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A review: effects of metformin on mice and rats

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Abstract

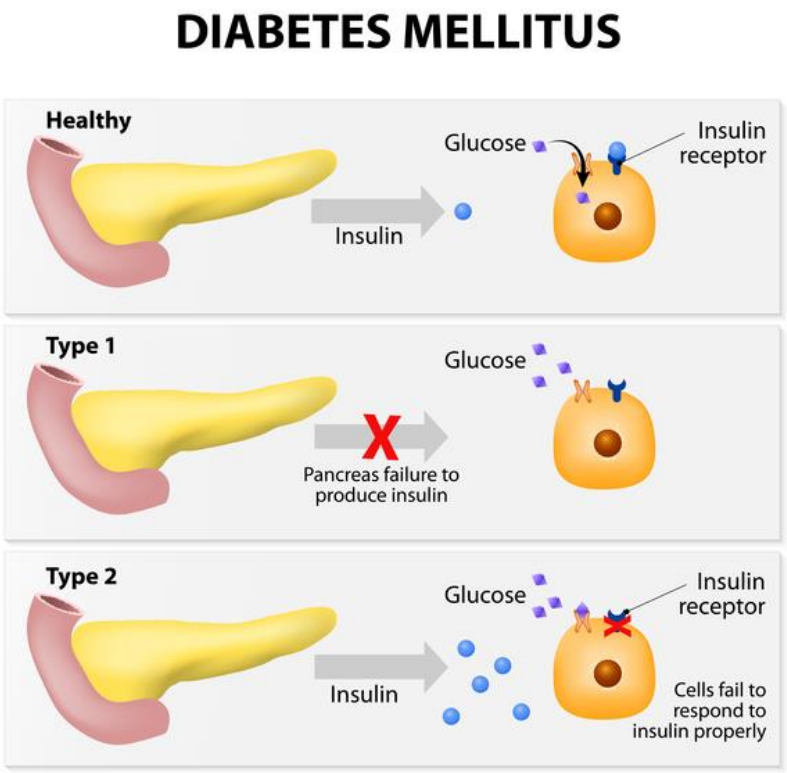
This review examines the effects of metformin on rat and mouse models, focusing on its role in metabolic regulation, aging, and associated physiological processes. Metformin, a widely used antidiabetic drug, has been extensively studied in rodent models to better understand its mechanisms of action and potential broader applications beyond glycemic control. Research consistently shows that metformin improves insulin sensitivity, reduces hyperglycemia, and mitigates weight gain in rodents, making it a valuable tool in studying type 2 diabetes and metabolic syndrome. Additionally, metformin's impact on aging has garnered significant attention. Studies suggest that metformin may extend lifespan and health span in rodents by enhancing mitochondrial function, reducing oxidative stress, and modulating key signaling pathways, such as mTOR. However, these effects are not universally beneficial, as some studies have reported adverse outcomes, including gastrointestinal issues and potential reproductive toxicity, particularly at higher doses. Overall, while metformin demonstrates considerable promise in rodent models for its metabolic and anti-aging effects, the translation of these findings to human health requires further investigation. This review highlights the need for continued research to fully elucidate metformin's mechanisms and long-term implications in both preclinical and clinical settings.

Keywords: Diabetes mellitus, Metformin, autoimmunity, pharmacokinetic, ketoacidosis

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycemia
that results from disturbed insulin secretion or function or both.[1] Diabetes mellitus (DM) is a metabolic disease with high morbidity. It significantly deteriorates the quality of health and life. Early diagnostic methods for diabetes remain lacking, resulting in patients losing the optimal treatment opportunity, which increases the risk of diabetic complications.[2] Current therapeutic options include oral hypoglycemic drugs or insulin injections, which provide temporary blood glucose level control; however, these therapies cannot prevent diabetic complications and are associated with adverse effects such as hypoglycemia.[3] Chronic hyperglycemia in DM is accompanied by

damage, dysfunction and failure of various organs and tissues, development of micro- (retinopathy, nephropathy, and neuropathy) and macrovascular (cardiovascular disorders)
Complications.[4] This type of diabetes mellitus is also called autoimmune diabetes and previously known as juvenile-onset or ketosis prone diabetes. The individual may also seek with other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, and Addison's disease.[5]



