ORIGINAL ARTICLE

# Development and Validation of a Novel Experimental Model for Quick Induction of Prediabetes and Studying Cardiometabolic Effects

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## Abstract

**Objective:** Prediabetes is a chronic metabolic disorder increasingly recognized as a major contributor to morbidity and mortality worldwide. Its gradual progression poses challenges for experimental modeling. This study aimed to establish and characterize a novel approach for inducing early and sustained prediabetes in rats. **Method:** Adult male rats were subjected to a high-fat diet, followed by a liquid diet composed of vanaspati ghee and coconut oil (3:1 ratio), along with 25% dextrose in drinking water for eight weeks. The onset of prediabetes was verified through biochemical assessments, including fasting blood glucose (FBG), HbA1c, insulin levels, HOMA-IR, and HOMA-β indices, complemented by histopathological and transmission electron microscopy analyses. **Results and Conclusion:** Biochemical evaluation confirmed prediabetes, as evidenced by significant elevations in FBG and HbA1c ($p < 0.05$). Additionally, increased insulin resistance (HOMA-IR) and impaired β-cell function (HOMA-β) were observed. Histopathological examination of the pancreas revealed structural alterations, while ultrastructural analysis demonstrated compromised insulin secretory granules, further validating β-cell dysfunction. This experimental model successfully achieved rapid and sustained progression of prediabetes, replicating the dual features of insulin resistance and early β-cell impairment characteristic of human disease. In conclusion, this study introduces a reliable and efficient method for inducing prediabetes in rodents. The model closely reflects the pathophysiological mechanisms underlying human prediabetes, offering a valuable platform for investigating disease progression and potential therapeutic interventions. **Significance:** Using this new approach of a novel animal model for prediabetes, we could expedite the development of prediabetes and demonstrate the role of insulin resistance and beta cell dysfunction in prediabetes.

**Key words:** Prediabetes, novel model, metformin, histopathological, ultrastructure change

## INTRODUCTION

Typically characterized by blood glucose levels that are higher than normal but below the thresholds for diabetes, prediabetes is a high-risk condition for the development of diabetes and is marked by impaired fasting glucose and impaired glucose tolerance. Approximately 5–10% of individuals with prediabetes develop diabetes each year, while the conversion rate varies depending on the definition of prediabetes and community variables.[1]

The pathogenesis of prediabetes is unique compared to frank hyperglycemic type 2 diabetes mellitus (T2DM) experimental models. The prediabetic stage is a relatively milder stage of diabetes compared to the frank diabetic stage.[2] Insulin resistance is considered the major pathogenesis of prediabetes, whereas diabetes is characterized by a combination of both insulin resistance and severe beta cell dysfunction. However, there is accumulating evidence that, besides insulin resistance, beta cell dysfunction also contributes to

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prediabetes. To elucidate the pathogenesis of prediabetes and know more about the exact role of insulin resistance and pancreatic β-cell failure, more authentic animal models that mimic both the causative pathogenesis and features of human prediabetes are very crucial. Moreover, the prediabetic model can also be used to study the disease-modifying effects of various antidiabetic materials.

Although various animal models of type 1 and T2DM are available, the number of animal models for pre-diabetes is very limited for exploring the disease pathogenesis and for drug development research.[2,4] Researchers Obrosova *et al*.,[5] Watcho *et al*.,[6] Shevalye *et al*.[7] have used mouse models fed on a high-fat/high-calorie diet to induce prediabetes. However, the impact of disease on the histopathological and ultrastructural changes in the pancreatic beta cell subjected to prediabetes was not elucidated. Moreover, literature on prediabetes experimental models demonstrated a long lag period (12–16 weeks) for the induction of prediabetes. Moreover, none of these models have been validated using anti-diabetic medications. Soares *et al*.[8] and Nunes *et al*.[9] have reported the sucrose-fed mouse model of prediabetes, which receives 35% sucrose solution *ad libitum* for 9 weeks. This approach exhibits similar characteristics to the high-fat diet (HFD) model, including a comparatively longer induction period of 9 weeks and no evaluation with an anti-diabetic medication.

Using a combination of a HFD and a liquid diet consisting of a 3:1 ratio of coconut oil and Vanaspati ghee, with an additional 25% dextrose in a drinking water bottle, this is the first report of a new model of prediabetes. The successful development of prediabetes in experimental rats that mimicked the disease's natural history was confirmed by biochemical, histopathological, and electron microscopic investigations. Biochemical markers: Serum insulin, C-peptide, derived parameters: Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-beta), and histopathological assessment of injury have been used in the present study to understand the disease pathogenesis and deleterious effects of Prediabetes.[2] In addition, for the first time, electron microscopy of the pancreas was undertaken to understand the ultrastructural changes of the pancreatic cells, which ultimately affects beta cell function. Study of the ultrastructural changes in cellular organelles was evaluated and the changes in Mitochondria, Endoplasmic Reticulum (ER), Vacuoles, Secretory Granules (Mature and Immature), and Nucleus were studied.

