Chapter-5

RESULTS

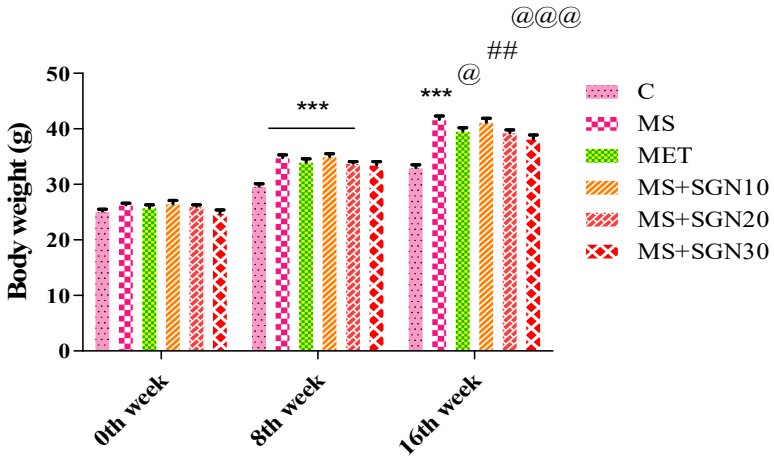
5.1 To study the effect of Sitagliptin on metabolic syndrome, fatty liver and hepatic oxidative stress in a high-fat diet-induced animal model of obesity (Objective I).

5.1.1 Effect of Sitagliptin on body weight, weight gain, and FBG level in obese mice

During the sixteen weeks of the experimental protocol, the body weight of the Swiss albino mice was recorded every week and is represented in **Fig. 5.1a**. After eight weeks and sixteen weeks, the body weight of the Swiss albino mice was found to be significantly increased in HFFW fed and MS groups (p<0.001), respectively, compared to the control group. However, we observed a significant reduction in the body weight after sixteen weeks in MS+SGN20 and MS+SGN30 groups (p<0.01, and p<0.001 respectively) relative to the MS group. At the end of the experiment, the weight gain of the Swiss albino mice was evaluated (**Fig. 5.1b)**, where the reduction potential of sitagliptin was found to be 16.4±0.6 in MS+SGN20 group and 16.3±0.6 in MS+SGN30 group equivalent to 16.5±0.4 in the MET group. The FBG level was measured at 0^{th}, 8^{th}, and 16^{th} week during the experimental protocol and is represented in **Fig. 5.1c**. After eight weeks and sixteen weeks of HFFW diet supplementation, a remarkable increase in FBG level was observed in the HFFW fed and MS groups, respectively, as compared to the control group (p<0.001). However, in the treatment groups, FBG levels improved significantly in MS+SGN10 (p<0.01), MS+SGN20, and MS+SGN30 groups (p<0.001;

for both) respective to the MS group, equivalent to MET group (p<0.001, relative to MS).

a.



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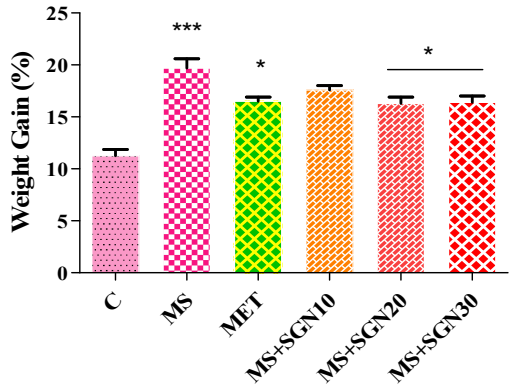

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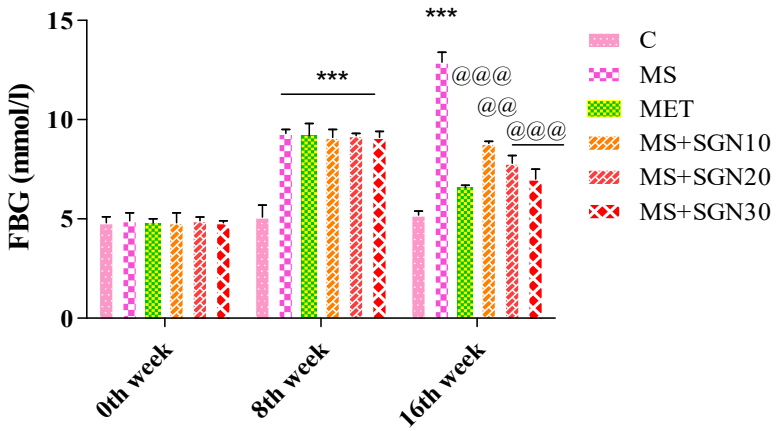


Figure 5.1: The effect of chronic sitagliptin treatment on a) Body weight; b) Weight gain; and c) FBG level. Results depicted as Mean ± SEM (n=6). *** indicates p<0.001 against C; * indicates p<0.05 against MS; ## indicates p<0.01 against MS; @ indicates p<0.05 against MS; and @@@ indicates p<0.001 against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups. Two-way ANOVA followed by Bonferroni post hoc test was applied to find statistical significance among the groups for body weight and FBG levels.

5.1.2 Effect of Sitagliptin on cumulative food intake, liver weight index and visceral fat index in obese mice

Cumulative food intake was evaluated at the end of sixteen weeks, where we found a significant increase in the cumulative food intake in the MS group (p<0.001) relative to the control group **(Fig.5.2a)**. But, surprisingly, we found that sitagliptin did not affect the cumulative food intake in the treatment groups. The liver weight index of the Swiss albino mice is represented in **Fig.5.2b.** It increased significantly in the MS group (p<0.01) relative to the control group after sixteen weeks of HFFW. However, a significant reduction was observed in the liver weight index of the Swiss albino mice after eight weeks of sitagliptin treatment in MS+SGN20 and MS+SGN30 groups (p<0.05; for both), relative to the MS group, equivalent to MET (p<0.05, versus MS group). Similarly, a significant increase was observed in the visceral fat index of the MS group (p<0.001) with respect to the control **(Fig.5.2c)**. It was found to be reduced significantly in MS+SGN20, MS+SGN30, and MET groups (p<0.05; for both) relative to the MS group**.**

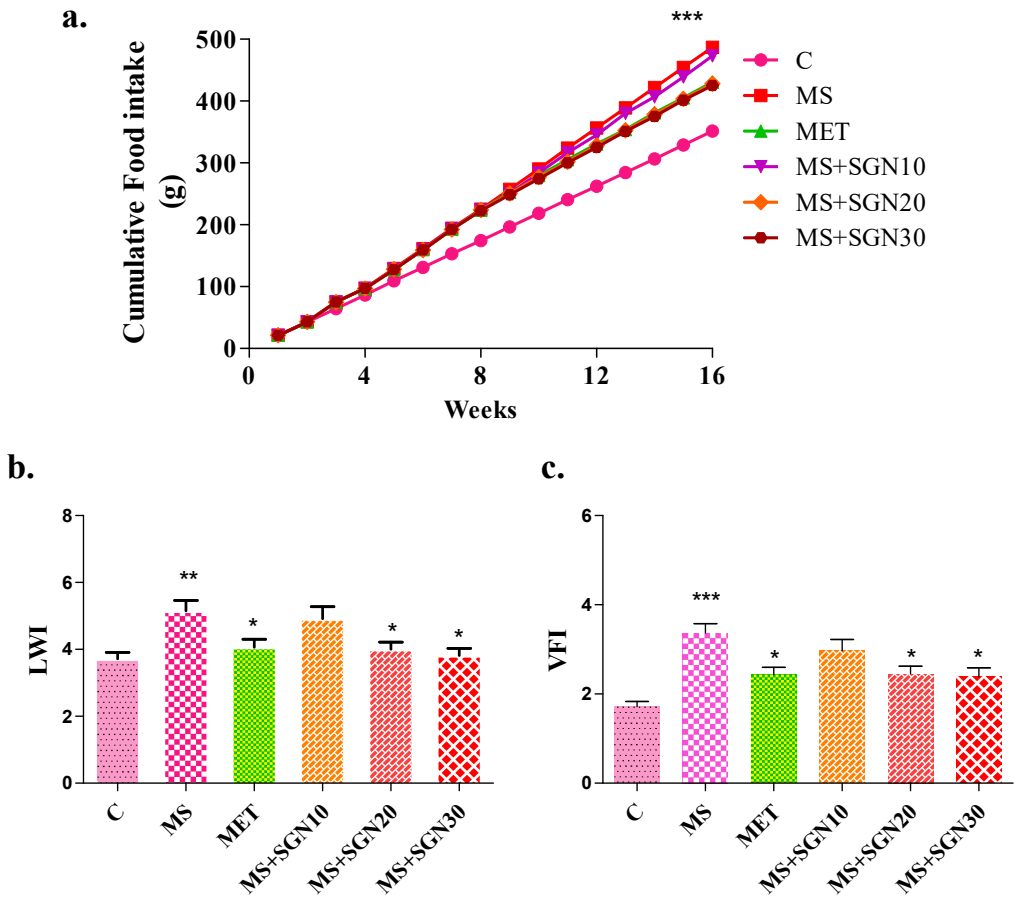


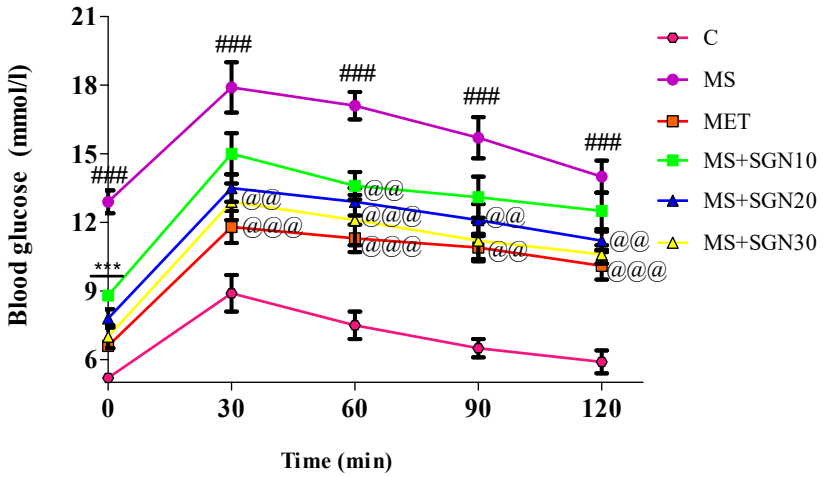
Figure 5.2: The effect of chronic sitagliptin treatment on a) cumulative food intake; b) LWI; and c) VFI. Results depicted as Mean ± SEM (n=6). *** indicates $p<0.001$ against C; and ** indicates $p<0.001$ against MS; and * indicates $p<0.05$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups. LWI- Liver weight index and VFI- Visceral fat index.