Metformin, an oral biguanide class of antihyperglycemic agent, is by far the most widely used glucose-lowering drug for type 2 diabetes mellitus (T2DM).[13] It has an excellent safety record in both children and adults. Over the past decades, accumulating evidence suggests that metformin has profound beneficial effects on human health span, e.g. reducing the incidence of T2D. [16] Recent studies have even suggested the multifunctional profiles of metformin, such as cardiovascular protection, anti-cancer, and anti-inflammatory actions.[17] Besides commonly application in T2DM, metformin has been proved beneficial to patients with T1DM, due to improvement of insulin sensitivity.[18] The full clinical context or metformin blood concentration is often not reported, making it difficult to distinguish metformin-associated from metformin induced lactic acidosis, respectively. Because of the risk to develop metformin-associated lactic acidosis (defined as lactate ≥5 mmol/L, pH <7.35 and metformin concentration >5 mg/L).[19] Metformin is currently contraindicated in patients with severe chronic kidney disease. Moreover, it is recommended to be used with caution when conditions are present that may reduce renal function.[20]

Moreover, recent clinical studies have suggested that, in patients with impaired glucose tolerance (IGT), metformin treatment helps to down-regulate various proinflammatory cytokines released from inflammatory cells.[21,22] Metformin belongs to the peroral (PO) antidiabetic drug class of biguanides. It improves glucose tolerance in patients with T2D, lowering both basal and postprandial plasma glucose levels by reducing hepatic neogenesis in non-insulin-dependent diabetes mellitus patients. Metformin is administered orally as an immediate release or sustained release tablet. It is administered in the form of a hydrochloride salt with an oral bioavailability of 50–60%.[23] Physiologically based metformin pharmacokinetics model of mice (Darta Maija Zake et. Al.), The Effects of Metformin Treatment on Diabetic Albino Rats' Pancreas, Liver, and Kidney Histology (Almutairi, R. S1 et.al.),

Metformin treatment of juvenile mice alters aging-related developmental and metabolic phenotypes (Yun Zhu et.al.). Streptozotocin-Induced Diabetic Models in Mice and Rats.