Prediabetes, the high-risk state for future development of diabetes and its cardiometabolic complications, is prevalent globally. To search for medications that would help with this co-morbidity, efforts were made to create a unique animal model of pre-diabetes coexisting with cardiometabolic alterations. It's interesting to note that the proposed model may well replicate the cardiometabolic alterations associated with prediabetes.

This novel experimental model of prediabetes was developed to overcome some of the limitations of the previously reported animal model of prediabetes. The developed model has an induction period that is far shorter than that of the prediabetes models used currently and is a useful model to study the disease pathogenesis and cardiometabolic effects. In addition, the developed model has been validated using the antidiabetic medication Metformin, which is the standard drug treatment for prediabetes.[3] Thus, the experimental model of prediabetes was successfully developed, validated, and has been copyrighted (No: L-142215/2024).

## MATERIALS AND METHODS

### Ethical approval

Institutional Animal Ethics Committee obtained (Approval No: 2023/10/03). CPCSEA Registration No.: 303/PO/Re/S/2000/CPCSEA.

### Experimental animal

The study employed adult male Wistar rats weighing 100–120 g and age 4–6 weeks. The rats were kept in the MGM Medical College's Central Animal Facility in Navi Mumbai, India. In the animal house, they were kept in standard laboratory conditions. The Institutional Animal Ethics Committee accepted the study protocol, which complies with the Guidelines for the Use and Care of Experimental Animals in Research and the Committee for the Purpose of Control and Supervision of Experiments on Animals and Indian National Science Academy. Rats were housed in air-conditioned rooms with natural light-dark cycles in 38 × 23 × 15 cm polyacrylic cages, with no more than four animals per cage. The animals were allowed free access to a standard diet or HFD and Water (25% dextrose) *ad libitum*.

### Development of a novel experimental model for prediabetes

Male Wistar rats having an average body weight of 100–120 g receive a commercially available high fat high carbohydrate diet for 9 weeks, were included in the study. Composition of diet included (Moisture, Crude Protein, Crude Fiber, Crude Fat, NFE, Calcium, and Phosphorous). In addition, 25% dextrose in drinking bottles and the Liquid HFD suspension were prepared in our laboratory by using a mixture of Vanaspati ghee and coconut oil (3:1) and administered to the rats by oral feeding (3 mL/kg) for 9 weeks for the induction of prediabetes. Every week, the rats were tested for blood glucose (100–125 mg/dL) to confirm the presence of prediabetes. At the end of the study, biochemical, histopathological, and electron microscopy assessment was undertaken.

### Validation of novel experimental model for prediabetes

To validate the experimental model of prediabetes, the blood glucose-lowering effects of a standard antidiabetic drug (Metformin) were studied in the developed model of prediabetes. The experimental rats were randomly divided into three groups. There were twelve rats in each group.

### Experimental groups

A total of 36 rats were used in this study. The animals were randomly divided into three experimental groups ($n$ = 12 per group). All rats in each group were assessed according to the designated experimental protocol.

1. Normal Control (NC) – Animals received standard pellet diet and purified water for 24 h *ad libitum* and were considered as NC group
2. Disease Control (DC) – Animals received a commercially available high-fat, high-carbohydrate diet, 25% dextrose water in bottles for 24 h, and 3 mL/kg liquid fat solution (Vanaspati ghee and coconut oil in the ratio of 3:1 [v/v]) each day over a period of 9 weeks and were considered as DC group
3. Metformin – Animals receiving commercially available high-fat, high-carbohydrate diet, 25% dextrose in bottles for 24 h and 3 ml/kg liquid fat solution (Vanaspati ghee and coconut oil in the ratio of 3:1 [v/v]) each day over a period of 9 weeks. Subsequently, after confirming prediabetes at the 3rd week, Metformin (100 mg/kg) was additionally administered orally to the experimental rats till the end of the experimental duration.

### Evaluation parameters

#### *Assessment of body weight changes and mortality, if any*

The animals' body weights (g) were noted at the beginning of the study and then weekly until the end of the study period. At the same time, any deaths that occurred throughout the oral administration period for the corresponding groups were also recorded.

#### *Estimation of biochemical parameters*

At the end of the study, the heart puncture technique was used to obtain rat blood samples from all experimental groups under light ketamine anesthesia (40 mg/kg) to estimate the biochemical parameters (Blood glucose, Glycated hemoglobin [HBA1c], Serum Insulin, C Peptide, Creatinine phosphokinase MB [CPK MB], Lipid Profile).