5.1.3 Effect of Sitagliptin on OGTT and area under the curve of blood glucose (AUG) in obese mice

During OGTT, a spike in blood glucose level was observed within 30 min in all the groups, as shown in **Fig. 5.3a**. In the MS group, at 60, 90, and 120 min, blood glucose levels were found to be significantly elevated respective to the control group ($p<0.001$). In contrast to this, glucose levels in MS+SGN20, MS+SGN30, and MET

groups were significantly decreased at 30, 60, 90, and 120 min (p<0.01, p<0.001, p<0.01, p<0.01, respectively; for all) versus the MS group. Accordingly, AUG was plotted based on the OGTT results (**Fig. 5.3b**). There was a significant increase in the AUG of the MS group relative to the control group (p<0.001). However, with respect to the MS group, a significant reduction was observed in the AUG of MS+SGN10 (p<0.01), MS+SGN20, and MS+SGN30 (both p<0.001) groups (equivalent to MET: p<0.001 versus MS group).

a.



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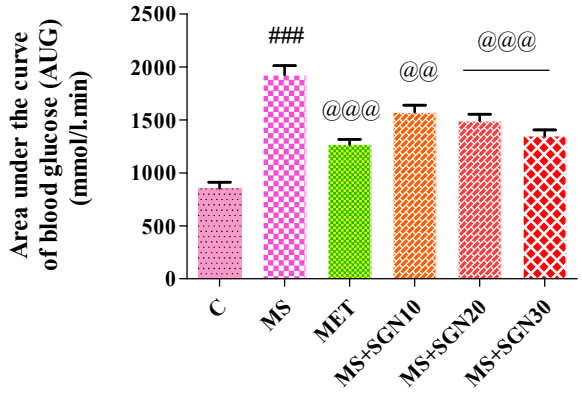


Figure 5.3: The effect of chronic sitagliptin treatment on a) OGTT; and b) AUG. ### indicates $p<0.001$ against C; @@@ indicates $p<0.001$ against MS; and @@ indicates $p<0.01$ against MS. The bar indicates the significance exhibited by MET, MS+SGN10, MS+SGN20, MS+SGN30 against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups. Two-way ANOVA followed by Bonferroni post hoc test was applied to find statistical significance among the groups for OGTT.

5.1.4 Effect of Sitagliptin on ITT in obese mice

A significant reduction in the blood glucose levels in the MS group was observed at 30 and 60 min ($p<0.001$) relative to the control group, as represented in **Fig. 5.4**. However, the blood glucose levels increased again significantly at 90 and 120 min ($p<0.001$) compared to the control group. In the treatment groups, we observed a significant decrease in the blood glucose levels at 30, 60, 90, and 120 min compared to the MS group. At 30, 60, 90, and 120 min, blood glucose levels were reduced significantly in MS+SGN20 ($p<0.01$, $p<0.01$, $p<0.001$, $p<0.001$), MS+SGN30 ($p<0.001$; for all intervals), and MET ($p<0.001$; for all intervals) groups, relative to the MS group.

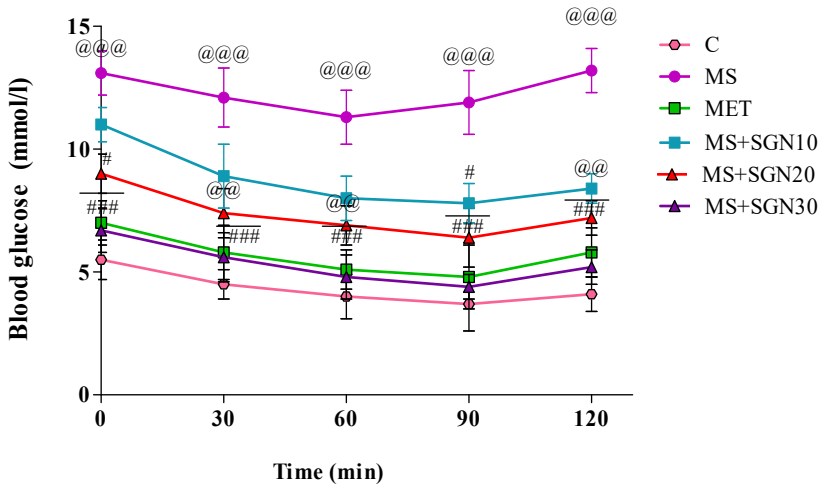


Figure 5.4: The effect of chronic sitagliptin treatment on ITT. @@@ indicates $p<0.001$ against C; ### indicates $p<0.001$ against MS; @@ indicates $p<0.01$ against MS; and # indicates $p<0.05$ against MS. The bar indicates the significance exhibited by MET, MS+SGN20, MS+SGN30 against MS. Two-way ANOVA followed by Bonferroni post hoc test was applied to find statistical significance among the groups.

5.1.5 Effect of Sitagliptin on FSI and HOMA-IR in obese mice

FSI and HOMA-IR are represented in **Fig. 5.5a and Fig. 5.5b** respectively. In the MS group, there was a significant increase in the FSI levels ($p<0.001$) as compared to the control group after sixteen weeks of the HFFW diet. However, we observed a significant decrease in the FSI levels in MS+SGN10 ($p<0.05$), MS+SGN20 ($p<0.01$), and MS+SGN30 ($p<0.001$) groups with respect to the MS group. Increased insulin resistance was observed in MS, as HOMA-IR was found to be significantly elevated ($p<0.001$) relative to the control group. However, as compared to the MS group, HOMA-IR decreased significantly in MS+SGN10 ($p<0.05$), MS+SGN20 ($p<0.001$), and MS+SGN30 ($p<0.001$) groups. The decrease in insulin resistance in MS+SGN30 was equivalent to the MET group ($p<0.001$; versus MS).

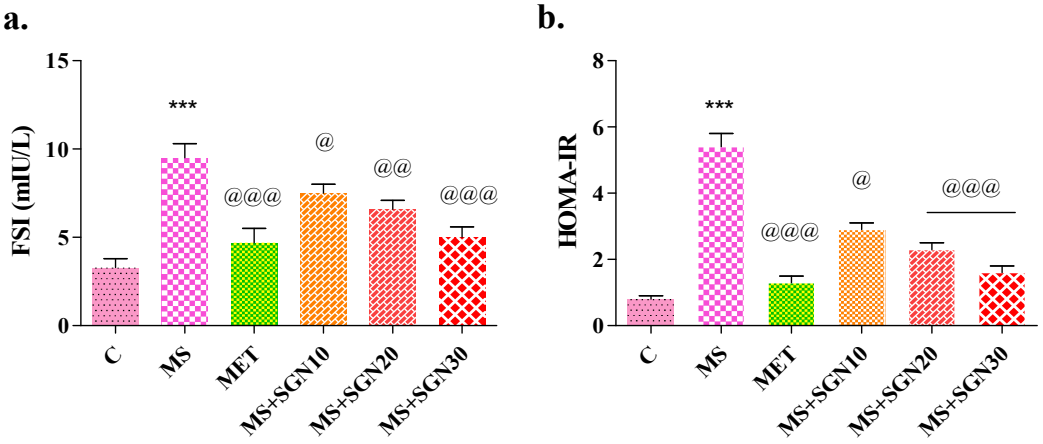
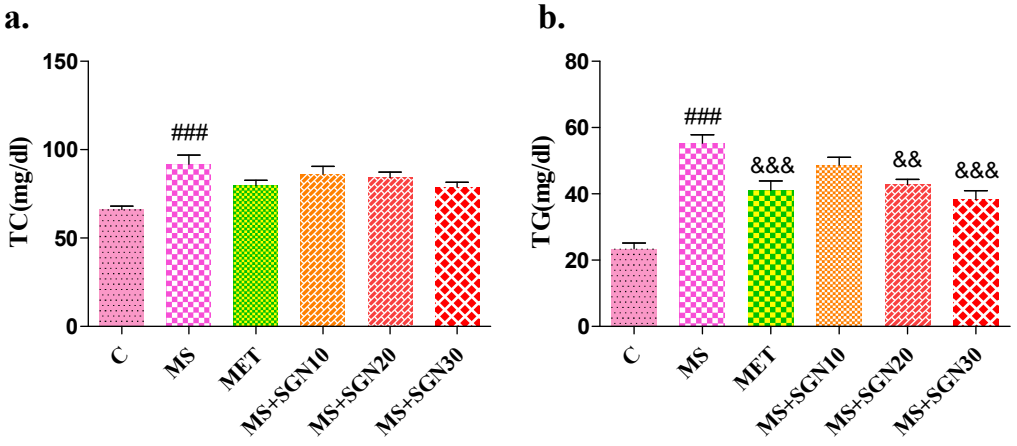


Figure 5.5: The effect of chronic sitagliptin treatment on a) FSI; and b) HOMA-IR. *** indicates $p<0.001$ against C; @@@ indicates $p<0.001$ against MS; @@ indicates $p<0.01$ against MS; and @ indicates $p<0.05$ against MS. One-way ANOVA followed

by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.1.6 Effect of Sitagliptin on lipid profile in obese mice

The effect of sitagliptin on the serum lipid profile of experimental animals is represented in **Fig. 5.6a-e.** After sixteen weeks of HFFW, a significant increase was observed in the TC (p<0.001; **Fig. 5.6a**), TG (p<0.001; **Fig. 5.6b**), LDL-C (p<0.001; **Fig. 5.6d**) and VLDL-C (p<0.001; **Fig. 5.6e**) levels in MS group with respect to the control group. In contrast to this, a marked decline in HDL-C levels (**Figure 5.6c**) was observed in the MS group (p<0.001) in comparison to the control group. However, the levels of TG, LDL-C, VLDL-C, and HDL-C were significantly improved in the treatment groups after eight weeks of oral administration of sitagliptin. In MS+SGN20 and MS+SGN30 groups, there was a significant decrease in the levels of TG (p<0.01 and p<0.001), LDL-C (p<0.05; p<0.01), VLDL-C (p<0.01; p<0.001) whereas increased levels of HDL-C (p<0.01; p<0.001) were observed relative to MS group. The improvement in lipid profile in MS+SGN30 group was equivalent to metformin.