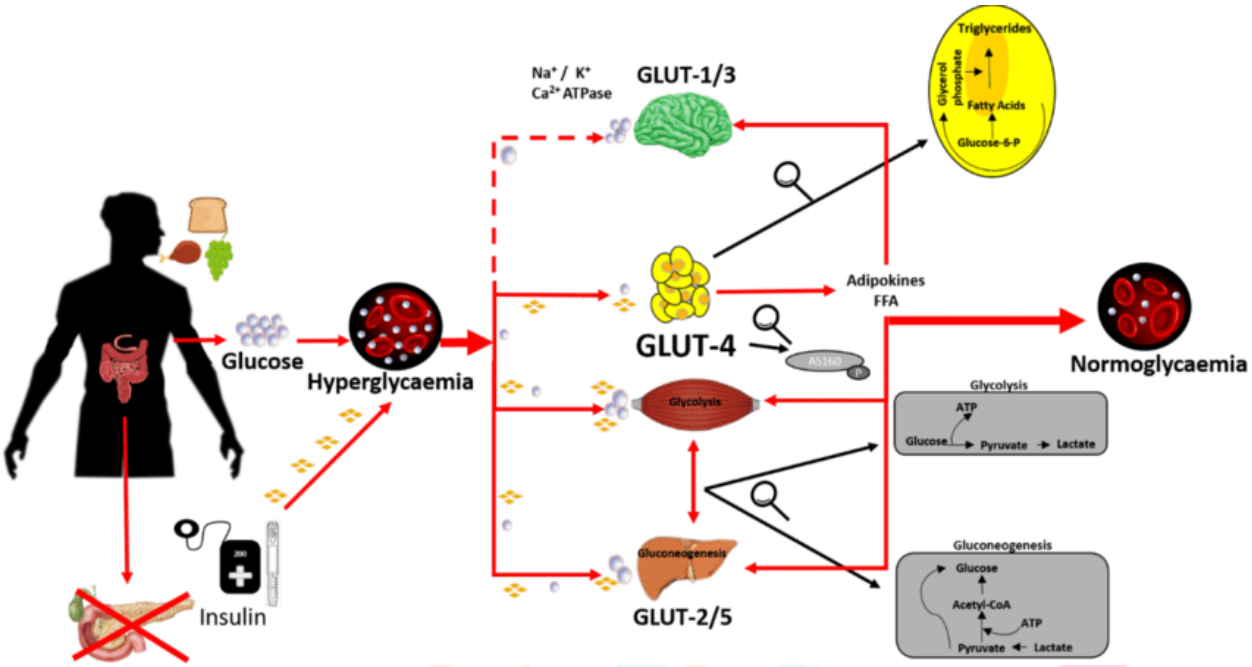
Classification of Diabetes-

(1) Type 1 DM.

(2) Type 2 DM.

Type 1 DM:- Type 1 DM also known as Insulin- Dependent Diabetes Mellitus (IDDS),Treatment with injections of insulin is required.[6] Type 1 DM (DM1) resulting from destruction of pancreatic β-cells usually leading to absolute insulin insufficiency.[1] T1DM accounts for 10% of DM cases and is characterized by absolute insufficiency of insulin, often presenting with symptoms such as thirst, weight loss, and polyuria.[7] The variability in the rate at which the immune-mediated destruction of the pancreatic β-cells occurs often defines the eventual progression of this disease. In some cases, children and adolescents, the β-cell destruction and subsequent failure occur suddenly, which can lead to diabetic ketoacidosis (DKA), often described as the first manifestation of the disease. In others, the disease progression is very slow with a mild increase in fasting blood glucose levels, which assumes a severe hyperglycemic form with or without ketoacidosis, only in the presence of physiological stress conditions such as severe infections or onset of other disorders. In some other cases, which include adults, β-cells may retain some degree of function to secrete only that quantity of insulin, which is only sufficient to prevent ketoacidosis for many years. However, due to progressive insulin deficiency, these individuals become insulin-dependent with the emergence of severe hyperglycemia and subsequent ketoacidosis. Despite the variable progression of this type of diabetes, the affected individuals in the beginning or in the middle or even in the later stages of their life become severely or absolutely insulin-deficient and become dependent on insulin treatment for their survival. This severe or absolute insulin deficiency irrespective of its occurrence at any age manifests itself as low or undetectable levels of plasma C-peptide.[12,13,14]

Type 2 DM:- Type 2 DM also known as non-insulin-dependent diabetes mellitus (NIDDS). Type 2 (DM2) mainly related to insulin resistance and relative insulin insufficiency or mainly violation of insulin secretion with or without insulin resistances.[8]Type 2 diabetes mellitus is also known as adult-onset diabetes.[9] T2DM progresses very slowly and asymptomatically with even mild hyperglycemia developing over years and as such remains largely undiagnosed until the appearance of classic symptoms associated with severe hyperglycemia such as weight loss, growth impairment, blurred vision, polyuria, and polydipsia in the advanced stages of the disease. T2DM has been more frequently associated with increasing age, obesity, family history of diabetes, physical inactivity, and adoption of modern lifestyles: with prior GDM in women and with pathophysiological conditions such as hypertension and dyslipidemia. It occurs more frequently in individuals belonging to certain racial or ethnic groups including Native Americans (American Indians), Asian Americans, African Americans, Hispanic, and Latino. The frequent occurrence of T2DM in the mentioned racial or ethnic groups and its observed strong association with first-degree blood relations point strongly toward the role of genetic factors in the etiology of this disease, but these factors are complex and remain largely unspecified. However, unlike T1DM, no association of this disease has been found with genes involved in the immune response including autoimmunity and consequently there is no immune-mediated pancreatic β-cell destruction.[10,11]



Schematic diagram of prandial glucose metabolism at rest in diabetes (both type 1 and type 2 DM). Red arrows indicate transport via the bloodstream. Black arrows indicate transport on a cellular level. White circles: Glucose; yellow diamonds: Insulin, GLUT: Glucose Transporter on a cellular level. White acids, ATP: adenosine triphosphate, A+/K+ : Sodium-potassium pump, Ca2+ : Calcium,AS160: TBC1D4. (A higher resolution / color version of this figure is available in the electronic copy of the article).

