumet) and (Januvia and Janumet) in Embryos: When comparing the frequencies of the total chromosomal aberrations (structural and numerical) in embryos treated with Galvus and embryos treated with Galvumet we found that embryos treated with Gavlus had more frequent in the chromosomal aberrations than those treated with Galvumet.

The total number of structural and numerical aberrations of Galvus (30.33 and 10.67) compared with (8.00 and 6.33) for Galvumet embryo group.

Also, embryos treated with Januvia had more frequent in the number of chromosomal aberrations than embryos treated with Janumet.

The total number of structural and numerical aberrations of Januvia (34.67 and 14.67) compared with (20.33 and 8.67) for Janumet embryo group.

DISCUSSION

Type 2 diabetes or non insulin dependent diabetes mellitus is a type of diabetes in which there are insufficient insulin levels in the body combined with insulin resistance (a situation where cells do not respond properly to insulin. They become resistant hence glucose does not enter the cells). Though there is insulin in the body, it is not enough to overcome the resistance of cells, resulting in increased levels of sugar in the blood. It is the most common type of diabetes, with around 90% of diabetics belonging in this type. It is also referred to as adult onset diabetes, due to its late onset in patients. Patients are usually 30 years old when this disease sets in.

The WHO has stated several risk factors for this disease, which include family history, race or ethnicity, obesity, age of greater than forty five, hypertension, hyper lipidemia, history of gestational diabetes, as well as lack of physical activity and excessive food intake.

Sadly, there is no cure of diabetes. Pharmacologic and non pharmacologic therapies are aimed at maintaining the quality of life of patients. Specifically, the treatment goals of diabetes therapy include maintenance of normal blood glucose levels and reduction or prevention of the incidence of complications, morbidity and mortality. Non pharmacologic therapy primarily consists of lifestyle modifications, which include exercise and diet. Pharmacologic therapy includes the use of oral hypoglycemic agents. There are several drugs available to treat diabetes, such as insulin.

Januvia and Galvus, are an oral hypoglycemic agent belonging to the novel class of drugs called Dipeptidyl peptidase-4 Inhibitors it has been approved for the treatment of type 2 Diabetes mellitus.

Januvia and Galvus have been shown to effectively control glucose levels both in animal and human [15].

In the present study we found that the treatment of pregnant females with Januvia from day 3 to 18 of gestation with a dose of (0.04 mg/kg/day) corresponding to the therapeutic dose for human caused a significant increase in the embryonic toxicity and chromosomal aberrations in maternal bone marrow cells and in the embryonic cells compared with those occurring under the effect of Galvus and control groups.

Also, there were significant increases in the chromosomal aberrations and embryonic toxicity in mothers and embryos treated with Galvus if compared with the control animals, but these increases were less than those occurring under the effect of Januvia.

These results are in agreement with Goldstein [16] who reported that Januvia had a mutagenic and carcinogenic effects on liver and pancreas of humans.

Also, positive results were obtained by Lignero *et al*. [17] who observed that Galvus has a slightly mutagenic effects on animals but this effect was slightly less than the mutagenic effect of Januvia.

On the other hand, negative results were observed by Davis *et al*. [18] who found that there was no evidence of mutagenic or clastogenic of Januvia or Galvus in human and animals.

Also, negative results were observed by Meghan and Kiwon [19] who found that Galvus was not mutagenic or clastogenic in mouse micronucleus test.

Also, negative results were obtained by Otterbeck and Banerji [20] who found that valdagliptin (Galvus) was not mutagenic in convert ional in vitro and in vivo tests for genotoxicity.

Also, negative results were observed by Pederson *et al*. [21] who found that in a fertility and embryonic development study in rats revealed no evidence of impaired fertility, reproductive performance on early embryonic development due to vildagliptin (Galvus).

While positive results were obtained by Yin *et al*. [22] who evaluated embryo foetal toxicity in rats and rabbits. An increased incidence of wavy ribs was observed in rats in association with reduced in maternal and foetal body weight with a dose of 75 mg/kg/day (10-fold human exposure).

Also positive results with Januvia were observed by Ann and Thornberry [23] who found that higher doses of Januvia in rabbits increased the incidence of rib malformations in offspring at 100 mg /kg/day.

While negative results were observed by Bergman *et al*. [24] who found that sitagliptin (Januvia) administered to pregnant female rats and rabbits from gestation day 6 to 20 (organogenesis) was not teratogenic at oral doses up to 250 mg/kg (rats) and 152 mg/kg in (rabbits).

Also, in the present study, we found that when pregnant females were administrated orally with a dose of Janument equal to the recommended dose in human caused a significant increase in the total chromosomal aberration (structural and numerical) in the pregnant females and in the embryos but this effect was less than the effect of Januvia alone.

