

Full length Research Article

# Comparative Therapeutic Effect of Single/Combined Administration of Saxagliptin, Metformin and Intranasal Insulin on Dexamethasone Induced Insulin Resistance in Wistar Rat Model

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**Summary:** Glucocorticoids have therapeutic benefits in the management of several inflammatory and immunological disorders. Despite these medicinal effects, they have the drawback of causing metabolic disorders such as hyperglycemia, insulin resistance etc., which is known to be a key indicator of metabolic syndrome. Metabolic syndrome is a major predisposing factor to type 2 diabetes mellitus and cardiomyopathy. This study was designed to compare and evaluate the effects of saxagliptin, metformin and intranasal insulin (when used singly or in combination) on dexamethasone induced insulin resistance. Fifty-six female rats were randomly assigned into eight groups. Group 1 represented the control; Group 2 was administered with dexamethasone (1mg/kg) and served as untreated group. Other groups were administered dexamethasone(1mg/kg) and treated with singly/combinations of intranasal insulin (2IU); metformin and (40mg/kg) and saxagliptin (8mg/kg). Treatments were given for period of one week. At the end of the study, blood samples were collected for biochemical assays such as lipid profile, serum insulin, glucagon, adiponectin, glucokinase enzymes and glucose-6-phosphatase enzymes. Representative pancreases were excised for histological examination. Results showed that dexamethasone (1mg/kg) induced hyperglycemia, hyperinsulinemia, dyslipidemia, increased glucose-6-phosphatase, decreased glucokinase, impaired glucose tolerance and disrupted the structural integrity of the pancreas. Treatment with saxagliptin, metformin and their combinations significantly decreased blood glucose level, decreased LDL Level, improved glucose tolerance and offered protection to the pancreatic islet cells. In conclusion, the selected hypoglycemic agents used in present study ameliorate the dexamethasone induced hyperglycemia and insulin resistance of which the combination of metformin with saxagliptin showed greater efficacy.

**Keywords:** Insulin resistance, Hyperglycemia, Dexamethasone, Type 2 Diabetes

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## INTRODUCTION

The burden of type 2 diabetes is a global health concern and the cost of managing this condition is worrisome especially to low- and middle-income countries. According to the International Diabetes Federation (IDF) estimation, about 415 million people worldwide were affected with Type 2 diabetes as of 2015, a number which is still on a rise and expected to reach around 642 million by 2040 (Cersosimo *et al.*, 2018).

Type 2 diabetes is usually known to develop more at adult age, affecting mainly the elderly and/or obese individuals. Unfortunately, this disease is increasingly prevalent in young adults. This is particularly in highly susceptible ethnic groups due to the increase in childhood obesity (Petrovick 2018). Some of the commonly known risk factors of type 2 diabetes include being overweight and

obesity (particularly of the android type), age, inactivity, family history, etc. Besides these, several drugs have been linked with an increased risk development of type 2 diabetes, among which includes Glucocorticoids (Pasięka & Rafacho 2016).

Glucocorticoids are among the most prescribed drugs worldwide for their therapeutic benefits in the treatment of several inflammatory and immunological diseases. Despite their therapeutic potentials in ameliorating these diseases, they have the drawback of inducing insulin resistance in humans. This can ultimately lead to steroid-induced diabetes or worsen previously diagnosed diabetes (Martinez *et al.*, 2016). The diagnosis and treatment of glucocorticoid induced diabetes mellitus are surprisingly underestimated by many health practitioners and the media disclosure of the benefit with the use of dexamethasone in patients with COVID-19 infection sets the habits for self-medication and inappropriate use of glucocorticoids for some individuals

(Alessi *et al.*, 2020). There are several drugs or combination of drugs with differing mechanisms of action that are used for treatment of type 2 diabetes mellitus (Davidson 2002). But despite advances in both our understanding of the pathophysiology of type 2 diabetes mellitus and the development of new treatment strategies, current management of patients with T2DM still remains sub-optimal (Lavernia *et al.*, 2015). Furthermore, there are limited studies that compare the effectiveness of different treatment therapies as a means of assessing the effectiveness of preventive measures taken against glucocorticoids induced diabetes mellitus.

This present study is designed to evaluate the effect(s) of saxagliptin, metformin and intranasal insulin used singly and in combination in glucocorticoids -induced insulin resistance.

## MATERIALS AND METHODS

**Drugs and Chemicals:** Recombinant human insulin (Actrapid, 100 IU/ml, Novo Nordisk) and Dexamethasone was purchased from Rotamedix Pharmacy Ilorin, Nigeria. Metformin (Glucophage) and Saxagliptin (Onglyza) was purchased from Momrata Pharmacy Ilorin, Nigeria. Isoflurane was obtained from Southern Anaesthesia & Surgical, (West Columbia).

**Animals:** housed in groups of seven and acclimatized for one week in the housing facility of the College of Health Sciences, University of Ilorin, Nigeria. Animals were housed under standard environmental conditions at a temperature of about 25°C. Animals had free access to water and formulated rat's chow ad libitum. All procedures involving animals were approved prior to the experimental phase. Ethical approval was obtained from the University Ethical Review Committee (UERC/ASN/2020/2032) and in compliance with the Helsinki declaration on the care and use of laboratory animals (World Medical Association 2020).

**Experimental design:** Fifty-six female wistar rats weighing between 180-230 grams were randomly distributed into eight groups (n=7). Dexamethasone was administered for a period of 7 days an hour before the administration of metformin, saxagliptin and intranasal insulin.

Group 1 - Control group received normal saline (0.9%)

Group 2 - Untreated group received intraperitoneal Dexamethasone injection (1mg/kg/day).

Group 3 was administered dexamethasone(1mg/kg) and were treated with intranasal insulin (2IU/day)

Group 4 was administered dexamethasone(1mg/kg/day) and were treated with the combination of intranasal insulin and orally administered Metformin (40mg/kg/day).