Material and Methods:-

Animal:-

Mice:- Metformin treatment on mice of alteration age and metabolic phenotypes.

From day 15 to day 56, pups were given 200mg/kg metformin (Cat. #151691, MP Biomedicals, Ohio, 44139) or same volume of saline daily via i.p. injection. In humans, to prevent T2D, the dosage is 850mg/day, according to FDA guidelines (https://www.fda.gov/media/72309/). The equivalent dose in mice is approximately 170mg/kg/day, based on body surface area. The maximum safe dosage for treating T2D in human children is 2000mg/day, which equals 400mg/kg/day. Based on these data, we determined to use 200 mg/kg/day to treat the pre-mature mouse pups. According to previous published research in mice, 200mg/kg i.p. may yield a serum concentration between 120 uM ~ 160 uM [25,26]. Although in most of the clinical studies and experiments using adult rodents, metformin was orally administered, we treated the pups via i.p. injection to ensure the accuracy of the dosage and minimize invasiveness. If metformin were mixed in the food, most of the metformin consumed by the pups would come from the dam's milk. It would be impossible to ensure the pups received the correct dose of metformin. Variation in milk intake by the pups may further complicate the study, because developmental traits, such as body weight, could be affected by the volume of milk consumed. Gavage feeding could cause serious intra-esophageal irritation or injury to pups, because the upper gastrointestinal tract of a pre-weaned mouse pup is fragile.[27]

Metformin PBPK model in mice:-

(i) Parameter estimation:- The mice models simulating single per-oral dose (Bio Models ID: MODEL2103020001) and single intravenous dose (Bio Models ID: MODEL2103020002) were deposited in *BioModels* data base.[28] In SBML L2V4 format and as COPASI files. The model parameter estimation of the plasma-tissue partition coefficients (Kt:p) was performed simultaneously

using both the intravenous (IV) dataset, including plasma, small intestine, and stomach measurements, as well as peroral (PO) dataset, including plasma, portal vein, small intestine, liver, kidney, heart, muscle, fat, and brain from Wilcock experiments of a 50mg/kg metformin dose. [29] All parameters of the mice model are presented in the supplementary file A Table. The estimated parameters were Kt:p and Vmax values, while physiological parameters were taken from literature data (see section 4.2.2). A single set of parameter values was obtained for both the IV and the PO experimental datasets. The experimentally determined concentration-time profiles were compared with the model simulations in Fig 1 for the PO and Fig 2 for the IV administration. The mice model was not validated due to the lack of appropriate experimental data.

Pharmacokinetic parameters:-

Key pharmacokinetic parameters–area under the curve (AUC24), half-life (T1/2), maximal concentration (Cmax), and time of maximal concentration (Tmax) were compared (Table 1) to evaluate the predictive capability of the model. The parameters were calculated for both the experimental and model simulations following a 50mg/kg dose. Due to the IV curves' decreasing nature, only AUC24 and T1/2 values were calculated for the IV experimental data (Table 2).