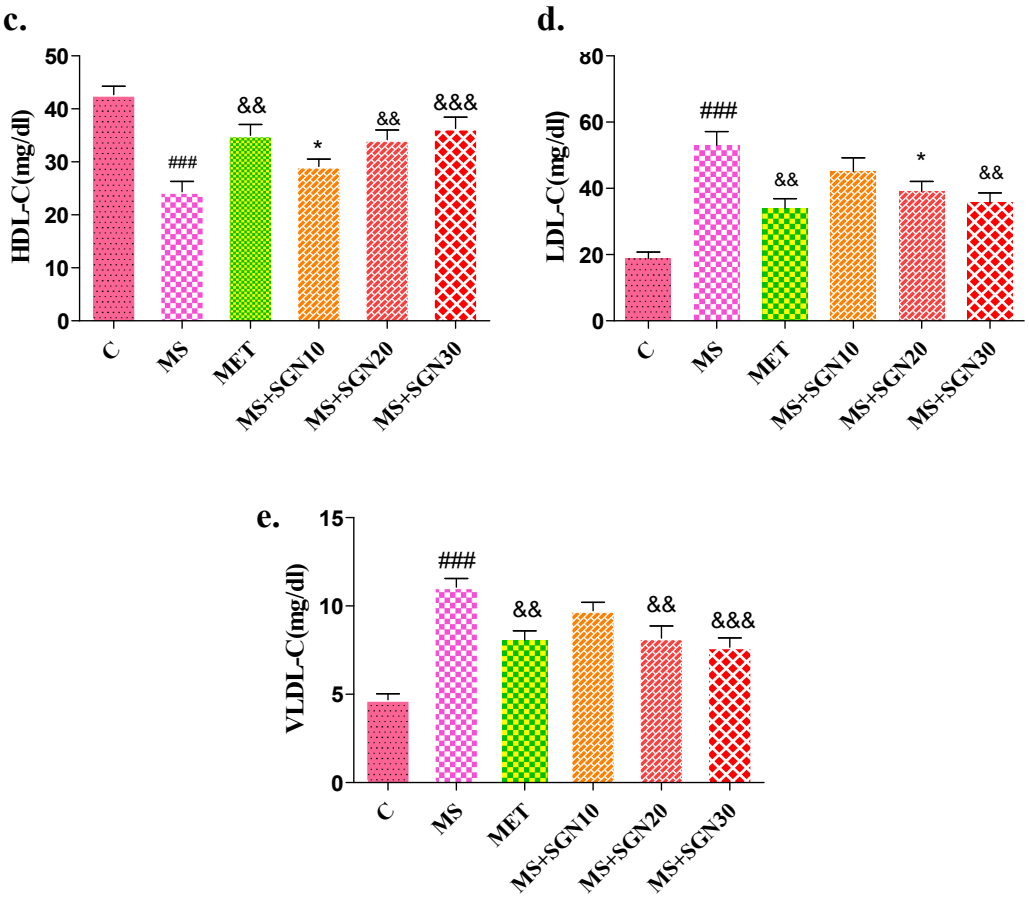


Figure 5.6: The effect of chronic sitagliptin treatment on a) TC; b) TG; c) HDL-C; d) LDL-C; and e) VLDL-C levels. Results depicted as Mean ± SEM (n=6). ### indicates $p<0.001$ against C; &&& indicates $p<0.001$ against MS; && indicates $p<0.01$ against MS; and * indicates $p<0.05$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.1.7 Effect of Sitagliptin on serum free fatty acid levels in obese mice

Fig. 5.7 represents the serum free fatty acid levels. We observed significantly increased free fatty acid levels in the MS group ($p<0.001$) after sixteen weeks of HFFW diet, relative to the control group. However, a marked reduction was observed in MS+SGN20 ($p<0.05$), MS+SGN30 ($p<0.01$), and MET ($p<0.05$) groups relative to the MS group.

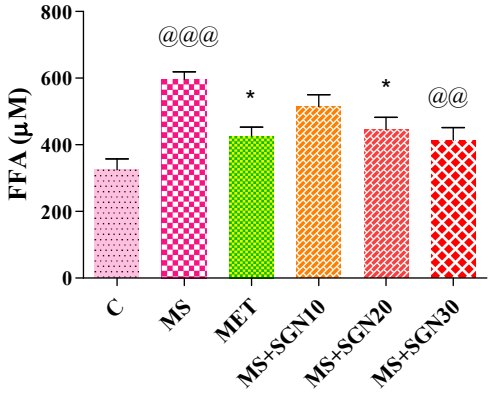


Figure 5.7: The effect of chronic sitagliptin treatment on serum free fatty acid levels. Results depicted as Mean ± SEM (n=6). @@@ indicates $p<0.001$ against C; @@ indicates $p<0.01$ against MS; and * indicates $p<0.05$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups. FFA- Free Fatty acid.

5.1.8 Effect of Sitagliptin on ALT and AST levels in obese mice

ALT and AST levels are shown in **Fig. 5.8a and 5.8b,** respectively. At the end of sixteen weeks of HFFW, ALT and AST levels were increased significantly in the MS group in comparison with the control group ($p<0.001$; for both). However, the levels of ALT and AST, both, were found to be significantly reduced in MS+SGN20 and MS+SGN30 groups ($p<0.05$; for both) against MS group. The observed effect of sitagliptin at 20mg/kg and 30mg/kg was equivalent to MET ($p<0.05$ versus MS). We observed non-significant changes among MET, MS+SGN20, and MS+SGN30 ($p>0.05$).

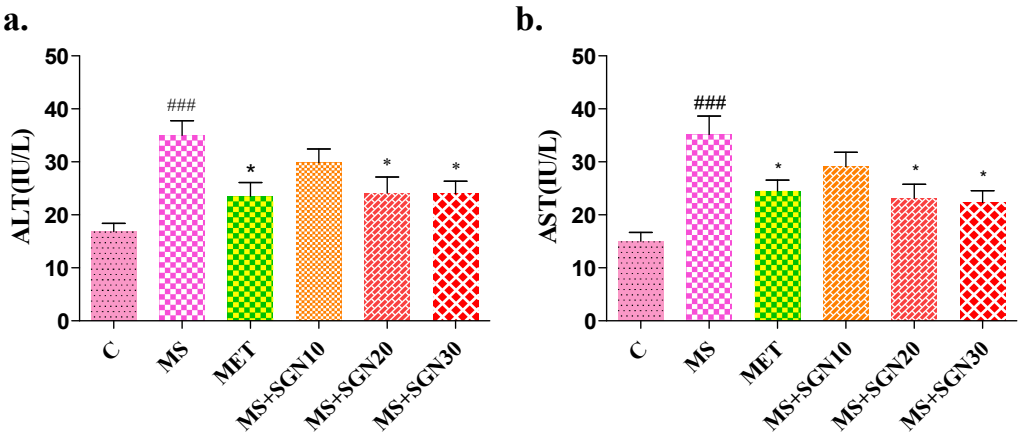


Figure 5.8: The effect of sitagliptin treatment on serum a) ALT; and b) AST levels. Results depicted as Mean ± SEM (n=6). ### indicates $p<0.001$ against C; * indicates $p<0.05$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.1.9 Effect of Sitagliptin on serum leptin and adiponectin levels in obese mice

The level of serum adipocytokines leptin and adiponectin are shown in **Fig. 5.9a and 5.9b,** respectively. They were found to be deregulated in MS group after HFFW intake: leptin levels were significantly increased ($p<0.001$), whereas adiponectin levels were significantly decreased ($p<0.001$) with respect to the control group. However, a significant increase was noticed in the leptin levels in the MET group ($p<0.05$), MS+SGN20, and MS+SGN30 ($p<0.01$; for both) when compared with MS group. The adiponectin levels were raised significantly in MET ($p<0.05$), MS+SGN20, and MS+SGN30 groups ($p<0.01$ and $p<0.001$, respectively). Surprisingly, no significant difference was observed between metformin and sitagliptin treated groups.

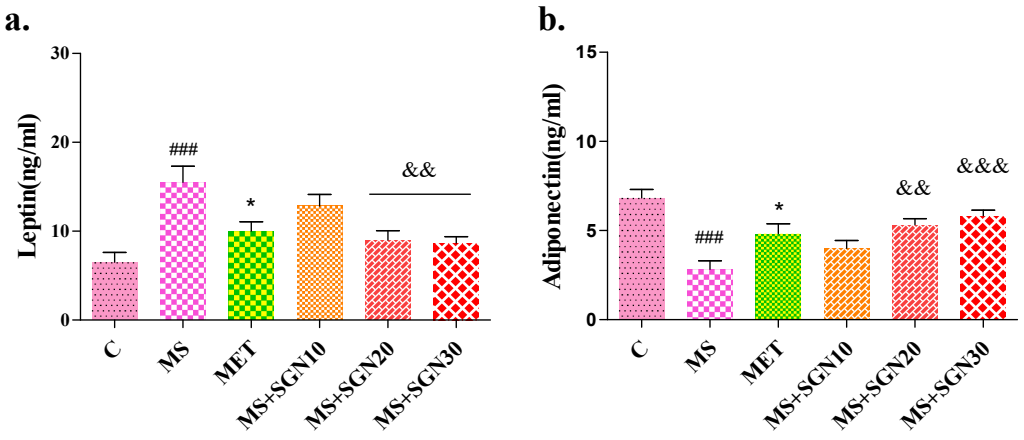


Figure 5.9: The effect of chronic sitagliptin treatment on a) Leptin; b) Adiponectin levels. Results depicted as Mean ± SEM (n=6). ### indicates $p<0.001$ against C; &&& indicates $p<0.001$ against MS; && indicates $p<0.01$ against MS; and * indicates $p<0.05$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.1.10 Effect of Sitagliptin on serum uric acid and GLP-1 levels in obese mice

Fig. 5.10a and **5.10b** represent serum uric acid and GLP-1 levels, respectively. Estimation of uric acid at the end of the experimental protocol showed a significant increase in the MS group ($p<0.001$) when compared with the control group (**Fig. 5.10a**). But it was found to be significantly decreased in MS+SGN20 and MS+SGN30 groups ($p<0.05$; for both) with respect to MS group, equivalent to metformin. GLP-1 levels were found to be significantly reduced in the MS group ($p<0.01$), relative to the control group after sixteen weeks of the HFFW diet. However, in the treatment groups, GLP-1 levels increased significantly in MS+SGN20 ($p<0.05$), MS+SGN30 ($p<0.01$), and MET ($p<0.05$) groups, relative to MS group.