#### *Histopathological assessment*

At the end of the study, experimental animals were sacrificed and organs were excised immediately, rinsed with ice-cold saline, and trimmed off with its respective connective tissue and fat. All the organs were instantly fixed in 10% buffered formalin solution and weighed. Cross sections (5 m thick) of the fixed myocardial tissues were cut. These sections were stained with hematoxylin and eosin and visualized under a light microscope. The investigators performing the histologic evaluation were blind to biochemical results.

#### *Electron microscopy*

For ultrastructural assessment, the Pancreatic tissue was studied using transmission electron microscopy (TEM). Small fragments of the pancreas were fixed in 2.45% glutaraldehyde and 2.45% paraformaldehyde in a 0.1M sodium cacodylate buffer (pH 7.4) at 4°C for 2 h. The tissue was washed in a 0.1 M sodium cacodylate buffer (pH 7.4) at room temperature for 10 min for 3 times. The tissue fragments had been embedded in TAAB embedding resin. Uranyl acetate and lead citrate were used to stain ultrathin (70–75 nm) sections of the tissue, which were then examined using a Zeiss EM 902 TEM.

### Statistical analysis plan

Statistical analysis was performed by student t-test, $P < 0.05$ was considered as statistically significant.

## RESULTS

### Standardization and validation of the experimental model of prediabetes

#### *Biochemical parameters*

Blood glucose values above 100 mg/dL confirmed the induction of prediabetes. The blood glucose levels in the DC group rats were significantly higher as compared to NC group rats at 8 weeks. Prediabetes was conformed at the 3rd week after the induction protocol. The Diabetic parameter HBA1c also increased significantly in the DC group as compared to the NC [Figure 1]. Metformin treatment demonstrated significant antidiabetic effects as indicated by a significant

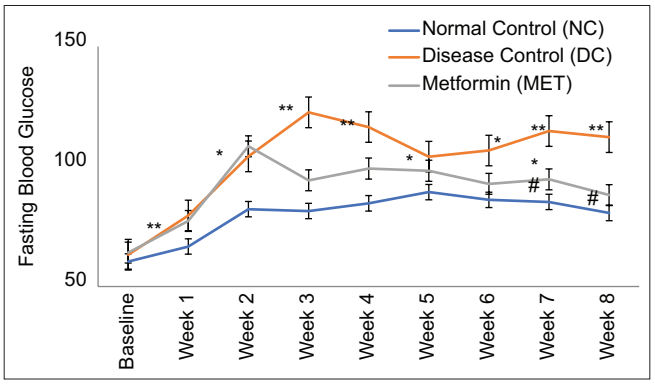


**Figure 1:** Change in fasting blood glucose in the various experimental groups. *$P$ < 0.05, **$P$ < 0.01, versus normal control; #$P$ < 0.01 versus disease control

decrease in blood glucose ($P < 0.001$) and HBA1c ($P < 0.01$) [Figure 2].

### *Histopathology of the pancreas*

The pancreas of the NC group rats was characterized by an organized pattern and showed normal architecture of islets of Langerhans and the beta cells [Figure 3a]. The DC group showed damaged islets of Langerhans, atrophy of beta cells, and reduced beta cell mass [Figure 3b]. In Metformin treated group, there was less inflammation and edema [Figure 3c].

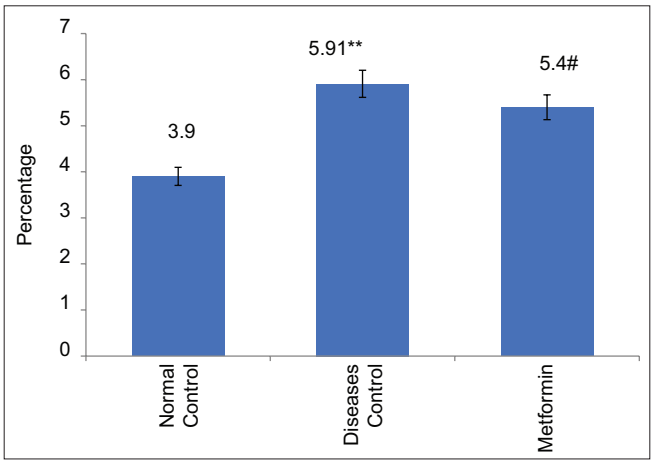


**Figure 2:** Change in glycated hemoglobin in the various experimental groups. $^{**}P < 0.01$, versus normal control; $^{\#}P < 0.01$, $^{\#}P < 0.01$ versus disease control

### *Ultrastructural changes in the pancreatic cell*

TEM is a valuable method to detect early changes in the ultrastructure of pancreatic cells during the development of prediabetes in rats fed with a High-Fat High-Carbohydrate (HFHC Diet). Rats fed with a HFHC Diet developed pathological ultrastructural alterations in the endocrine cells of the pancreas. The ultrastructural structural changes in cellular organelles were evaluated and the presence of changes in mitochondria, ER, vacuoles, secretory granules (Mature and Immature), nucleus and overall preservation of Pancreatic beta cell ultrastructure and beta cell mass were noted.