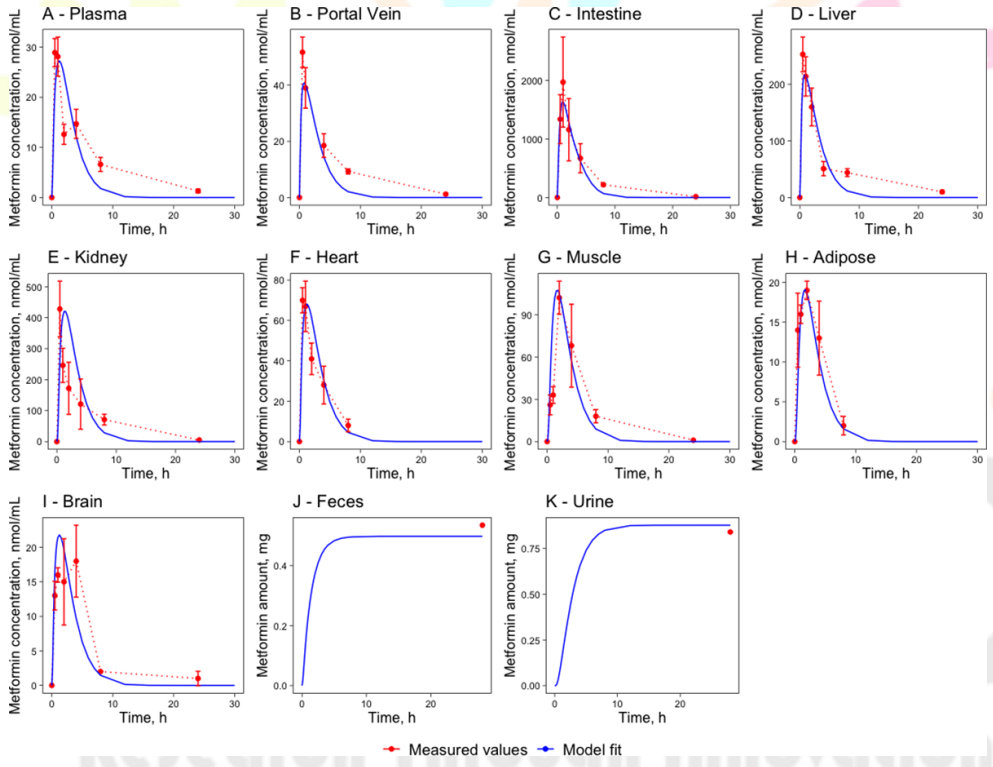


Fig 1. Metformin pharmacokinetics in major compartments of metformin action. Venous plasma (A), portal vein (B), small intestine (C), liver (D), kidney (E), heart (F), muscle (G), adipose (H), brain (I), feces (J) and urine (K) following a single PO 50 mg/kg dose in mice. The red marks represent the experimental data's concentration-time profiles with error bars representing standard deviation [1] and the blue lines represent the model simulations.

Generally, the pharmacokinetic parameters have a good agreement between experimental and simulated curves. There are discrepancies in the T-max values between the experimental data and the model simulations for the PO dataset. That is partly due to the sampling frequency in the experimental dataset, where the maximum may be situated between the sample.