Group 5 was administered dexamethasone(1mg/kg/day) and were treated with the combination of intranasal insulin and orally administered Saxagliptin (8mg/kg/day)

Group 6 was administered dexamethasone(1mg/kg/day) and were treated with oral administration of metformin (40mg/kg/day).

Group 7 was administered dexamethasone(1mg/kg/day) and were treated with oral administration of metformin (8mg/kg/day).

Group 8 was administered dexamethasone(1mg/kg/day) and were treated with the combination of orally administered metformin (40mg/kg/day) and saxagliptin (8mg/kg/day).

**Induction of Insulin Resistance:** Induction of insulin resistance was done using the method employed by Martinez *et al.* (2016). Insulin resistance was induced by intraperitoneal injection of dexamethasone (1 mg/kg) for 7 days before the administration of saxagliptin, metformin and intranasal insulin.

**Intranasal Insulin Administration Procedure:** Intranasal insulin administration was done in line with the method employed by Njan *et al.*, (2018). Rats were anesthetized by placing them in tightly sealed transparent glass jars containing isoflurane (5%) for brief period (< 30 s). A total of 2 IU (1 IU/ 10 µl; 10 µl/nostril) of rapid acting insulin was quickly administered intranasally using a micropipette (P-10, Eppendorf). The procedure lasted for about 10 to 15 seconds. Animals that regain consciousness was monitored and returned to their respective cages.

**Oral Glucose Tolerance Test:** The Oral Glucose Tolerance Test (OGTT) was performed on overnight fasted rats and after which basal glycemia measurement on the 6th day. Each animal received orally, 2 g/kg of glucose and their glycemia was further measured at 30, 60, 90 and 120 min after glucose load (Chaimum-aom *et al.*, 2017). The blood glucose from the animals were measured using blood glucose test strips and glucometer (Accucheck).

**Fasting Blood Glucose Measurement:** Fasting blood glucose levels were measured with an ACCU check glucometer on the 8th day for all groups using samples collected from the tail vein after an overnight fast (12 hours).

**Tissue collection:** At the end of the experiments (on the 8th day), rats from each group were subjected to general anaesthesia by ketamine. Following decapitation, blood was collected in plain tubes. The rats were dissected, and the pancreas and liver were rapidly removed. Representative pancreatic fragments were taken and used for histological examination (Mohamed *et al.*, 2014). Pancreas were removed and fixed in 10% buffered formalin and dehydrated by graded series of alcohol, embedded in paraffin, sectioned at 5 µm in thickness, and mounted on glass slides. Pancreatic sections were stained with Hematoxylin and Eosin (H & E) and assessed for tissue injury. Acinar damage was assessed based on the appearance of lining cells, its pyramidal structure, presence, or absence of inflammatory cells, and pyknotic nuclei. Damage to the islets of Langerhans was assessed based on the presence or absence of intra-islet hemorrhages, cellular infiltrates and nuclear pyknosis. The liver was removed and homogenized with 0.25M sucrose solution in the liver weight: volume ratio of 1:4 of the weight of the liver to that of the volume of the 0.25M sucrose solution and evaluated for the levels of glucokinase and glucose-6-phosphatase enzymes (Njan *et al.*, 2018).

**Biochemical Analysis:** Serum high density lipoprotein (HDL)-cholesterol, triglycerides (TG), low density lipoprotein (LDL) and total cholesterol (TC) concentrations

*Saxagliptin, metformin and intranasal insulin ameliorate dexamethasone induced hyperglycemia and insulin resistance.*

were analyzed by enzymatic determination, using the kits purchased from Randox laboratories Ltd, United Kingdom. Serum insulin levels were analyzed by enzymatic determination using ELISA kit purchased from CalBiotech. Serum adiponectin levels were analyzed by enzymatic determination using rat adiponectin ELISA kit purchased from bioassay technology laboratory. Values were expressed in mg/dl. Serum glucagon levels were analyzed by enzymatic determination using rat glucagon ELISA kit purchased from bioassay technology laboratory. Values were expressed in ng/L.

**Statistical Analysis:** Data collected were cleaned and statistical analysis was performed with GraphPad Prism (version 8.0) statistical software using the one-way/repeated ANOVA with Turkeys multiple comparison and Dunnet comparison test. Values of  $p \leq 0.05$  were considered significant. Values are expressed as Mean  $\pm$  SEM; \*P-value  $\leq 0.05$ , \*\*P-value  $< 0.002$ , \*\*\*P-value  $< 0.001$ .

## RESULTS

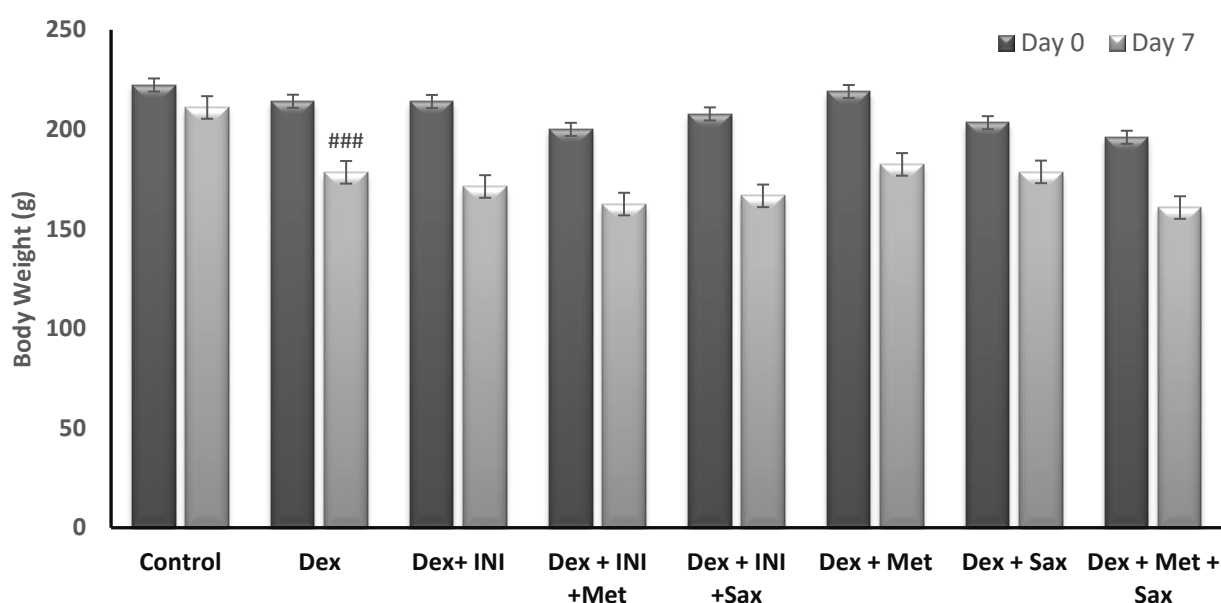
**Effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on Body Weight:** Repeated intraperitoneal administration of dexamethasone (1mg/kg) for period of 7 days significantly decreases the body weight (grams) of the animals when compared to control group #. Oral administration of metformin (40mg/kg), saxagliptin (8mg/kg) or intranasal insulin (2IU) and their combinations could not significantly reverse the weight loss caused by dexamethasone (Figure 1).

**Effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on Fasting Blood Glucose Level.:** From the chart (Figure 2),

intraperitoneal administration of dexamethasone (1mg/kg) increased the fasting glucose levels in comparison with the control group. The administration of metformin (40mg/kg) and saxagliptin (8mg/kg) alone significantly reduced the fasting glucose levels when compared with the dexamethasone group\*. Also, the combination of metformin (40mg/kg) with saxagliptin (8mg/kg), and combination of intranasal insulin (2IU) with saxagliptin (8mg/kg) significantly reduced the fasting blood glucose levels when compared to the dexamethasone group\*. Combination of metformin (40mg/kg) and saxagliptin (8mg/kg) was significantly lower compared to intranasal insulin alone group.

### Effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combinations on Oral Glucose Tolerance Test and Area Under the Curve.:

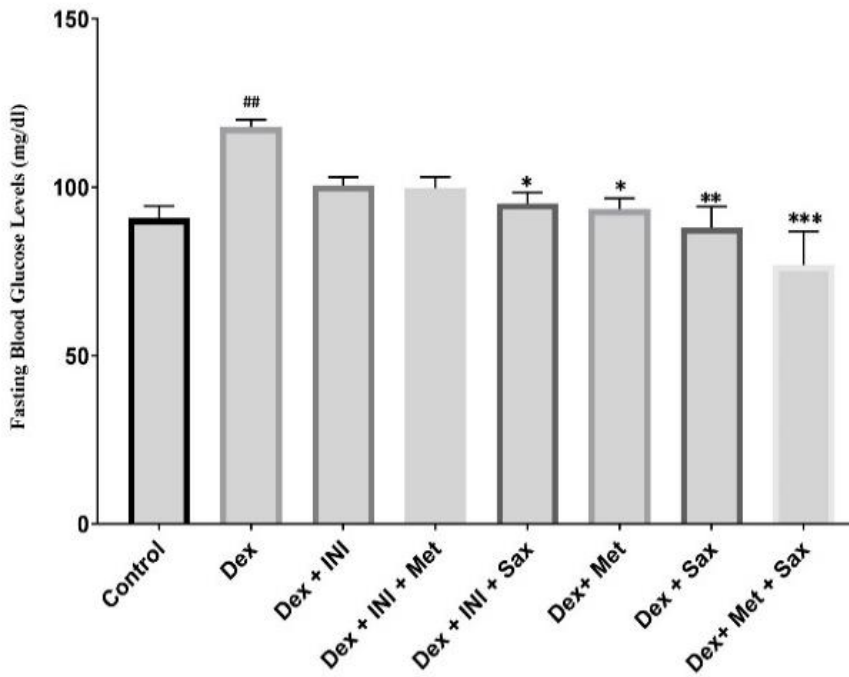
Figure (3a) shows the OGTT curve in control and experimental animals following oral administration of 2g/kg of 40% glucose solution. Glucose peaked in the normal group and returned to baseline within 120 minutes. Dexamethasone treatment caused elevated fasting blood glucose relative to the normal control. Further glucose impairment was observed as blood glucose level did not return to normal at 120 minutes. Treatment with intranasal insulin also impaired glucose tolerance as blood glucose level was higher than dexamethasone at 120 minutes. Metformin treatment reduced blood glucose to levels comparable to normal control whether administered alone or in combination with intranasal insulin. Also, saxagliptin treatment reduced blood glucose to levels comparable to normal control whether administered alone or in combination with intranasal. Combination of metformin and saxagliptin improved glucose tolerance as blood glucose peaked at 60 minutes and fell to base line at 120 minutes.



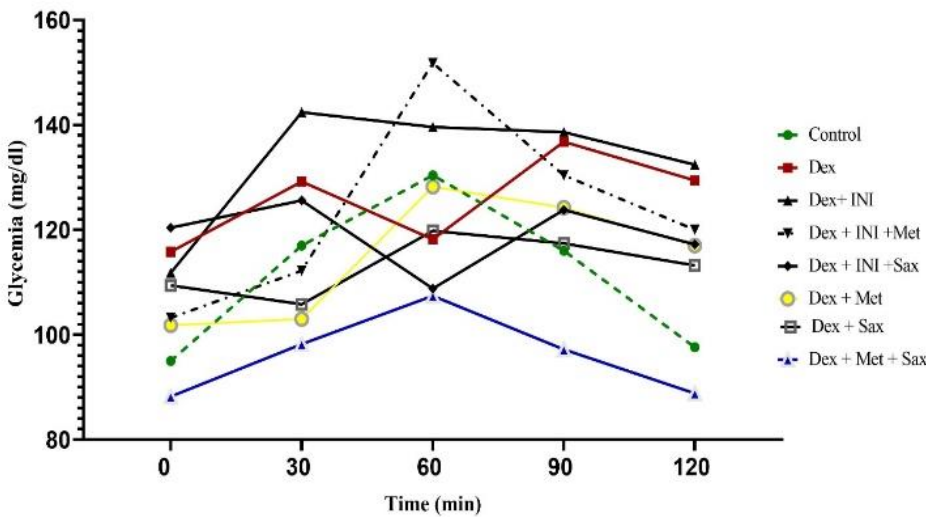
**Figure 1.**

Showing the effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on body weight measured at day 0 and day 7 of drug administration.