- Ultrastructural Changes in Mitochondria: Normal ultrastructure of pancreatic cell was found to contain a high number of mitochondria [Figure 4a], whereas in the DC group, changes in morphology and number were seen in mitochondria as compared to NC [Figure 4b]. There was an increase in the number of mitochondria and a decrease in mitochondrial damage after administering Metformin [Figure 4c].
- Ultrastructural Changes in ER: The normal structure of pancreatic cell was studied in the control group with intact morphological structure of ER [Figure 5a]. In the DC group morphological changes, no linear structure lesser number of ribosomes were seen in the ER in the disease condition [Figure 5b]. In the Metformin group, there was an increase in the number of ER and morphologically, the ER appeared similar as NC [Figure 5c].
- Ultrastructural Changes in Nucleus: The normal structure of pancreatic cell was studied in the control group [Figure 5a], whereas in the disease condition,

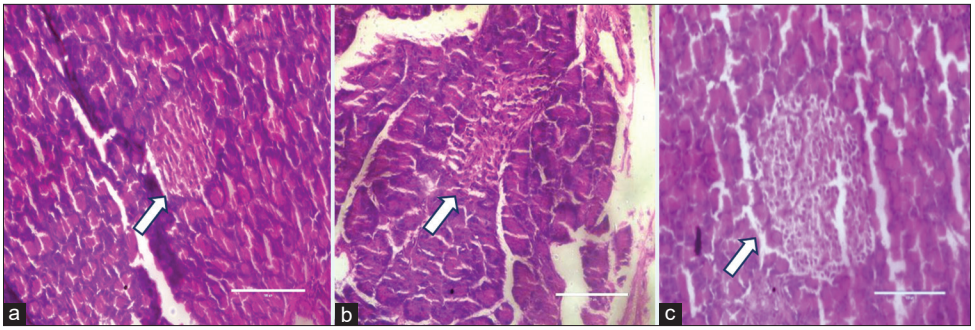


**Figure 3:** Histopathological picture of pancreas; (a) Normal control, (b) Disease control, (c) Metformin

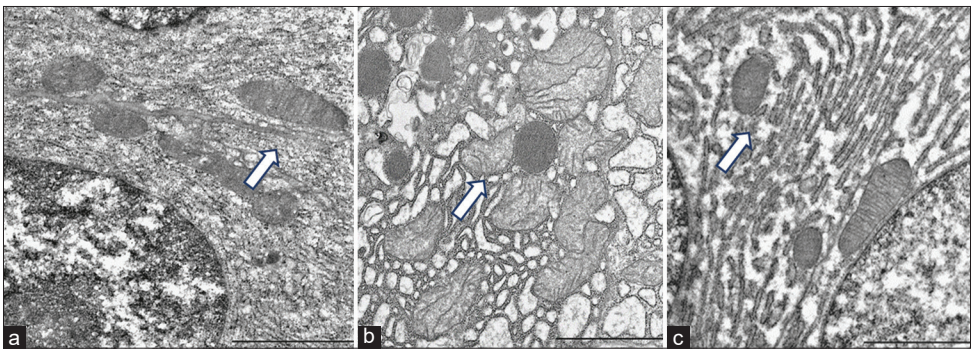


**Figure 4:** Mitochondrial ultrastructure in pancreatic islets beta cell; (a) Normal control, (b) Disease control, (c) Metformin

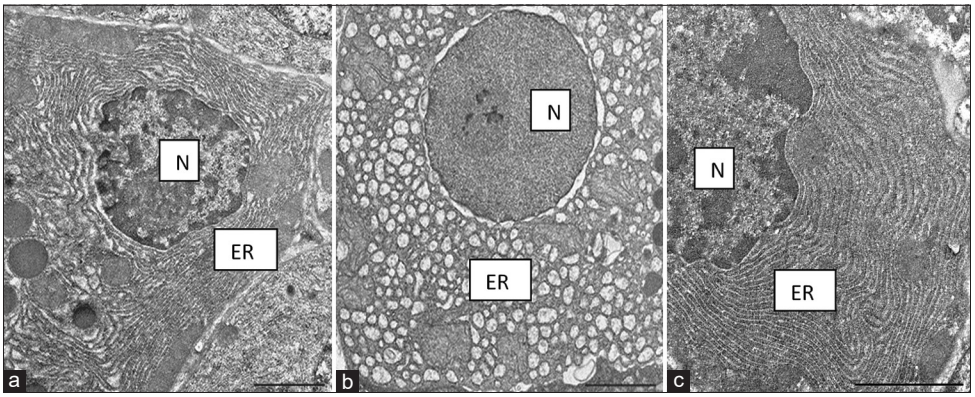


**Figure 5:** Endoplasmic reticulum and nucleus ultrastructure in pancreatic islets beta cell; (a) Normal control, (b) Disease control, (c) Metformin