This result is in agreement with that of Aleksey and Matveyenk [25] who reported that there was no evidence of mutagenic potential of metformin alone or in combination with Januvia or Galvus in human or animals.

Also, positive results we observed by Bailliar *et al*. [26] who found that metformin alone or in combination was not teratogenic in rats and rabbits at a dose up to 1000 mg/kg/day.

In our study, we found that when pregnant females were administrated orally with a dose of Galvumet equal to the recommended dose for human, there was a slight significant increases in the chromosomal aberrations but these increases were less slightly than Galvus alone and also less than Janumet mothers and embryos treated groups.

These finding was agreement with Bernd and Dlizabeth [27] who reported that Galvus has a safety profile than Januvia in humans and animals and when administrated orally did not cause any side effects like the other antidiabetic drugs.

Also, positive results were observed by Bolli *et al*. [28] who found that Galvus have a better safety profile than the other antidiabetic agents, alone or with a combination with metformin.

Also, similar results were obtained by Yaris *et al*. [29] who reported that no evidence of carcinogenicity with metformin alone was found in either male of female mice.

While negative results were obtained by Bosi *et al*. [30] who found when Galvus used in combination with other diabetes medications, it actually reduced the potential side effects of the other diabetes medications for example when Galvus combined with metformin, it reduced the chance of stomach upset and reduction of HbA among older patients.

CONCLUSION

In conclusion, Januvia and Galvus had a mutagenic effect on both mothers and their embryos when administrated orally to the pregnant females from day 3 to 18 of pregnancy at a doses equal to the recommended doses of human and these effects more significant in animals treated with Januvia than that treated with Galvus drug.This may be due to that Galvus has a safety profile and a less side effects than that of Januvia drug.

Also, Janumet (Januvia/metformin) and Galvumet (Galvus/metformin) caused a slightly increased in the developmental and chromosomal aberrations in mothers and their embryos if administrated during pregnancy in a recommended doses for human but these increases were less than that of Galvus and Januvia alone without metformin.These results may be due to that metformin alone has no mutagenic effects, so metformin may minimize or lower the mutagenic effects of Januvia and Galvus in Janumet and Galvumet drugs.

Also, the effect of Janumet on pregnant females and their embryos were became very close to the effect of Gavlumet, this may be as a result of the effect of metformin on the two drugs which lower the cytotoxic effects of Januvia to be the same effect of Galvus.

So from the above data we concluded finally that Galvus and Januvia should not be taken during pregnancy but Galvumet and Janumet could be used during pregnancy only under medical control and after consideration of the risk/benefit.

REFERENCES

1. Fukushima, M., H. Snzuki and Y. Seino, 2004. Insulin secretion capacity in the development from normal glucose tolerance to type 2 diabetes. Diabetes Res., 66(1): 537-543.
2. Ishibashi, Y., T. Matsni, M. Takenchi and S. Yamagishis, 2011. Sitagliptin augments protective effects of GIP-1 against advanced glycation end product receptor axis in endothelial cells. Horm Metab Res., 43: 731-734.
3. Drucker, D.J., 2006. The biological of incretin hormones. Cell Metab, 3: 153-165.
4. Signorovitch, J.E., E. Swallow, E. Kanto, L. Fan and J.B. Grenenberger, 2011.Comparative efficacy of vildagliptin and sitagliptin in Japanese patients with type 2 diabetes mellitus: a matching- adjusted indirect comparison of randomized trials. Clin Drug Investig. 31: 665-674.
5. Thoms, S.M. and R.K. Compblee, 2003. Metformin hydrochloride in the treatment of type 2 *diabetes mellitus*. A chemical review with a focus on dual therapy. Clin. Ther., 25(12): 2991-3026.
6. Raz, I., Y. Chen and M. Wu, 2008. Efficacy and safety of sitagliptin added to an going metformin therapy in patients with type 2 diabetes. Current medical Research & Opinion, 24: 537-50.
7. Pagat, G.G. and J.M. Barnes, 1946. Evaluation of drug activities, Vol 1. Academic Press.
8. Hus, I.T.C. and J,.L. Patton, 1969. Bone marrow preparations for chromosome studies, In Bermischek Comparative mammalian cytogenetics springerver Lag., pp: 454-460.
9. Yosida, T.H., K. Truchiya and K. Moriwaki, 1971. Frequency of chromosome polymorphism of Ruttus Rattus Collected in Japan. Chromosoma, 33: 30-37.
10. Evans E., M.D. Byattshaw and E.C. Ford, 1972. Chromosomes of mouse embryos and newborn young preparations from membranes and tails Tips-stain Technol., 47: 229-234.
11. Romagnano, A., G.L. Richer and M.A. Perrone, 1995. A direct technique for the preparation of chromosomes from early equine embryos. Can. J. Gent. Cytol., 27: 365-369.
12. Siegal, S., 1956. Non parametic statistics for the behavioural sciences MC. Graw, Hill, New York, pp: 116-127.
13. Snedecor, G.W. and W.G. Cochran, 1990. Statistical Methods, 9th ed. Lowa State Univ. Press-Lova, U.S.A.
14. Waller, A. and D.B. Duncan,1969. Multiple range and multiple test. Bionetries, 11: 1-24.
15. Drucker, D.J. and M.A. Nauck, 2006. The incretin system: Glucagon-like peptides-4 receptor agonists and dipeptidyl peptidase-4- inhibitors in type 2 diabetes. Lancet, 368: 1696-1705.
16. Goldstein, M.R., 2007. DDP-4 inhibitors and cancer. Ann Int Med. Sept., pp: 21.
17. Lignero, S., M. Saylan. J.E. Foley and A. Schweizer, 2010. An assessment of adverse effects of vildagliptin versus comparators on the liver, the pancreas, the immune system, the skin and in the patients with impaired renal function. Diabetes,Obesity & Metabolism. 12: 495-509.
18. Davis, J.A., S. Singh, S. Sethi, S. Roy, R. Mittra, G. Rayasam, V. Bansoi and A. Ray, 2010. Nature of action of sitagliptin the dipeptidyl peptidase-IV inhibitor in diabetic animals. Indian J. Pharmaco L. 42: 229-233.