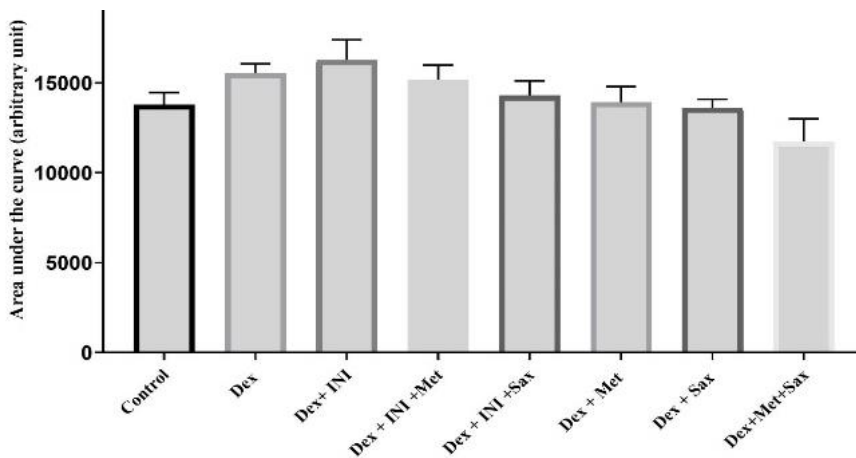
*Saxagliptin, metformin and intranasal insulin ameliorate dexamethasone induced hyperglycemia and insulin resistance.*



**Figure 2:** Showing the effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on Fasting Blood Glucose level.



**Figure 3(a):** Showing effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combinations on Oral Glucose Tolerance Test. Values are expressed in mean ± SEM (n=5).



**Figure 3 (b):** Showing the effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combinations on AUC from oral glucose tolerance test.

The figure (3b) shows the glucose Area under the Curve (AUC) in control and experimental animals treated with or without dexamethasone, intranasal insulin, metformin and saxagliptin. Dexamethasone treatment led to non-significant

increase in AUC. The combination of metformin and saxagliptin however significantly reduced glucose AUC when compared with intranasal insulin alone group.

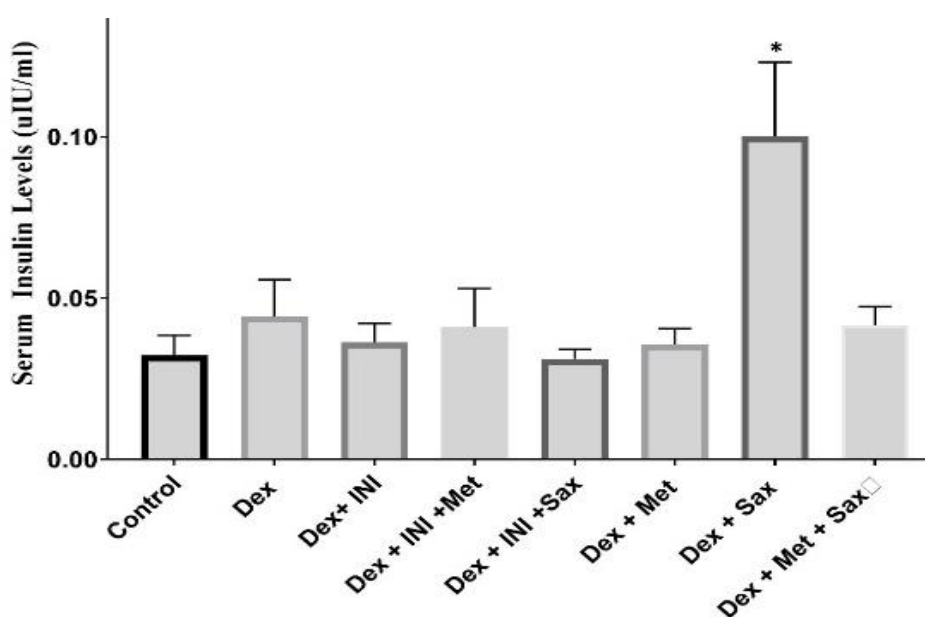
*Saxagliptin, metformin and intranasal insulin ameliorate dexamethasone induced hyperglycemia and insulin resistance.*

**Effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on Fasting Serum Insulin Level.:** Intraperitoneal administration of dexamethasone (1mg/kg) resulted in non-significant increase in serum insulin levels in comparison with the control group (mean values  $0.044 \pm 0.01$  vs  $0.032 \pm 0.01$ ). Oral administration of saxagliptin (8mg/kg) significantly increased serum insulin levels in comparison to dexamethasone alone and all other administered hypoglycemic drugs with p-values respectively (Figure 4).