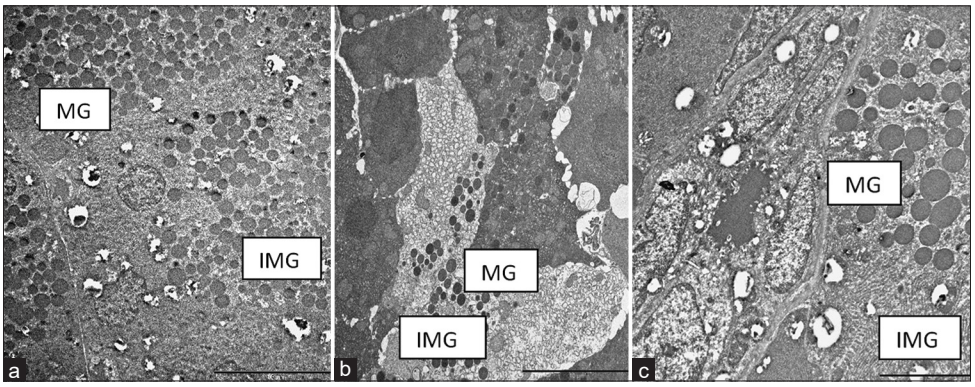


**Figure 6:** Insulin Secretory Granules (Mature & Immature) ultrastructure in pancreatic islets beta cell; (a) Normal control, (b) Disease control, (c) Metformin

morphological structural changes were seen in the nucleus [Figure 5b]. In the Metformin group, the morphological changes improved as compared to DC group [Figure 5c].

- Ultrastructural Changes in Insulin Secretory Granules (Mature and Immature): The pancreatic beta cells of the control group contained numerous secretory mature and immature granules scattered throughout their cytoplasm [Figure 6a], whereas in the DC group, mature secretory granules less in numbers were observed [Figure 6b]. In comparison, in the Metformin group, there was an increase in secretory mature granules [Figure 6c].

## Cardiometabolic alternations in prediabetes

### *Metabolic changes*

- Insulin and C-Peptide: Serum insulin and C-Peptide increased in the DC group, which reduced after Metformin treatment
- HOMA IR and HOMA-Beta: HOMA IR increased in DC and after Metformin treatment. Insulin resistance was significantly reduced as compared to the DC group. HOMA β decreased in the DC group and post-Metformin, it did not significantly change [Table 1].

**Table 1:** Biochemical parameters in the various experimental groups

| Parameters | Normal control | Disease control | Metformin |
|---|---|---|---|
| Insulin | 0.25±0.024 | 0.433±0.487 | 0.16±0.06 |
| C peptide | 0.013±0.001 | 0.019±0.004 | 0.01±0.001 |
| HOMA IR | 0.04±0.004 | 0.11±0.14 | 0.06±0.008 |
| HOMA β | 11.49±5.19 | 0.69±0.29 | 0.4±0.084 |

HOMA-IR: Homeostatic model assessment for insulin resistance

- Lipid profile: Total cholesterol and triglycerides levels were elevated in the DC group, which decreased in the Metformin group. No significant change in high-density lipoprotein levels was noted in the various groups [Figure 7].

### *Cardiac changes*

There was a significant increase in serum CPK-MB levels in DC rats at the 9$^{th}$ week, which reduced with Metformin, though the results were not statistically significant. The other cardiac markers, hs-CRP were measured on the 9$^{th}$ week of study and were found to be significantly raised in the DC group, which decreased post-Metformin treatment [Table 2].

### *Histopathological changes in prediabetes*

a. Histopathology of the liver

Histological assessment of the liver of the NC group rats shows normal architecture of the central vein, the peripheral vein, and hepatocytes [Figure 8a]. The DC group showed degeneration, scattered necrotic cells, congestion in the central vein, and inflammation, edema, and fatty change [Figure 8b].

In Metformin Less granular degeneration, inflammation and necrosis [Figure 8c].