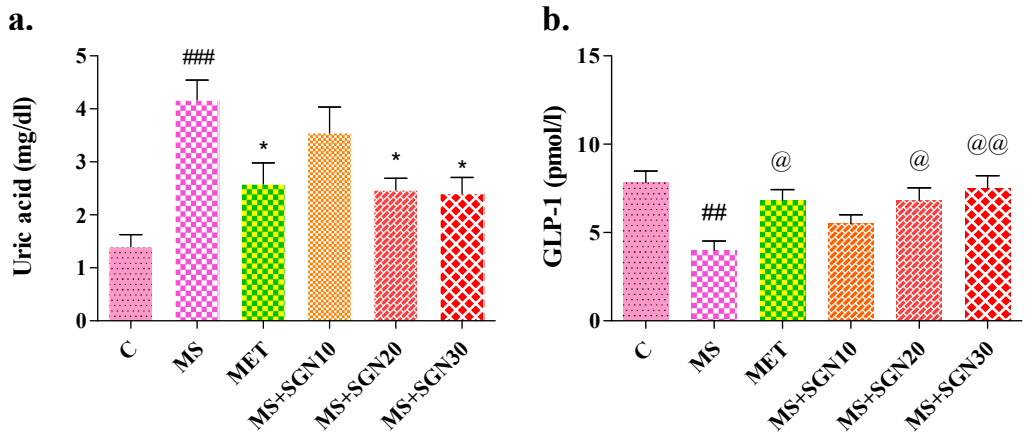


Figure 5.10: The effect of sitagliptin treatment on serum a) Uric acid; and b) GLP-1 levels. Results depicted as Mean ± SEM (n=6). ### indicates $p<0.001$ against C; ## indicates $p<0.01$ against C. @@ indicates $p<0.001$ against MS; @ indicates $p<0.05$ against MS; and * indicates $p<0.05$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.1.11 Effect of Sitagliptin on hepatic fat content in obese mice

Hepatic TG and TC content are represented in **Fig. 5.11a and Fig. 5.11b**, respectively. Prolonged supplementation of the HFFW diet significantly increased the hepatic TG and TC content in the MS group ($p<0.001$ and $p<0.01$, respectively) compared with the control group. However, in MS+SGN20 ($p<0.01$), MS+SGN30 ($p<0.001$), and MET ($p<0.01$) groups, there was a significant reduction in TG levels (**Fig. 5.11a**). Also, we observed a decrease in TC levels in MS+SGN20 and MS+SGN30 groups ($p<0.05$; for both), relative to the MS group, which was equivalent to metformin (**Fig. 5.11b**). We did not observe any difference among the treatment groups.

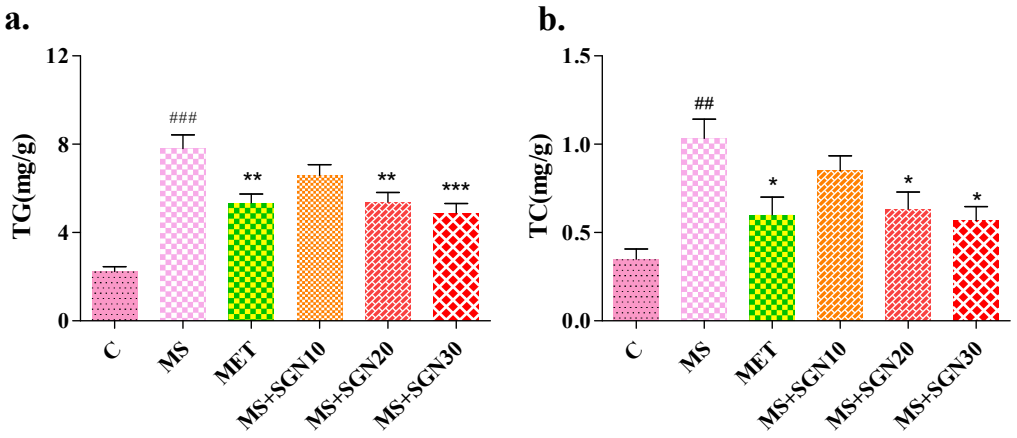


Figure 5.11: The effect of chronic sitagliptin treatment on hepatic fat content a) TG; and b) TC levels. Results depicted as Mean ± SEM (n=6). ### indicates $p<0.001$ against C; ## indicates $p<0.01$ against C; *** indicates $p<0.001$ against MS; ** indicates $p<0.01$ against MS; and * indicates $p<0.05$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.1.12 Effect of Sitagliptin on hepatic oxidative stress in obese mice

Hepatic MDA, SOD, and GSH levels estimated at the end of the experimental protocol are shown in **Fig. 5.12a-c**. MS group demonstrated significantly enhanced levels of MDA ($p<0.001$) and reduced levels of both enzymatic antioxidants SOD and GSH ($p<0.01$; for both) relative to the control group. Hepatic MDA levels decreased significantly after sitagliptin treatment in MS+SGN20 and MS+SGN30 groups ($p<0.01$; for both) relative to MS group (**Fig. 5.12a**). Contrarily, SOD and GSH levels increased significantly in MS+SGN20 and MS+SGN30 groups ($p<0.05$; for both) relative to MS group (**Fig. 5.11b** and **Fig. 5.11c** respectively). The effects observed with sitagliptin were equivalent to metformin.

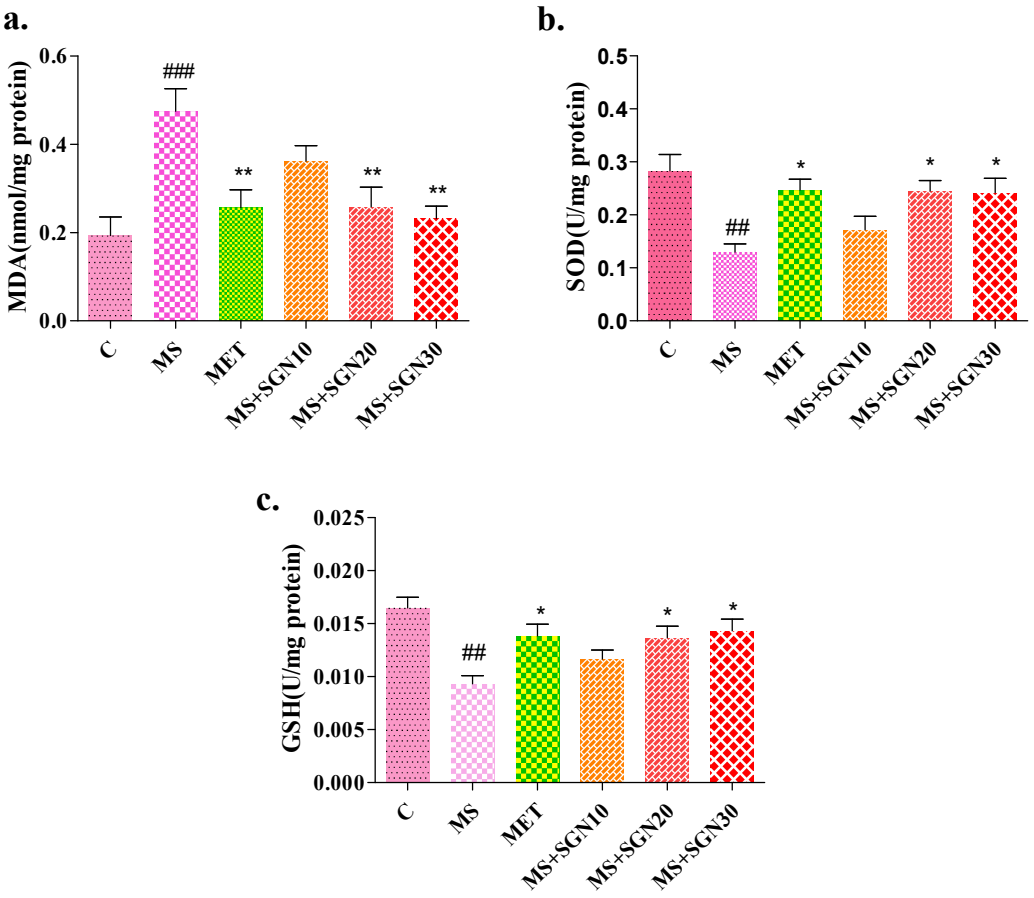


Figure 5.12: The effect of chronic sitagliptin treatment on hepatic oxidative stress a) MDA; b) SOD; and c) GSH levels. Results depicted as Mean ± SEM (n=6). ### indicates $p<0.001$ against C; ## indicates $p<0.01$ against C; ** indicates $p<0.01$ against MS; and * indicates $p<0.05$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.1.13 Hepatic histopathological evaluation

To examine the effect of sitagliptin on lipid accumulation in the liver, we performed H & E staining, illustrated in **Fig. 5.13**. Sixteen weeks of the HFFW diet depicted increased lipid droplets in the MS group compared to the C group (marked by arrows). However, in MS+SGN20, MS+SGN30, and MET groups, we observed a reduction in the hepatic lipid accumulation.

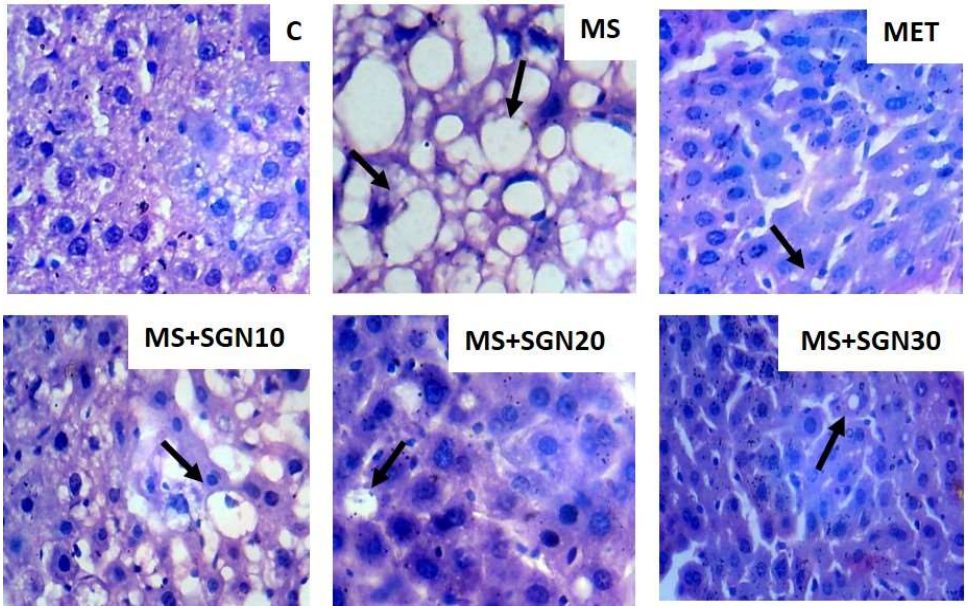
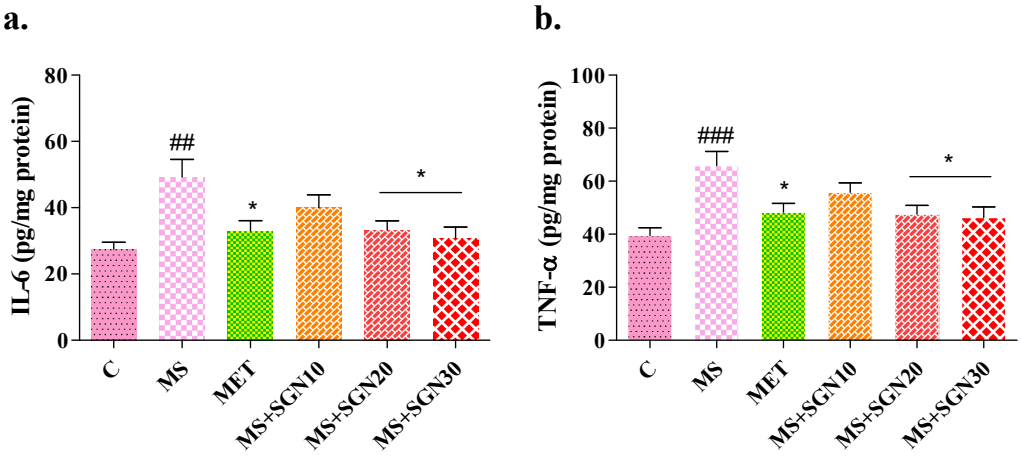


Figure 5.13: Histopathological evaluation of the liver section by H & E staining. The marked arrows indicate increased lipid deposition in liver in MS, which are reduced in treatment groups. Scale bar = 200 µm.