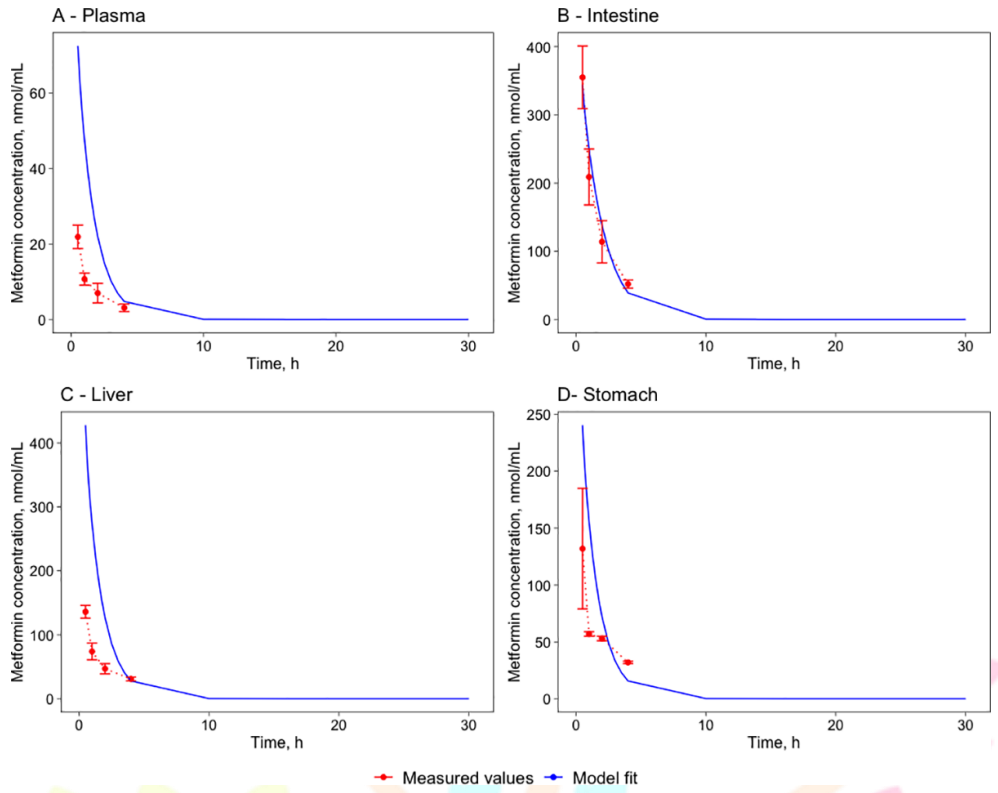


Fig 2. Metformin pharmacokinetics. Venous plasma (A), small intestine (B), liver (C), and stomach (D) following a single intravenous 50 mg/kg dose in mice. The red marks represent the experimental data's concentration-time profiles with error bars representing standard deviation and the blue lines represent the model simulations.

Table 1. Metformin pharmacokinetic parameter comparison of experimental data and model simulations in plasma, portal vein, intestine, liver, kidney heart, muscle, adipose tissues, and brain following a single 50mg/kg PO dose in mice**.**

Collection time points (e.g., 0.5; 1; 2; 3; 8 and 24h). The most significant T-max discrepancies are in the plasma, heart, liver, kidney, and brain tissues. The differences in the pharmacokinetic parameters for the portal vein compartment are in the C-max and AUC24 values where C-max is lower by 22% in the model simulations, causing the difference in the AUC24 by 34%. The model can simulate the dynamic tendencies of the portal vein concentration-time profile. Differences are also noticeable in the intestinal compartment, where the AUC24 and T1/2 values are lower for the model simulations by 22% and 35% correspondingly. These differences can be expected as the intestinal kinetic parameters are scaled from Proctor.[30]

Where experiments are carried out in cell cultures. Still, the kinetic parameters could differ under physiological circumstances. The kidney's differences are in the T1/2 values, where 0.9h measured vs.2.5h simulated leads to a 180% difference (just 1.6h in absolute numbers). The underlying cause of this difference is that the parameters concerning active excretion of metformin–parameters were estimated by simultaneously running simulations for the PO and IV experiments. Since the IV dataset elimination is much quicker than in the PO dataset, the resulting active excretion parameters are a compromise between the two. Differences in the C-max values for metformin concentration in the brain tissues could indicate a more complex transport mechanism across the blood-brain barrier that is not depicted in the model. On the other hand, the brain tissue concentration time-curve shows a surprising concentration fall at

the 2nd hour and an increase at the 5th hour–this is uncommon for metformin concentration time profiles as they usually have only one peak for a single-dose regimen. A similarly unusual fall in the concentration-time shapes can be noticed in the venous plasma and portal vein experimental concentration-time curves. The differences between the model simulations are more extensive for the IV dataset than in the PO dataset due to rapid excretion. In the model simulations, metformin is excreted slower than in experimental results. Therefore, the AUC24 and the T1/2 values are more significant than in the literature dataset, but the curve dynamics are very similar (see Fig 2). The biggest difference can be observed in plasma: T1/2 is measured at 0.50h while model simulations show 0.9h giving a 24-minute delay. The even bigger difference is in the AUC values: the measured value is 26.1 nmolh/mL, and the simulated value is 91.4 nmolh/mL. The differences in both cases are caused by a delayed concentration decrease in plasma as the model simulations are required to create a single compromise parameter set by considering the stomach and experimental intestine concentrations and the PO dataset.