19. Meghan, S. and B. Kiwon, 2010. Genetic deletion or pharmacological inhibition of dipeptidyl peptidase-4 improves cardiovascular outcomes after myocardial infection in mice. Diabetes, 59(4): 1063-1073.
20. Otterbeck, P.E. and M.A. Banerji, 2012. The Efficacy and safety of vildagliptin in the galant trial: chronic kidney disease and other applications. Expert Review of endocrinology and Metabolism, 6(2): 143-151.
21. Pederson, RA., H.A. White, D. Schlenzing, R.P. Panly, C.H. Mclntosh and H.U. Demnth, 1998. Improved glucose tolerance in zucker fatty rats by oral administration of the dipeptidyl peptidase IV inhibitor isoleucine thiazolidide. Diabetes, 47: 1253-8.
22. Yin, M., M. Meissner, W.H. Van Gilst and R.A. De Boer, 2011. Early and late effects of the DPP-4 inhibitor viladgliptin in a rat model. Cardiovase Diabetol., 10: 85.
23. Ann, E. and N. Thornberry, 2007. Januvia (sitaglipth) a selective Dipeptidyl Peptidale IV inhibitor for the treatment of type 2 diabetes. Annual Reports in Medicinal Chemistry, 42: 95-109.
24. Bergman, A.J., C. Stevens, M. Zhony laethem, M. De smet, K. Snyder, W. Tanaka, M. Tanen. A.Q. Wang, L. Chen, M.J. Davies and S. Ramoel, 2006. Pharmacokinetic and pharma co dynamic properties of multiple oral doses of sitagliptin adouble- blind randomized placebo- controlled study in healthy male volunteers. Clin Ther., 28: 55-72.
25. Aleksey, V. and O. Matveyenk, 2009. Beneficial endocrine but adverse exocrine effects of sitagliptin in the human islet amyloid polypeptide transgenic rat model of type 2 diabetes interactions with metformin. Diabetes, 58(7): 1604-1615.
26. Bailliar, T.P., D. Jakubowicz and M.J. Luomo, 2004. Effect of metformin and Rosightazone alone and in combination in monobese women with polycystic ovary syndrome and normal indices of insulin sensitivity. Ferti. Streil, 42(4): 893-902.
27. Bernd, B. and E. Dlizabeth, 2009. Emerging role of dipeptidyl peptidase-4 inhibitor in the management of type 2 diabetes. Vascheath Risk Mang., 4(4): 753-768.
28. Bolli, G., F. Dotta.and E. Rochotte, 2008. Efficacy and tolerability of vildagliptin vs pioglitazone when added to metformin: a24-week randomized, double-blind study Diabetes, Obesity and Metabolism, 10: 82-90.
29. Yaris, F., E.Yaris, N.L. Kadioglum and N.L. Kalyoucu, 2004. Normal pregnancy outcome following in advertent exposure to Roriglitazone, gliclazide and atorvastatin in a diabetic and hypertensive women. Reprod. Toxiol, 18(4): 619-21.
30. Bosi, E., R.P. Camisasca and C. Collober, 2007. Effects of vildagliptin on glucose control over 24- weeks in patients with type 2 diabetes in adequately controlled with metformin. Diabetes Care, 30: 390-5.