5.2 To analyze the potency of Sitagliptin on white adipose tissue inflammation, adiponectin expression and hepatic fatty acid metabolism in experimentally induced obese mice with focus on AMPK signaling (Objective II).

5.2.1 Effect of Sitagliptin on pro-inflammatory cytokine levels in eWAT in obese mice

IL-6, TNF-α, and MCP-1 levels are shown in **Fig. 5.14a-c**. The level of pro-inflammatory cytokines was increased in eWAT in MS group after prolonged HFFW diet intake, IL-6 (p<0.01), TNF-α (p<0.001), and MCP-1 (p<0.001) relative to the control group. However, we observed a significant reduction in their levels in the treatment groups. IL-6 and TNF- α levels decreased significantly in MS+SGN20 (p<0.01; for both) and MS+SGN30 groups (p<0.01; for both) with respect to the MS group (**Fig. 5.14a and 5.14b respectively**). The effect was equivalent to metformin. MCP-1 level was found to be decreased significantly in MS+SGN30 (p<0.01) and MET (p<0.05) groups relative to the MS group (**Fig. 5.14c**).The lower dose of sitagliptin was found to be ineffective.



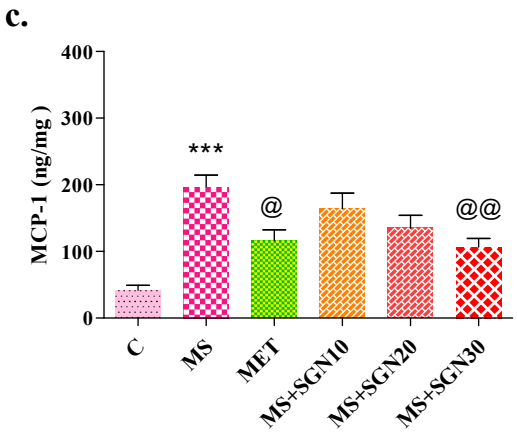


Figure 5.14: The effect of chronic sitagliptin treatment on eWAT a) IL-6; b) TNFα; and c) MCP-1 levels. Results depicted as Mean ± SEM (n=6). ### indicates $p<0.001$ against C; ## indicates $p<0.01$ against C; * indicates $p<0.05$ against MS, @@ indicates $p<0.01$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.2.2 eWAT histopathological evaluation

The histopathological evaluation of eWAT is depicted in **Fig. 5.15**. We observed an increase in the size of the adipocytes in the MS group after sixteen weeks of the HFFW diet compared to the control group. Whereas, in the treatment groups, there was a reduction in the adipocyte size in MS+SGN30 and MET groups with respect to the MS group. At lower doses of sitagliptin, no notable change could be observed.

5.2.3 Effect of Sitagliptin on hepatic and eWAT Adiponectin mRNA expression in obese mice

Fig. 5.16a and **5.16b** represent hepatic and eWAT adiponectin mRNA expression, respectively. Sixteen weeks of HFFW diet demonstrated a significant decrease in the ADIPOQ mRNA levels in the liver and eWAT in the MS group ($p<0.01$; for both), with respect to the control group. However, a significant increase in hepatic

ADIPOQ mRNA level was observed after eight weeks of sitagliptin administration in MS+SGN20 and MS+SGN30 (p<0.05; for both), with respect to MS group (**Fig. 5.16a**). Similar results were obtained for eWAT, where ADIPOQ mRNA levels improved significantly in MS+SGN20 (p<0.05) and MS+SGN20 groups (p<0.01) with respect to the MS group (**Fig. 5.16b**). There was no significant improvement in the adiponectin expression in both the tissues at the lower dose of sitagliptin.

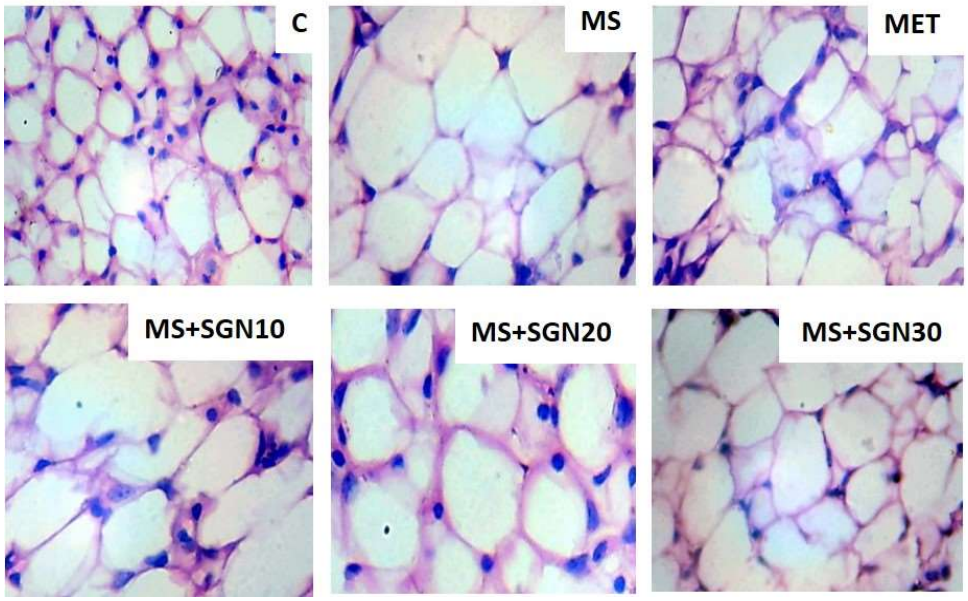


Figure 5.15: Histopathological evaluation of the eWAT section by H & E staining. In MS group, an increase in the size of the adipocytes is observed, which was decreased at the higher dose of sitagliptin. Scale bar = 200 µm.

5.2.4 Effect of Sitagliptin on hepatic AMPK and ACC phosphorylation in obese mice

The effect of sitagliptin on phosphorylation of AMPK and ACC proteins is represented in **Fig. 5.17a** and their relative expression in **Fig. 5.17b-c**. In the MS group, the relative protein expression of P-AMPK and P-ACC was reduced notably with respect to the control group (p<0.01; for both) (**Fig. 5.17c)**. However, in the treatment

groups, there was a significant increase in the relative levels of both the proteins; P-AMPK: MS+SGN20 (p<0.05), MS+SGN30 (p<0.01), and MET (p<0.05) (**Fig. 5.17b**); P-ACC: MS+SGN20, MS+SGN30, and MET (p<0.05; for all) (**Fig. 5.17b**) in comparison to the MS group. P-AMPK to β-actin ratio was increased by 1.7 and 1.8 folds in MS+SGN20 and MS+SGN30 groups, respectively. P-ACC to β-actin ratio was increased by 1.5 in the MS+SGN20 group and 1.6 in the MS+SGN30 group. In the MS+SGN10 group, no significant change was observed in the P-AMPK and P-ACC relative protein levels.

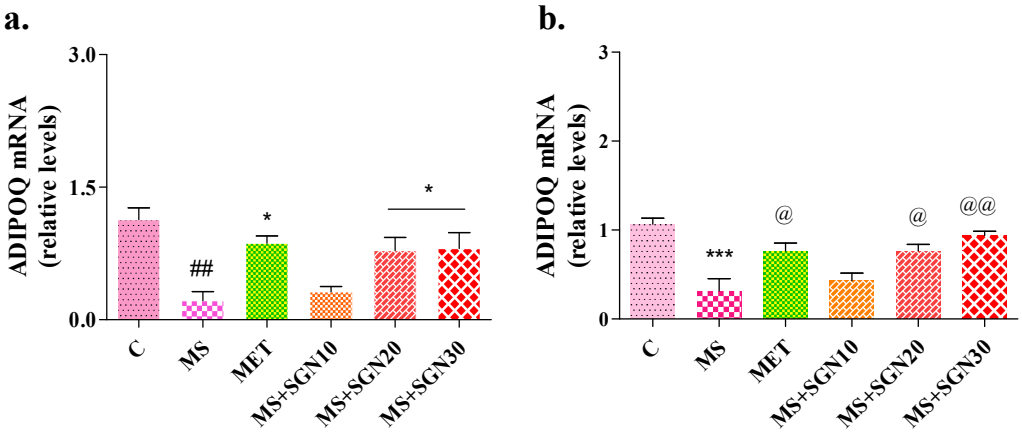


Figure 5.16: The effect of chronic sitagliptin treatment on relative mRNA levels of ADIPOQ with respect to β-actin in: a) liver and b) eWAT. Results depicted as Mean ± SEM (n=6). *** indicates p<0.001 against C; ## indicates p<0.01 against C; @@ indicates p<0.01 against MS; * indicates p<0.05 against MS; and @ indicates p<0.05 against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups. ADIPOQ- Adiponectin