Tissue	**Type**	**$T_{1/2}$ h**	**$T_{1/2}$ Measured Fitted,%**	**AUC_{24} Measured, nmol*h/mL**	**AUC Measured Fitted %**
Intestine	Measured	0.8	46	452.5	21
	Fitted	1.2		549.5	
Plasma	Measured	0.5	72	26.1	250
	Fitted	0.9		91.4	
Stomach	Measured	1.0	-13	182.9	190
	Fitted	0.9		529.5	
Liver	Measured	0.7	17	191.2	173
	Fitted	0.8		523.5	

Type	Cmax. nmol/mL	Cmax measured -fitted,%	Tmax, h	Tmax,measured -fitted,%	AUC24,nmol*h/mL	AUC,measured fitted, %	$T_{1/2}$,h	$T_{1/2}$,measured -fitted,%
Measured	29.0	-6	0.5	150	160.8	-35	3.6	-31
Fitted	27.2		1.3		105.3		2.5	
Measured	52.0	-22	0.5	50	217.6	-34	2.1	16
Fitted	40.7		1.8		142.9		2.4	
Measured	1971.0	-17	1.1	0	7291.6	-22	1.7	35
Fitted	1636.5		1.0		5652.8		2.3	
Measured	253.0	-14	0.5	75	1112.9	-31	2.1	15
Fitted	216.6		0.9		768.7		2.4	
Measured	428.0	-2	0.5	150	1541.3	4	0.9	180
Fitted	420.7		1.3		1603.1		2.5	
Measured	70.0	-3	0.5	150	236.7	11	2.4	4
Fitted	68.1		1.3		263.2		2.5	
Measured	102.0	5	2.0	-13	501.0	-14	3.3	-27
Fitted	107.3		1.8		431.2		2.4	
Measured	19.0	0	2.0	-13	93.8	-18	3.1	-22
Fitted	19.1		1.8		76.8		2.4	
Measured	18.0	21	4.0	-69	107.7	-22	2.2	21
Fitted	21.8		1.3		84.2		2.7	

Table 2. Metformin pharmacokinetic parameter comparison of experimental data [1] and model simulations in plasma, intestine, and stomach following a single 50mg/kg intravenous dose in mice.

Rat :- Material and method of effect of metformin drugs anti-diabetic effect on Albino rat ,this metformin drugs effected on these Albino rats of the liver, pancreas, and kidney. 20 adult white male albino rats (8-10 weeks old) weighing 170-200 g were obtained from the house of animals in the University of Babylon/ college of science. The rats were given two weeks to acclimate before beginning the experiment. These animals were divided into two main groups. The first ten rats were used to develop diabetes mellitus (type II) by injecting alloxan monohydrate as a single dosage of 130 mg/kg body weight intraperitoneally after 72 hours. Their fasting blood sugar levels were evaluated using a glucometer after 9-12 hours of fasting. The second set of ten rats received intraperitoneal injections of normal saline. Then both groups were split into four groups: Group 1: In the control group, as an alternative to the treatment given to the other groups, non-diabetic rats were given DW orally using an orogastric tube. Non-diabetic animals in Group 2: were given 1000 mg/kg/day of Metformin, administrated orally

by oral gavage tube. Group 3: Animals in the diabetic control group were given alloxan i.p. and administrated distilled water orally but have not been given any medications. Groups 4: Diabetic rats with Metformin after seven days of DM induction, diabetic rats received Metformin 1000 mg/kg/day orally. After 1 month of treatment, the animals were sacrificed, and the liver, pancreas, and kidney were collected for histological studies according to Bancroft's procedure[24]

CONCLUSION

In reviewing the effects of metformin on rats and mice, it is evident that this drug exhibits significant physiological and biochemical impacts, particularly in the context of metabolic and age-related disorders. Metformin, widely recognized for its glucose-lowering properties, has demonstrated notable effects in improving insulin sensitivity and reducing hyperglycemia in rodent models of type 2 diabetes. Beyond its glycemic control, metformin has also shown promising results in attenuating weight gain and reducing visceral fat, which are critical factors in metabolic syndrome.

Furthermore, studies have indicated metformin's potential role in extending lifespan and enhancing health span in rodents. The drug appears to mitigate age-associated declines in health, possibly through mechanisms involving reduced oxidative stress, improved mitochondrial function, and modulation of the mTOR signaling pathway. However, the effects of metformin are not uniformly positive across all studies, with some reports suggesting adverse outcomes in specific contexts or at high doses, including gastrointestinal disturbances and potential reproductive toxicity.

In conclusion, while metformin shows considerable promise in rodent models, particularly in metabolic and aging research, further studies are essential to fully elucidate its long-term effects and underlying mechanisms, especially in translating these findings to human health and therapeutics.

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