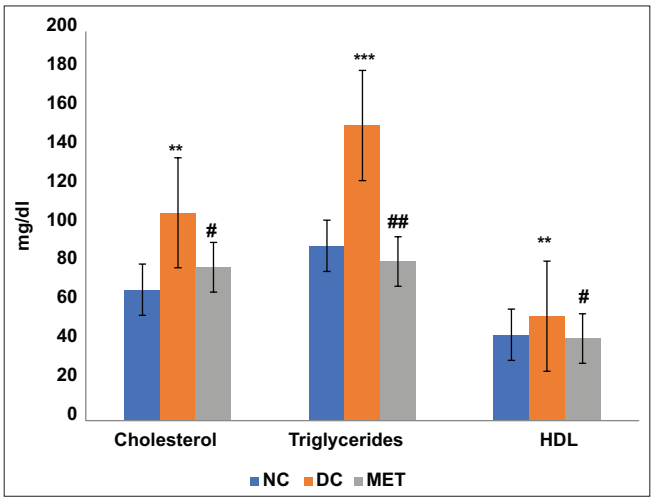


**Figure 7:** Change in Lipid Profile in the various experimental groups. $^{**}P < 0.01$, $^{***}P < 0.001$ versus normal control; $^{\#}P < 0.01$, $^{\#\#}P < 0.001$ versus disease control

b. Histopathology of the kidney

Histopathology of the NC group kidney showed absence of congestion of glomerular blood vessels, tubular necrosis, edema, and inflammation [Figure 9a]. The DC group demonstrated mild congestion of glomerular blood vessels, tubular necrosis, edema, inflammation, and cloudy degeneration [Figure 9b].

In the Metformin group, there is less congestion of glomerular blood vessels, tubular necrosis, inflammation, and focal area [Figure 9c].

c. Histopathology of the heart

**Table 2:** Cardiac, liver, and kidney markers in the various experimental groups

| Cardiac marker | Normal control | Disease control | Metformin |
|---|---|---|---|
| CPK MB | 1278.57±255.26 | 1531.85±271.36 | 1363±211.76 |
| HS CRP | 40.32±3.58 | 49.84±2.75** | 45.4±3.68 |
| SGPT | 60.28±17.22 | 68.42±8.95 | 52.8±14.72# |
| Creatinine | 0.178±0.0402 | 0.15±0.031 | 0.188±0.046 |
| Pancreatic Lipase | 6.3±1.99 | 5.9±1.45 | 5.52±0.40 |

$^{**}P<0.01$ versus normal control; $^{\#}P<0.01$ versus disease control, CPK MB: Creatinine phosphokinase MB, SGPT: Serum glutamic pyruvic transaminase, HS CRP: High-sensitivity C-reactive protein