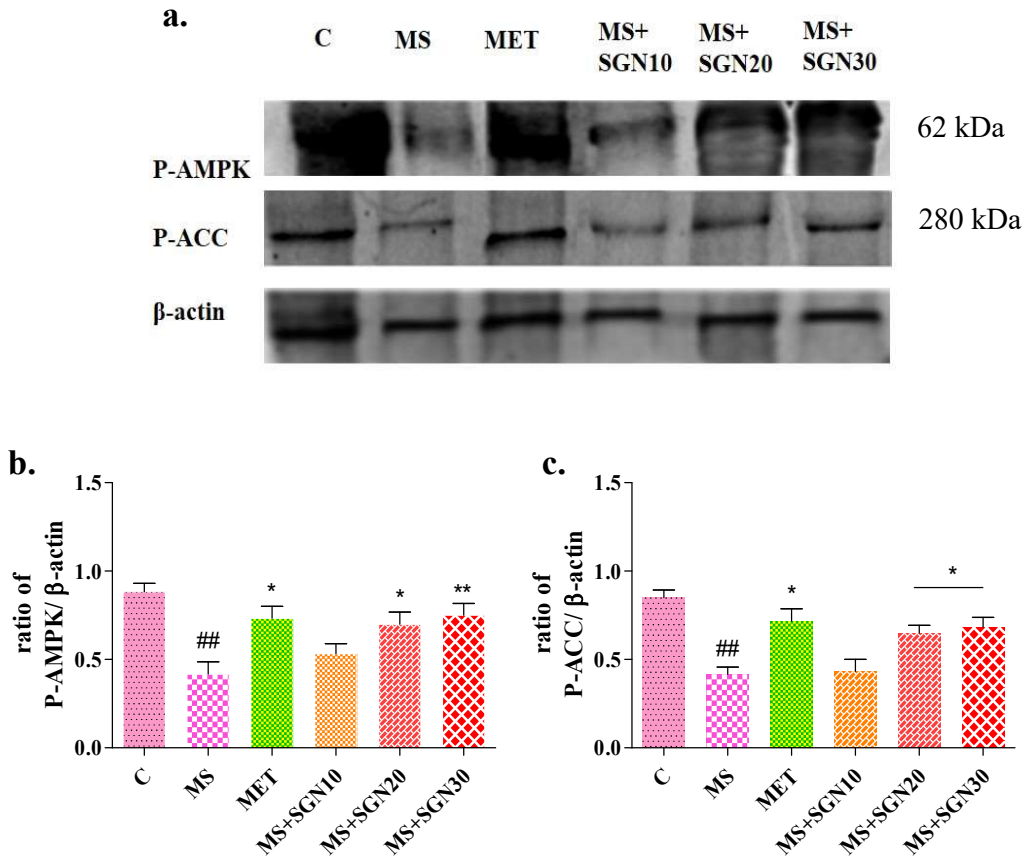


Figure 5.17: The effect of chronic sitagliptin treatment on a) Protein expression of hepatic P-AMPK and P-ACC; b-c) relative protein expression of P-AMPK and p-ACC with respect to β-actin. Results depicted as Mean ± SEM (n=6). ## indicates $p<0.01$ against C; ** indicates $p<0.01$ against MS; and * indicates $p<0.05$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.2.5 Effect of Sitagliptin on hepatic mRNA expression of fatty acid metabolism genes in obese mice

Fig. 5.18a-c represents the hepatic mRNA expression of CPT-1A, FASN, and PPARα in HFFW fed obese mice. There was a significant decrease in the expression of CPT-1A, PPARα, and an increase in the expression of FASN in the MS group ($p<0.01$; for all) with respect to the control group. However, in the treatment groups, there was a

significant improvement in the mRNA expression of fatty acid metabolism genes. CPT-1A and PPARα levels were increased significantly in MS+SGN20, MS+SGN30, and MET groups (p<0.05; for all), relative to MS group, whereas the expression of FASN was found to be reduced significantly in MS+SGN20, MS+SGN30, and MET groups (p<0.05; for all) relative to the MS group. The lower dose of sitagliptin was found to be ineffective in inducing any significant change in the expression of CPT-1A, FASN, and PPARα.

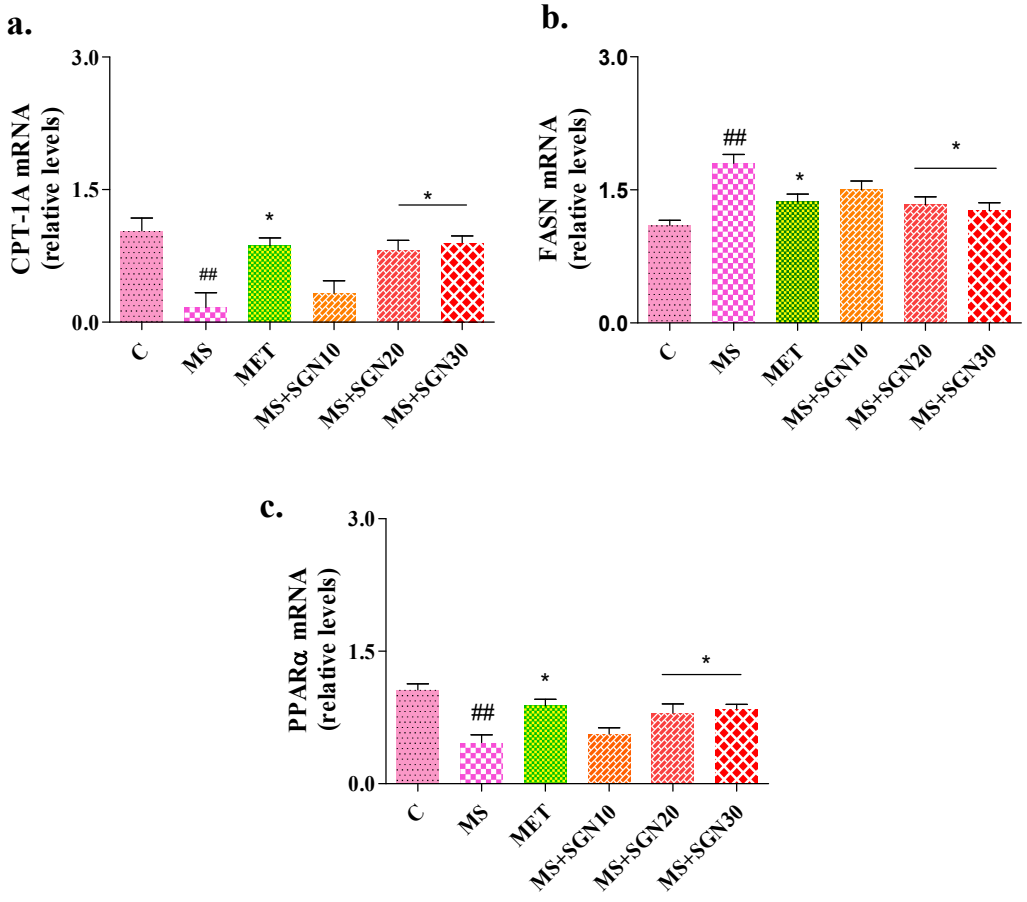


Figure 5.18: The effect of chronic sitagliptin treatment on hepatic mRNA expression a) CPT-1A; b) FASN; c) PPARα with respect to β-actin. Results depicted as Mean ± SEM (n=6). ## indicates p<0.01 against C; and * indicates p<0.05 against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.3 To investigate the effect of Sitagliptin on oxidative stress and mitochondrial biogenesis markers in white and brown adipose tissues in metabolically compromised obese mice (Objective III).

5.3.1 Effect of Sitagliptin on WAT and BAT weight in metabolically compromised obese mice

Sixteen weeks of the HFFW diet significantly increased WAT and BAT weight in the MS group (p<0.001 and p<0.01 respectively) compared to the control group, as shown in **Fig. 5.19**. However, sitagliptin treatment significantly decreased the WAT weight in MS+SGN20, MS+SGN30, and MET groups (p<0.05; for all), with respect to the MS group. Similarly, there was a decrease in the BAT weight in MS+SGN30 and MET groups (p<0.05; for both), relative to the MS group.

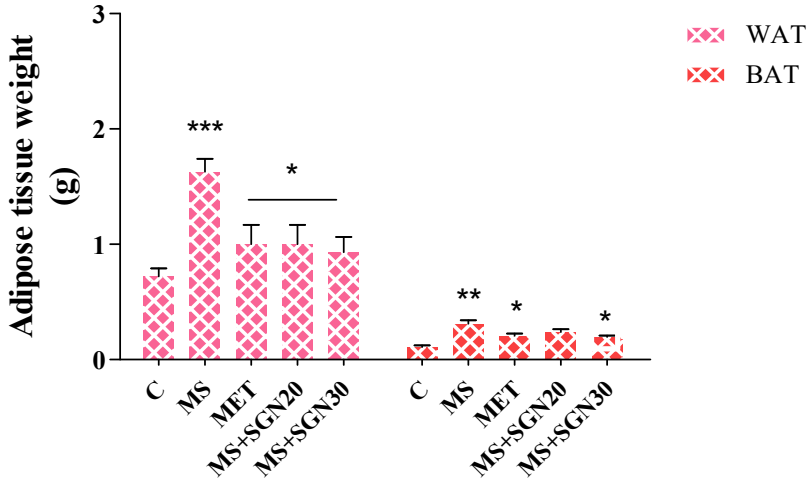
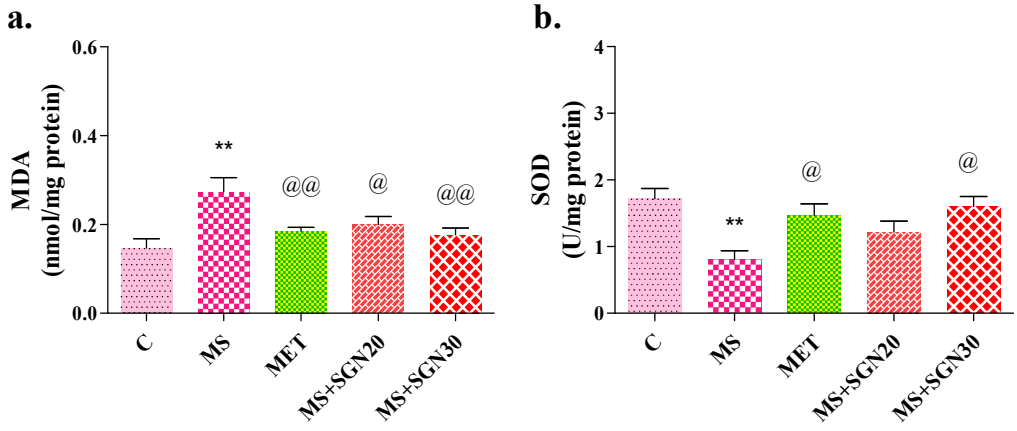


Figure 5.19: The effect of chronic sitagliptin treatment on WAT and BAT weight. Results depicted as Mean ± SEM (n=6). *** indicates p<0.001 against C; ** indicates p<0.01 against C; and * indicates p<0.05 against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.3.2 Effect of Sitagliptin on oxidative stress in eWAT in metabolically compromised obese mice

Fig. 5.20a-c represents the levels of MDA, SOD, and GSH in eWAT at the end of the experimental protocol. Sixteen weeks of the HFFW diet significantly increased MDA levels in the MS group (p<0.01) in comparison with the control group. However, in MS+SGN20 (p<0.05), MS+SGN30 (p<0.01), and MET (p<0.01) groups, there was a significant reduction in MDA levels relative to the MS group (**Fig. 5.20a**). SOD levels were decreased significantly in the MS group (p<0.01) relative to the control group. But, after respective treatments, an increase in SOD levels was observed in MS+SGN30 and MET groups (p<0.05; for both) relative to the MS group (**Fig. 5.20b)**. Also, there was a significant reduction in GSH levels in the MS group (p<0.05), relative to the control group. However, it was found to be increased significantly in MS+SGN30 and MET groups (p<0.05; for both) relative to the MS group (**Fig. 5.20c**). There was no significant improvement in the oxidative stress in eWAT at the lower dose of sitagliptin.