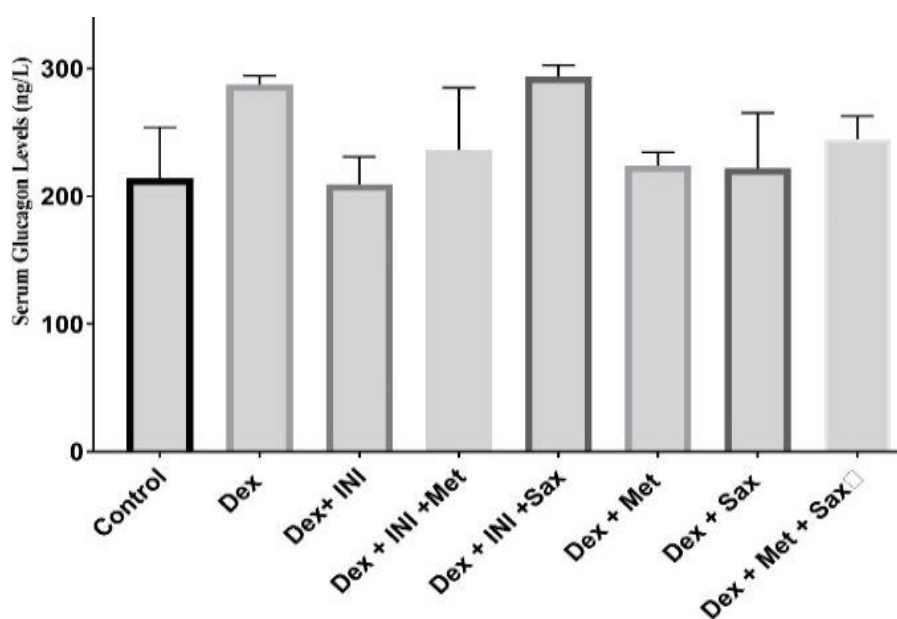
**Effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on Fasting Serum Glucagon Level:** From the chart below (Figure 5), intraperitoneal administration of dexamethasone (1mg/kg) increased serum glucagon levels in comparison with the control (mean values  $287.50 \pm 6.81$  vs  $213.91 \pm 40.11$ ). Except for combination of insulin with saxagliptin,

other hypoglycemic agents administered reversed the dexamethasone induced hyperglucagonemia.

**Effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combinations on fasting serum Adiponectin level:** The serum level of adiponectin was not significantly affected by dexamethasone (1mg/kg) administration (Figure 6). Although a slight decrease in adiponectin was observed when compared to the control group (mean values of  $3.58 \pm 0.17$  vs  $3.77 \pm 0.11$ ). All hypoglycemic agents administered were able to reverse the effect caused by dexamethasone. Combination of metformin (40mg/kg) with saxagliptin (8mg/kg) significantly increased adiponectin level in comparison to dexamethasone only group\* intranasal insulin alone group, intranasal insulin + metformin intranasal + saxagliptin metformin alone group and saxagliptin alone group.

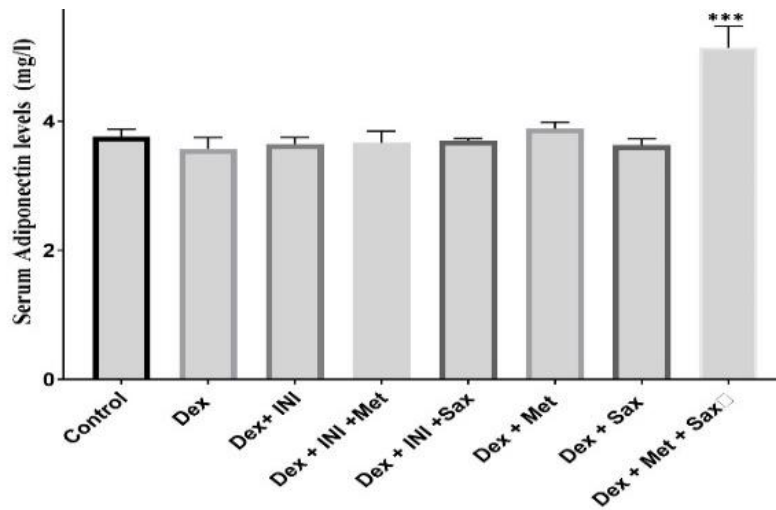


**Figure 4.** Effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on fasting serum insulin level

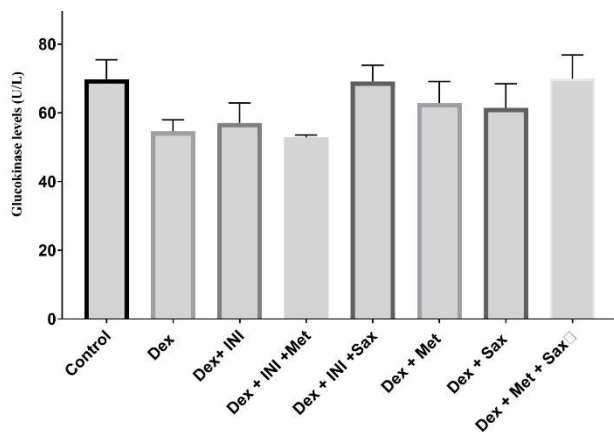


**Figure 5.** Showing the effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on fasting serum glucagon level.

*Saxagliptin, metformin and intranasal insulin ameliorate dexamethasone induced hyperglycemia and insulin resistance.*

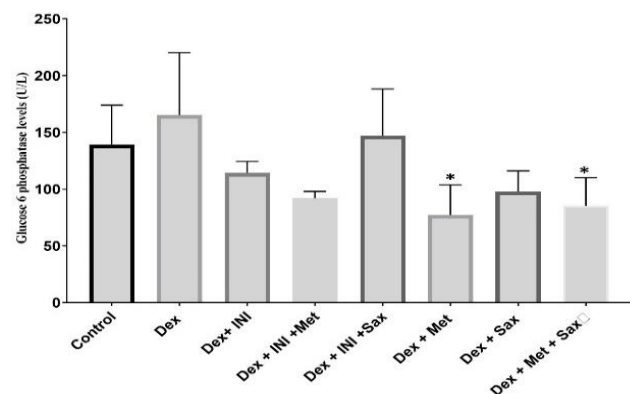


**Figure 6:** Showing the effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on fasting serum Adiponectin level.



**Figure 7a:**

Showing the effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on glucokinase enzyme levels.



**Figure 7b:**

Showing the effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on glucose-6-phosphatase enzyme levels.

#### Effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on liver enzymes:

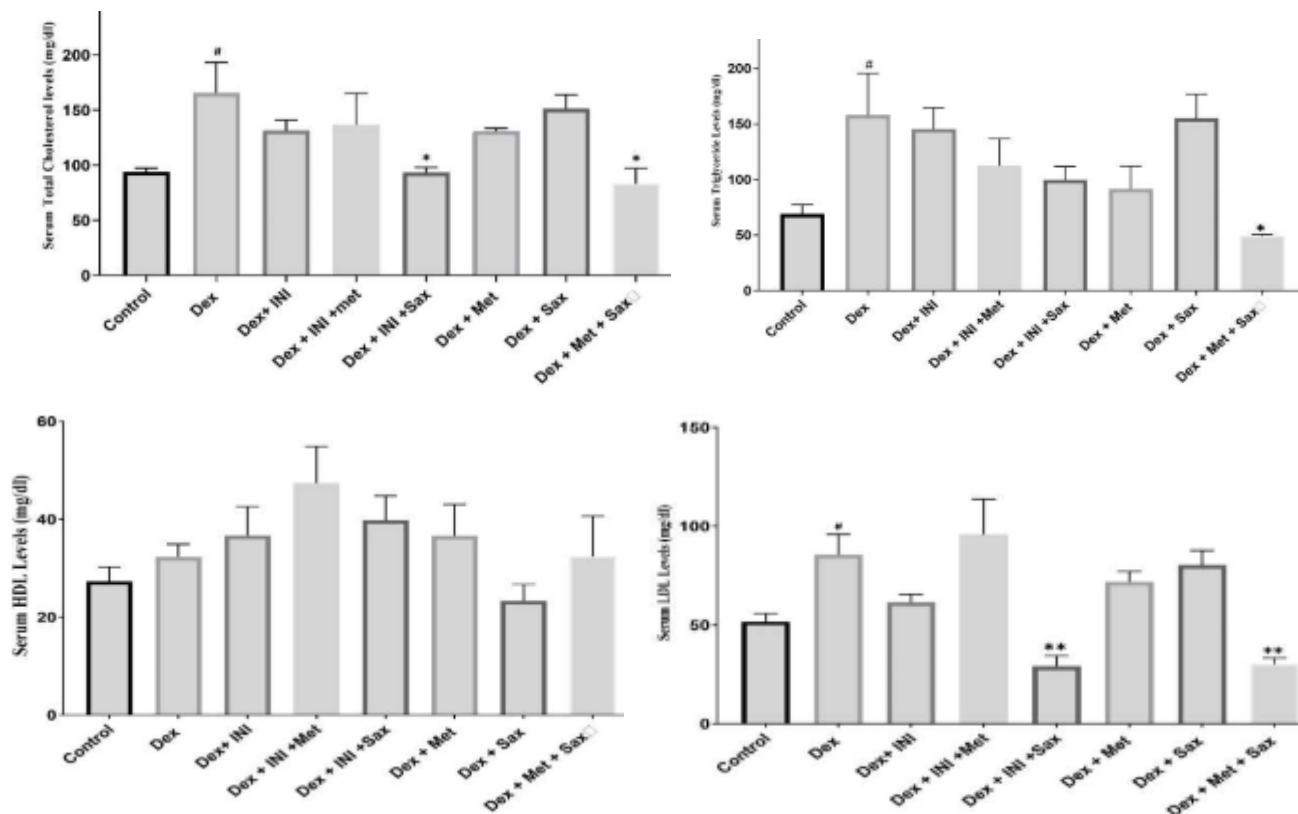
Intraperitoneal administration of dexamethasone decreased the level of glucokinase enzyme in comparison with the control (mean values  $54.5787 \pm 3.39$  vs  $69.7707 \pm 5.64$ ). All hypoglycemic agents increased the level of glucokinase enzyme in comparison to dexamethasone alone group, although none of the increase was significant (Figure 7a). Combination of insulin and saxagliptin was able to normalize the levels of the enzymes in comparison to control (mean values of  $69.09 \pm 6.99$  vs  $69.77 \pm 5.64$ ). Also, intraperitoneal administration of dexamethasone (1mg/kg) increased the levels of glucose -6- phosphatase enzyme (figure 7b) in comparison with the control group (mean values  $165.224 \pm 27.50$  vs  $139.362 \pm 34.55$ ). All hypoglycemic agents decreased the levels of glucose -6-phosphatase in comparison to dexamethasone alone group. Among which the administration of metformin (singly) and the combination of metformin (40mg/kg) with saxagliptin (8mg/kg) caused significant lowering of the level glucose-6- phosphatase compared to dexamethasone group \*.

#### Effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on Lipid Profile:

Administration of dexamethasone significantly increased the serum total cholesterol level (Figure 8a) in comparison with the control #. The hypoglycemic agents

reduced the level of total cholesterol compared to the dexamethasone group, among which the combination of intranasal insulin (2IU) with saxagliptin (8mg/kg) and combination of metformin (40mg/kg) with saxagliptin (8mg/kg) significantly reversed the increase in total cholesterol level induced by dexamethasone. Administration of dexamethasone significantly increased the serum Triglyceride levels (Figure 8b) in comparison to the control group. Combination of metformin (40mg/kg) with saxagliptin (8mg/kg) significantly decreased the serum triglyceride levels in comparison with the dexamethasone group # and saxagliptin alone group respectively. Administration of dexamethasone increased the serum HDL levels in comparison with the control (Figure 8c). The hypoglycemic agents employed in this study also increased the serum levels of HDL. Administration of dexamethasone (1mg/kg) significantly increased the serum LDL level (Figure 8d) in comparison with the control #. Except for the combination of metformin (40mg/kg) with intranasal insulin (2IU), all other hypoglycemic agent reversed the increased serum LDL caused by dexamethasone. The level to which the combination of intranasal insulin + saxagliptin and metformin + saxagliptin reversed this effect was significant compared to the group that received combination of intranasal insulin with metformin, saxagliptin alone and metformin alone group.

*Saxagliptin, metformin and intranasal insulin ameliorate dexamethasone induced hyperglycemia and insulin resistance.*



**Figure 8:**

- Effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on serum total cholesterol level.
- Effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on serum triglyceride level.
- Effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on serum HDL level.
- Effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on serum LDL level.

**Effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on the Histology of the Pancreas:** No visible lesion or damage was observed in the structure and architecture in the Hematoxylin and Eosin-stained sections of the pancreas of the control group (Figure 9a). Dexamethasone 1mg/kg showed pancreatic islet cells multifocal nuclear hypochromasia, cytoplasmic degeneration and individual nuclear loss necrosis (Figure 9b). Intranasal Insulin (2IU) administration showed pancreatic parenchyma acinar cell hyperchromasia with intact islet (Figure 9c). Treatment with combination of intranasal insulin (2IU) and Metformin showed mild to severe pancreatic parenchyma acinar cell hyperchromasia with intact pancreatic islet (Figure 9d). Combination of intranasal insulin (2IU) and Saxagliptin (8mg/kg) showed pancreatic acinar cell nuclear hyperchromasia and islet cell degeneration and necrosis with regeneration (Figure 9e). Metformin (40mg/kg) alone showed severe pancreatic parenchyma acinar cell hyperchromasia with intact pancreatic islet cells (Figure 9f). Saxagliptin (8mg/kg) alone showed pancreatic islet cell degeneration and necrosis (Figure 9g). Combination of metformin and saxagliptin showed moderate pancreatic parenchyma acinar cell nuclear hyperchromasia with intact acinar cells and islet cells (Figure 9h)

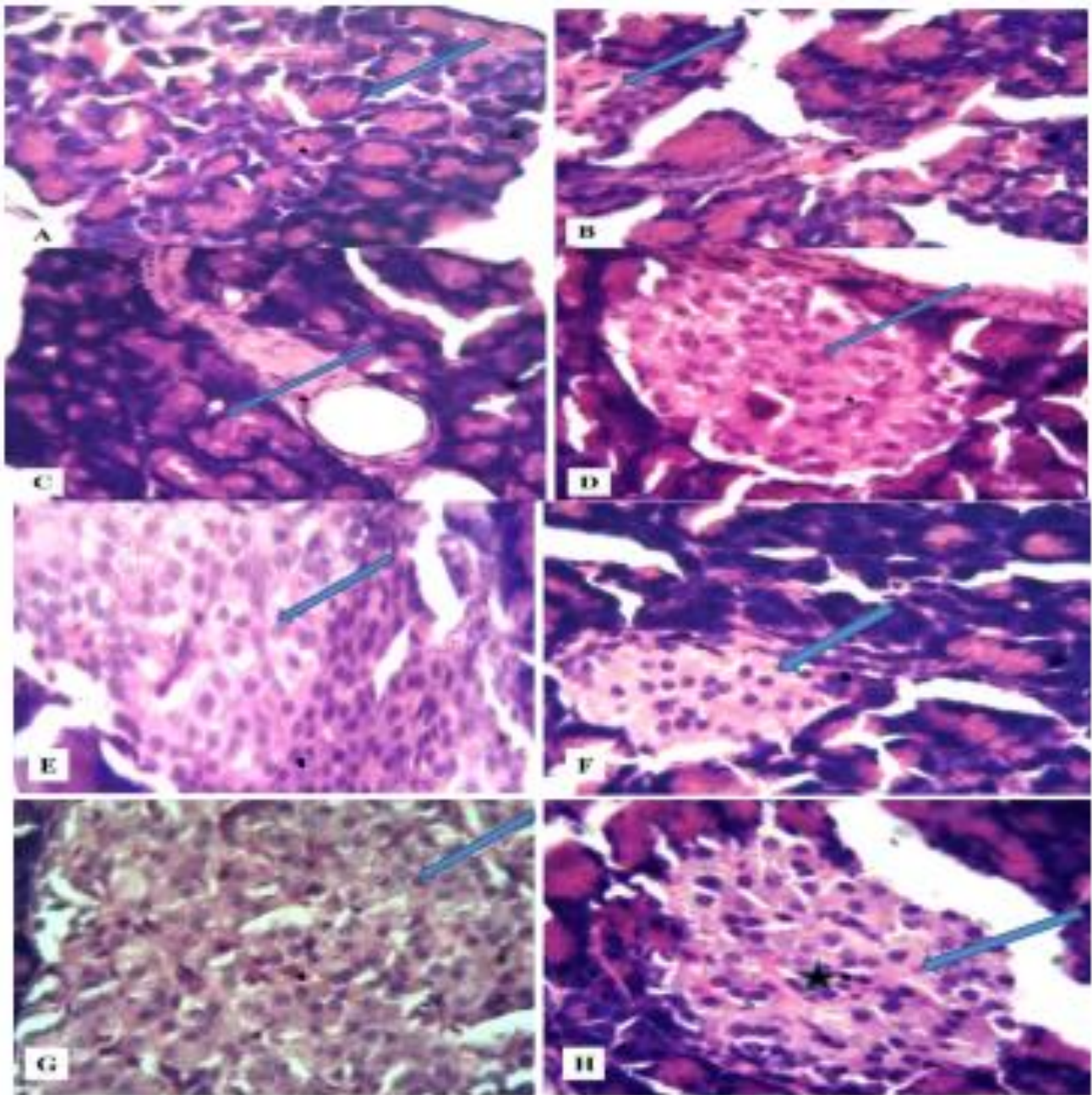
## DISCUSSION

A key challenge in the excessive use of dexamethasone is its ability to promote adverse metabolic effects which

include insulin resistance and can as such induce or worsen previously diagnosed diabetes (Pasioko & Rafacho 2016). The present study was carried out to compare the effect of selected hypoglycemic agents on dexamethasone induced insulin resistance. Dexamethasone confers a risk of weight gain in humans (Wang *et al.*, 2012). However, in this study, repeated administration of dexamethasone caused a significant reduction in body weight of animals. In support of the observed weight loss, Malkawi *et al.*, (2018), suggested that the mechanism of dexamethasone effect could be due to muscle wasting.