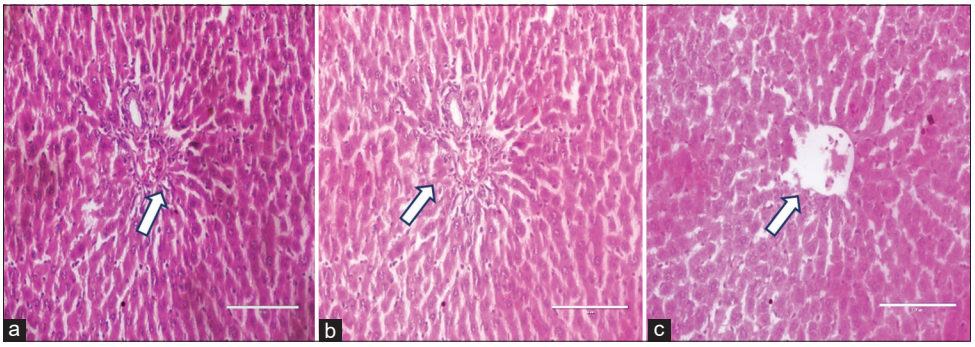


**Figure 8:** Histopathological picture of the liver. (a) Normal control, (b) Disease control, (c) Metformin

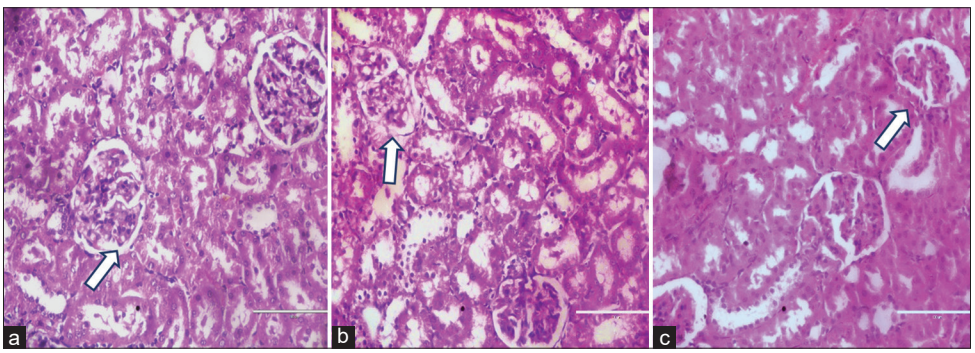


**Figure 9:** Histopathological picture of the kidney. (a) Normal control, (b) Disease control, (c) Metformin

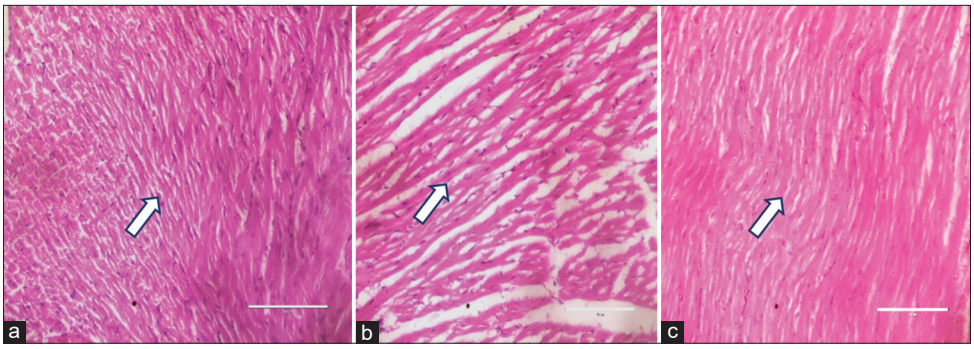


**Figure 10:** Histopathological picture of the heart. (a) Normal control, (b) Disease control, (c) Metformin

Histopathological assessment of the NC group rat heart revealed the non-infarcted architecture of the myocardium [Figure 10a]. In contrast, the DC group rats subjected to HFD demonstrated marked edema, and separation of myofibers, congested blood vessels, and mild inflammation [Figure 10b]. In the Metformin group, there was less inflammation and edema as compared to the DC group [Figure 10c].

### Effect of prediabetes on body weight, food, and water intake

The DC group rats showed a significant ($P < 0.001$) decrease in body weight every week as compared with the NC group rats. The drop-in body weight of the DC group rats did not last until at the end of the 9$^{th}$ week. Rats' body weight decreased after treatment with Metformin when compared to NC. Food and water Intake were noted every day for every group for the induction of prediabetes (HFHC Diet). The intake of food was decreased in the NC and Metformin group as compared to the DC group. The intake of water was modestly increased in the DC group as compared to the NC group. A decrease in water intake was seen in the Metformin group at the 3$^{rd}$ and 4$^{th}$ week of study.

## DISCUSSION

An intermediate stage of dysglycemia between normoglycemia and diabetes is known as prediabetes. The prediabetic state is an important risk factor for developing diabetes in the future, as well as associated with a high burden of cardiometabolic risk factors. The increasing prevalence of prediabetes globally is a major public health concern and does not bode well for the growing epidemic of diabetes and its cardiometabolic complications.[9,10] Therefore, research focusing on prediabetes and its sequelae is the need of the hour.

Several approaches have been used to develop the animal models of prediabetes, such as high-fat or high-calorie diet-fed models, HFD-fed STZ-injected models, and high sucrose-fed model.[2] Although many of these models have been successfully developed for prediabetes they share several limitations: long lag time for induction and inclusion of a few evaluation parameters to study the exact role of Insulin resistance and beta cell dysfunction in the pathogenesis of prediabetes. In addition, none of these models have not been scientifically validated by using the standard anti-diabetic drug, questioning the suitability of these models.[2]

To overcome some of the drawbacks of the previously published animal model of prediabetes, an experimental model was developed. This is the first report of a unique model of prediabetes induced by adding 25% dextrose to a drinking water bottle and combining a HFD with a liquid diet (Vanaspati ghee and coconut oil in the ratio of 3:1 [v/v]), useful to study the disease pathogenesis as well as cardiometabolic complications.

### Standardization and validation of the experimental model of prediabetes

In the present study, an increase in fasting blood sugar and HBA1c was observed in the DC group rats as compared to NC group rats, confirming the induction of prediabetes. Results of the present study were consistent with the results demonstrated by Obrosova *et al*.,[5] Watcho *et al*.,[6] Shevalye *et al*.,[7] Soares *et al*.,[8] and Nunes *et al*.[9] The histopathological assessment of the pancreas, demonstrating damaged islets of Langerhans, atrophy of beta cells and reduced beta cell mass confirmed the induction of prediabetes. Previous studies have not undertaken the histopathological assessment of the pancreatic cells in the prediabetic model.