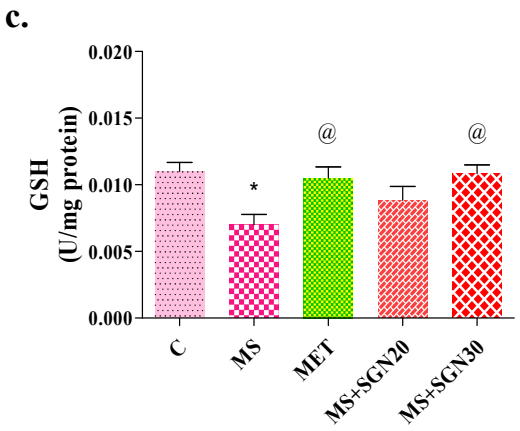


Figure 5.20: The effect of chronic sitagliptin treatment on oxidative stress in eWAT a) MDA; b) SOD; and c) GSH levels. Results depicted as Mean ± SEM (n=6). ** indicates $p<0.01$ against C; * indicates $p<0.05$ against C; @@ indicates $p<0.01$ against MS; and @ indicates $p<0.05$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.3.3 Effect of Sitagliptin on oxidative stress in iBAT in metabolically compromised obese mice

MDA, SOD, and GSH levels were estimated at the end of the experimental protocol in iBAT and are represented in **Fig. 5.21a-c**. There was a significant increase in the lipid peroxidation as determined with the MDA content in the MS group ($p<0.01$), with respect to the control group. The level of both enzymatic antioxidants were significantly decreased in the MS group, SOD ($p<0.001$), and GSH ($p<0.01$), relative to the control group. However, in treatment groups, MDA levels were reduced significantly in MS+SGN30 and MET groups ($p<0.01$; for both), relative to the MS group (**Fig. 5.21a**). In contrast to this, SOD and GSH levels were reversed significantly in MS+SGN30 and MET groups ($p<0.05$; for both), relative to the MS group (**Fig. 5.21b** and **Fig. 5.21c** respectively). The effects observed with sitagliptin were

equivalent to metformin. However, no significant effect was observed in MS+SGN10 and MS+SGN20 groups.

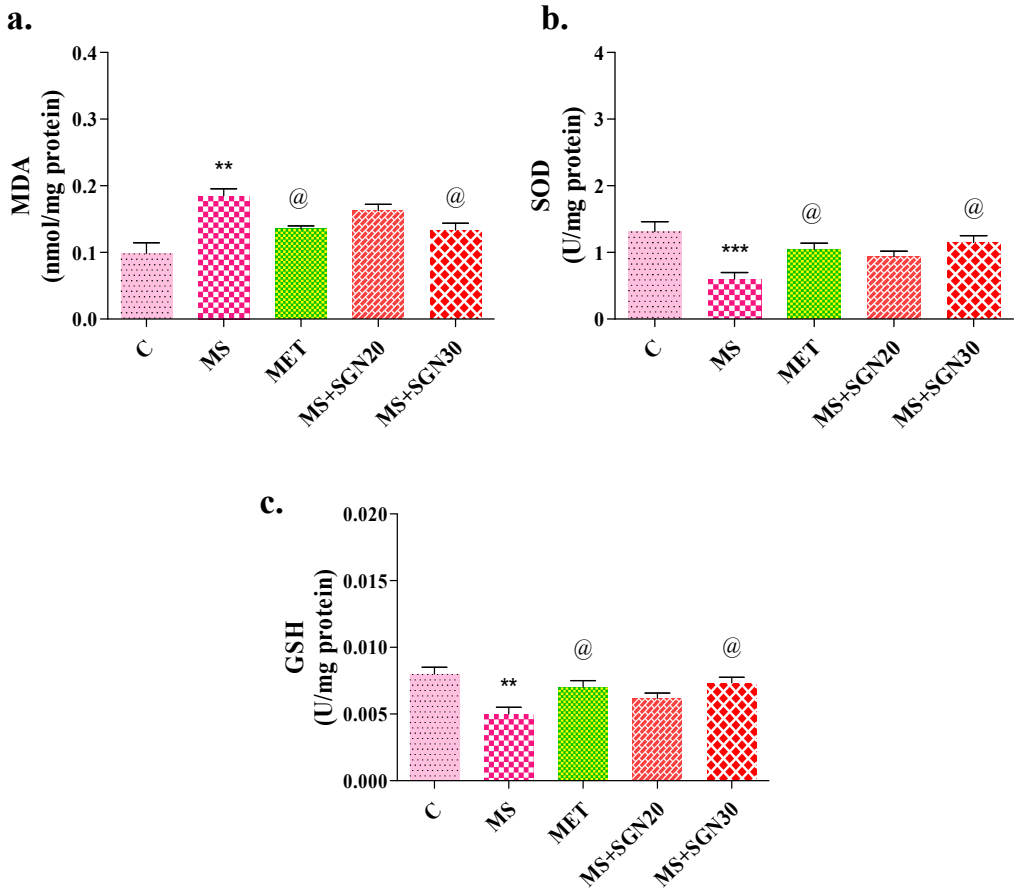


Figure 5.21: The effect of chronic sitagliptin treatment on oxidative stress in iBAT a) MDA; b) SOD; and c) GSH levels. Results depicted as Mean ± SEM (n=6). ### indicates $p<0.001$ against C; ## indicates $p<0.01$ against C; ** indicates $p<0.01$ against MS; and * indicates $p<0.05$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.3.4 iBAT histopathological evaluation

Fig. 5.22 represents the histopathological evaluation of iBAT in mice at the end of sixteen weeks of the experimental protocol. In the MS group, increased lipid droplets were observed as compared to the control group. However, in MS+SGN30 and MET groups, a reduction was found in the lipid droplets.

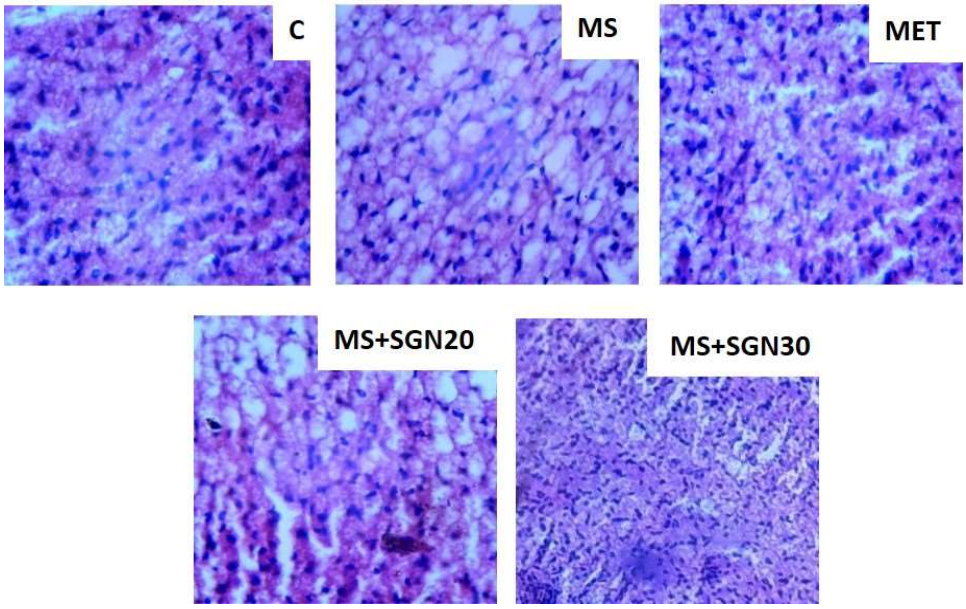


Figure 5.22: Histopathological evaluation of the iBAT section by H & E staining. There is a decrease in the lipid droplets in MS+SGN30 group. Scale bar = 200 μm.

5.3.5 Effect of Sitagliptin on eWAT P-AMPK levels in metabolically compromised obese mice

Fig. 5.23a represents the P-AMPK protein expression and **Fig. 5.23b** depicts the quantification data of protein levels in Swiss albino mice, after sixteen weeks of HFFW diet and eight weeks of treatment. The P-AMPK/β-actin relative expression ratio was found to be decreased in the MS group ($p<0.05$) in comparison to the control group (**Fig. 5.23b)**. However, interestingly we observed an increase in P-AMPK/β-actin

relative expression ratio in MS+SGN30 (p<0.05) and MET group (p<0.01) relative to the control group. There was no significant change in the P-AMPK relative protein levels at the lower doses of sitagliptin.

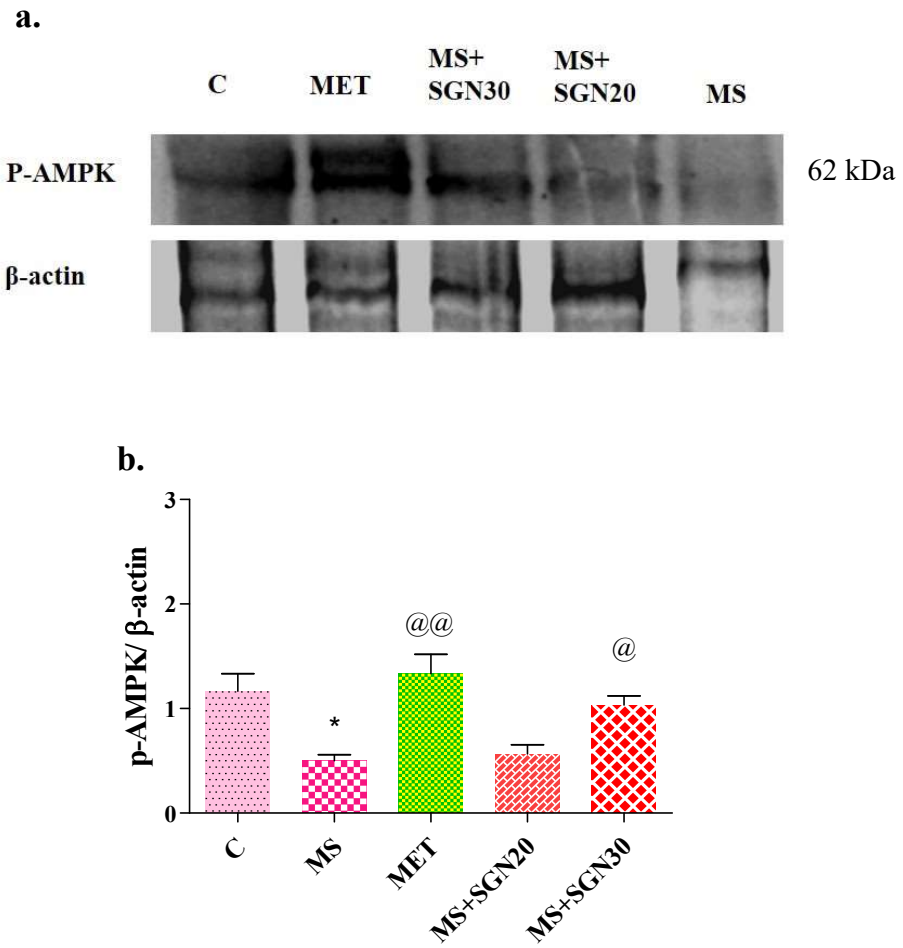


Figure 5.23: The effect of chronic sitagliptin treatment on a) Protein expression of P-AMPK; and b) relative protein expression of P-AMPK with respect to β-actin in eWAT. Results depicted as Mean ± SEM (n=6). *** indicates p<0.001 against C; @@ indicates p<0.01 against MS; and @ indicates p<0.05 against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.3.6 Effect of Sitagliptin on iBAT P-AMPK levels in metabolically compromised obese mice

Fig. 5.24a and Fig. 5.24b represents the P-AMPK protein expression and P-AMPK/β-actin relative levels in iBAT of mice, respectively. A marked decrease was observed in the relative expression level of P-AMPK/β-actin in the MS group (p<0.001) relative to the control group (**Fig. 5.24b)**. However, in the treatment groups, there was an increase in the P-AMPK/β-actin relative expression ratio in MS+SGN30 and MET group (p<0.01; for both) relative to the MS group. There was no significant change in the P-AMPK relative protein levels at the lower doses of sitagliptin.