Alterations in glucose homeostasis, insulin resistance and hyperglycemia are amongst the adverse effects that have been tied to glucocorticoids therapy (Pasioka and Rafacho 2016). In line with this, present study showed that dexamethasone significantly increased fasting blood glucose levels compared to control. Except for the administration of intranasal insulin alone and its combination with metformin, other hypoglycemic agents showed a significant reduction in blood glucose. Metformin has been the most preferred first line agent employed in the management of Type 2 diabetes mellitus (Pretrovick 2018), but the current study indicated that saxagliptin administered may be superior to metformin and intranasal insulin at lowering fasting blood glucose. The combination of metformin and saxagliptin offered better therapeutic benefits compared to the other combination/single regimen used in this study.

*Saxagliptin, metformin and intranasal insulin ameliorate dexamethasone induced hyperglycemia and insulin resistance.*



**Figure 9.**

H & E-stained microscopic photomicrograph of the different studied groups in Magnification x400. Arrows indicating the pancreatic islet cells.

To assess insulin sensitivity, oral glucose tolerance test done in present study indicated an impaired glucose tolerance in the dexamethasone group as blood glucose level peaked within 30 to 90 minutes following glucose load and did not return to baseline after a period of 2 hours. The mechanisms by which glucocorticoids impair glucose tolerance are not yet fully clarified in humans and merit continuous research. However, metformin, saxagliptin and their combination improved glucose tolerance compared to the dexamethasone group. This result reflects the ability of metformin and saxagliptin to ameliorate insulin resistance when used alone and in synergy when combined (Lindsay *et al.*, 2005). Intranasal insulin administered as monotherapy did not improve glucose tolerance, which is consistent with an early study (Njan *et al.*, 2018) and reflects the inability of intranasal insulin to improve insulin resistance within a

short term of treatment. However, combining intranasal insulin with metformin or saxagliptin improved glucose tolerance which could possibly due the additive effects of metformin or saxagliptin on glucose homeostasis when combined with intranasal insulin. Furthermore, daily administration of dexamethasone increased the activity of Glucose-6-phosphatase (Gluconeogenic enzyme) which is known to evoke gluconeogenesis (Jitrapakdee 2012). Intranasal insulin, metformin, saxagliptin and their combinations was able to reduce the activity of Glucose-6-phosphatase enzyme of which metformin alone and its combination with saxagliptin proved to be significant. This may be one of the possible mechanisms to justify the combination of metformin with saxagliptin at preventing dexamethasone-induced hyperglycemia and impaired glucose tolerance.

*Saxagliptin, metformin and intranasal insulin ameliorate dexamethasone induced hyperglycemia and insulin resistance.*



Dexamethasone -induced increase in total cholesterol level, triglyceride level and LDL observed in present study is line with those reported by Arab and Mahboubi (2015) except that, dexamethasone administered in this study increased HDL concentration. The mechanism of dexamethasone induced increase in HDL is still not entirely clear, but this is consistent with previous study by Wang *et al.*, 2012. Intranasal insulin, metformin, saxagliptin and their combinations used in present study was able to reduce Total cholesterol levels, Triglyceride levels, LDL and increase HDL of the combination of saxagliptin with intranasal insulin and its combination with metformin proved to be more efficacious. This result gives an insight of the potentials of these hypoglycemic drugs at reducing or preventing cardiovascular related diseases such as retinopathy, nephropathy, stroke, and coronary artery disease (CAD).

Several prospective studies have linked low levels of adiponectin with insulin resistance, metabolic syndromes and as a contributing factor to the progression from a prediabetes state to frank type 2 diabetes mellitus (Duncan *et al.*, 2004; Han *et al.*, 2009). In this regard, dexamethasone administered in present study had no significant modifiable effect on adiponectin levels and neither of intranasal insulin, saxagliptin or metformin was able to increase the levels of adiponectin within the short period of administration. Interestingly, combination of metformin with saxagliptin significantly increased adiponectin levels in comparison to all other groups. Glucokinase enzyme which has a very powerful control on glucose disposal by promoting the storage of glucose in liver cells as glycogen (Wilamowitz *et al.*, 2013) was explored in present study to further justify the effect of selected hypoglycemic agents at ameliorating dexamethasone induced hyperglycemia. Dexamethasone reduced the activity of this enzyme amongst which the combination of saxagliptin with intranasal insulin and the combination of saxagliptin with metformin was able to normalize this effect. In line with a previous study (Narendar *et al.*, 2015), administration of dexamethasone in present study increases serum insulin levels suggesting enhancement of beta-cell function to compensate for the increased glucocorticoid-induced peripheral insulin demand. Saxagliptin alone also significantly increased the level of insulin compared to all other hypoglycemic agents employed in present as an evident of its mechanism of action (Li *et al.*, 2016). To further support the concept of dexamethasone-induced hyperglycemia (Sofie *et al.*, 2018), result from this study also indicated an increase in glucagon activity. Except for the combination of saxagliptin with intranasal insulin, all other hypoglycemic agents explored in present study were able to reduce the level of glucagon relatively closer to the control group. Correlating with the biochemical assays conducted in this study, hyperglycemia and hyperlipidemia have been showed to fuel alterations in certain biochemical pathways, such as oxidative stress, low-grade inflammation, and apoptosis which could precipitate the development of insulin resistance and beta-cell dysfunction (Tangvarasittichai 2015). Results from histological investigation of the pancreas using H&E stain, indicated that our short-term administration of dexamethasone caused an atrophy of pancreatic islet cells. The goal of hypoglycaemic therapy is to achieve a tight glycaemic control and further prevents cardiovascular

complications with possible preservation of beta-cell function. Except for saxagliptin alone group of which sectioned part showed degeneration of the islet cell, all other hypoglycaemic agents employed in present study offered protection to the pancreatic islet cells. Previous reports from three cross-sectional studies (Scirica *et al.*, 2013, Cheinfeng lee *et al.*, 2014, Raz *et al.*, 2014,) have linked the extended use of saxagliptin to be associated with small but significant recurrent acute pancreatitis despite its efficacy and long clinical success. This present study establishes the effect of dexamethasone on hyperglycemia induction and impaired glucose tolerance and the role of selected hypoglycemic agents in ameliorating these effects and of which addition of saxagliptin to metformin therapy proved to be most effective.

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