The major advantage of this novel experimental model of prediabetes that has been developed is the quick induction time of 3–4 weeks. In contrast, other model for prediabetes developed by Obrosova *et al*.,[5] Watcho *et al*.,[6] Shevalye *et al*.,[7] Soares *et al*.,[8] and Nunes *et al*.[9] reported prediabetes induction time as 9–12 weeks. Metformin, the standard antidiabetic drug used to treat prediabetes has also been used to validate this model. No other researcher has used any antidiabetic drug for the validation of the prediabetic model.

In addition, this is the first study to include electron microscopic evaluation to demonstrate changes in the pancreas ultrastructure. Certain pancreatic cell organelles, such as the mitochondria, ER, nucleus, and secretory granules, exhibited

ultrastructural alterations. Rats in the NC group show a large number of mitochondria with their shape maintained, while in the DC group's mitochondria, the numbers were less with altered morphological structure. It may be hypothesized that in prediabetes, less energy is available as fewer mitochondria are present and their structures are altered, which prevents proper ATP production because there will be fewer oxysomes (a part of mitochondria which are primarily responsible for the ATP production) due to the reduced number of mitochondria.

While morphological abnormalities were observed in the DC group, the ER exhibited an intact morphological structure in NC. The two parts of the ER are the smooth ER and the rough ER. Because the ER is important for protein synthesis with the help of ribosomes (protein factories), changes in the ER structure, as seen in prediabetic rats, will interfere with the formation of new proteins. Following ER synthesis, proteins are matured and packaged in the Golgi complex. When the ER undergoes modifications, the protein cannot be produced properly, which prevents normal maturation and prevents the protein from being used by the cells.

Normal pancreatic cell nucleus structure was observed in NC, but morphological structural alterations were noted in diseased conditions. The nuclear membrane was found to be ruptured in DC during the formation of the ER and nucleolus, a part of the nucleus that produces ribosomes. The nucleus is known as the master cell organelle due to its stores all the information required for protein synthesis. Protein synthesis may be disrupted by structural modifications in the nucleus, as observed in the prediabetic rats, because it plays a significant role in transcription (DNA to mRNA), a process that occurs during protein synthesis.

The cytoplasm of the pancreatic beta cell contains many mature and immature Insulin secretory granules in the NC group, which significantly reduce in the DC group. It is well known that Insulin secretion is carried out by these secretory granules.[13] In DC conditions, there are less mature secretary granules, which affects the synthesis of insulin. Mature secretary granules are transformed from immature to mature and produce insulin, which helps in the breakdown of glucose.[13] In the Metformin group, an increase in mature secretory granules was seen. Thus, in the Metformin-treated group, the deleterious ultrastructural changes in the Pancreases were significantly less as compared to DC. Similar results were seen in a study done by Kelman *et al*. year in type 2 diabetes in mice.[13] There are no reports of pancreatic beta cell ultrastructural findings in prediabetes.

The experimental model was also used to assess the cardiometabolic complications of prediabetes. Metabolic abnormalities and some of the long-term cardiac complications of diabetes may already exist in prediabetes several years before the onset of T2DM. Recognition of these cardiometabolic changes may help to better understand the underlying etiology of prediabetes, which provides a window of opportunity for the development of T2DM.

### Cardiometabolic alternations in prediabetes

#### *Metabolic alterations*

Insulin and C peptide levelswas increased in DC because of hyperinsulinemia as compared to NC. Krit *et al*.,[11] Tanajak *et al*.[12] A decrease in Insulin and C-peptide levels was seen in Metformin treated group. Furthermore, HOMA IR, the marker of Insulin resistance, increased in the DC group as compared to the NC group. This elevated IR was positively modulated by Metformin.[8] In addition to an increase in IR, a decline in beta cell function, as assessed using HOMA β, was seen in prediabetes. Thus, a beta cell dysfunction and insulin resistance together plays a role in the pathogenesis of prediabetes.

The present study revealed that prediabetes was associated with an increase in total cholesterol and triglycerides levels. Metformin demonstrated hypolipidemic effects, which is consistent with findings from Shevalye *et al*.,[7] Soares *et al*.,[8] and Nunes *et al*.,[9] Krit *et al*.[11]

#### *Cardiac alterations*

Prediabetes was associated with cardiac damage as assessed using biochemical and histopathological studies. Histopathological pictures of the normal group rat heart revealed the non-infarcted architecture of the myocardium and in the prediabetic group rats showed that edema, and separation of myofibers, congested blood vessels, and inflammation. In the Metformin group, there was less separation of myofibers, inflammation, and edema as compared to DC.

The animal model may also be helpful to screen the drugs with cardiometabolic potential, as significant cardiac damage and elevation in lipid levels was observed in the present study.

## CONCLUSION

The novel experimental model of prediabetes was successfully developed and validated. The models successfully mimed the disease pathogenesis and the adverse cardiometabolic effects of prediabetes.

## ACKNOWLEDGMENTS

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