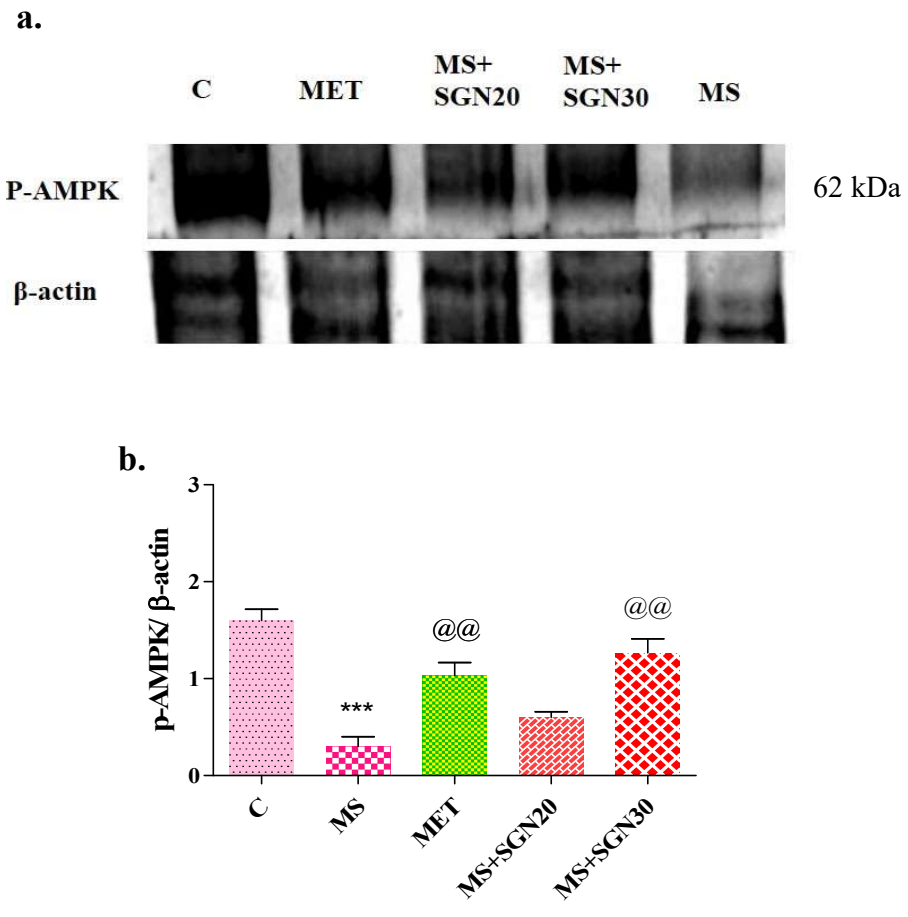


Figure 5.24: The effect of chronic sitagliptin treatment on a) Protein expression of P-AMPK; and b) relative protein expression of P-AMPK with respect to β-actin in iBAT. Results depicted as Mean ± SEM (n=6). *** indicates $p<0.001$ against C; @@ indicates $p<0.01$ against MS; and @ indicates $p<0.05$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.3.7 Effect of Sitagliptin on mRNA expression of thermogenesis and mitochondrial biogenesis markers in eWAT in metabolically compromised obese mice

The mRNA expression levels of thermogenesis and mitochondrial biogenesis markers in eWAT with relative to the standard β-actin is represented in **Fig. 5.25**. After sixteen weeks of HFFW diet, a significant decrease was observed in the mRNA levels by fold change of 0.3 for PPARα, 0.4 for PGC-1α, 0.4 for NRF-1, 0.4 for TFAM, and 0.5 for UCP-1 in the MS group relative to the control group ($p<0.05$; for all). However, sitagliptin considerably augmented the mRNA expression levels of PPARα by fold change of 2.1 ($p<0.05$), PGC1-α by 1.8 ($p<0.05$), NRF-1 by 1.8 ($p<0.05$), and TFAM by 2 ($p<0.01$) in MS+SGN30 with respect to the MS group. But we did not find any increase in UCP-1 levels in the treatment groups. The lower doses of sitagliptin did not improve the thermogenic and mitochondrial biogenesis markers in the treatment groups.

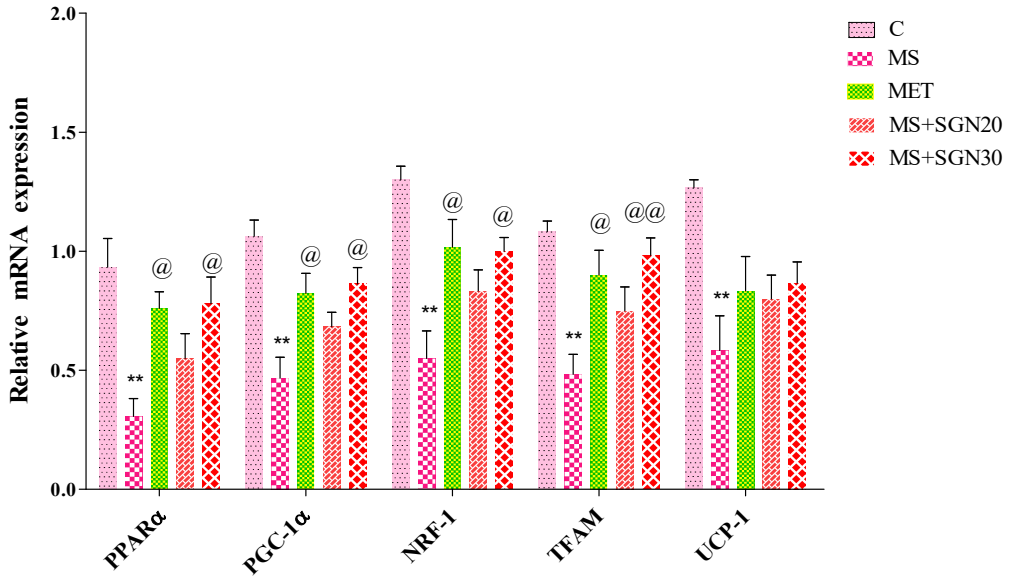


Figure 5.25: The effect of chronic sitagliptin treatment on relative mRNA levels of PPAR1α, PGC-1α, NRF-1, TFAM, and UCP-1 in eWAT with respect to β-actin. Results depicted as Mean ± SEM (n=6). ** indicates $p<0.01$ against C; @@ indicates $p<0.01$ against MS; and @ indicates $p<0.05$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.

5.3.8 Effect of Sitagliptin on mRNA expression of thermogenesis and mitochondrial biogenesis markers in iBAT in metabolically compromised obese mice

Fig. 5.26 represents the mRNA expression of thermogenic and mitochondrial biogenesis markers in iBAT. There was a significant decrease in their levels by a fold change of 0.4 for PPARα, 0.5 for PGC-1α, 0.4 for NRF-1, 0.5 for TFAM, and 0.4 for UCP-1 in MS group ($p<0.01$; for all) relative to C group. However, in the treatment groups, we observed a significant increase in the expression of these markers by a fold change of 2.1 for PPARα, 1.7 for PGC-1α, 2 for NRF-1, 1.9 for TFAM, and 2 for UCP-1 in MS+SGN30 group with respect to MS group ($p<0.05$; for all), similar to

metformin. There was no significant change in the thermogenic and mitochondrial biogenesis markers in the treatment groups at the lower doses of sitagliptin.

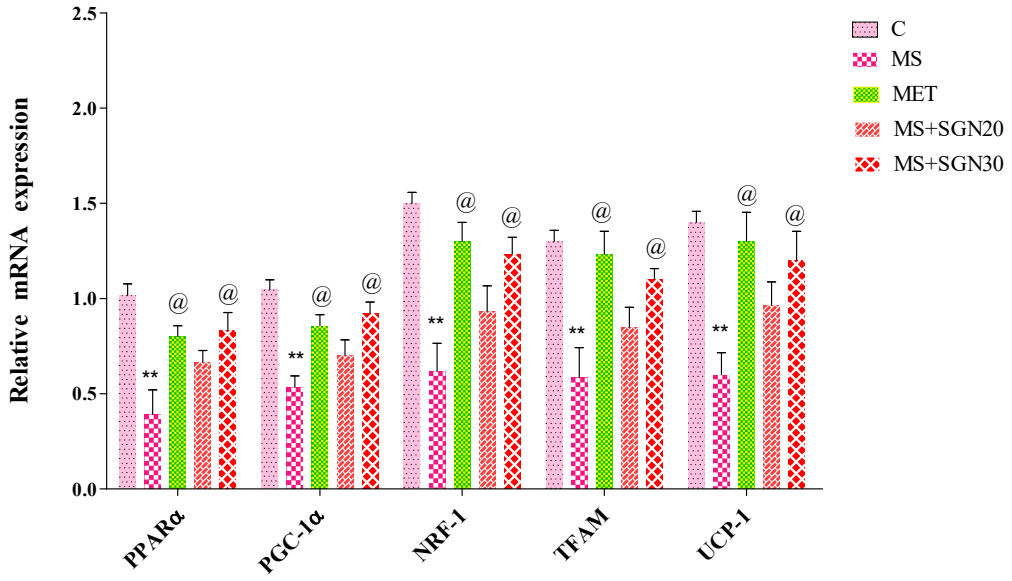


Figure 5.26: The effect of chronic sitagliptin treatment on relative mRNA levels of PPAR1α, PGC-1α, NRF-1, TFAM, and UCP-1 in iBAT with respect to β-actin. Results depicted as Mean ± SEM (n=6). ** indicates $p<0.01$ against C; @ indicates $p<0.05$ against MS. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to find statistical significance among the groups.