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Research Article

Combined effects of metformin and honey on lipid metabolism in diet-induced hyperlipidemic rats

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hyperlipidemia, Metformin, Honey, Lipid profile, Fasting blood glucose

ABSTRACT
The study was designed to evaluate the combined effect of metformin and honey treatments on lipid metabolism in diet-induced hyperlipidemic rats. Eighty-five male Wistar rats (80-100g): twenty normal and sixty-five hyperlipidemic rats were used. Sixty-five rats were fed with high fat diet for 30 days to induce hyperlipidemia. Five rats each from control and hyperlipidemic were randomly selected to evaluate fasting blood glucose (FBG) level and hyperlipidemic state. Fifteen normal rats (control) formed Group 1(received 0.3ml/100g b.w distilled water) and the hyperlipidemic rats were divided into 4 groups of fifteen rats. Group 2 (hyperlipidemic control), Group 3 (hyperlipidemic plus metformin-100mg/kg), Group 4 (hyperlipidemic plus honey-1ml/100g); Group 5 (hyperlipidemic plus metformin-100mg/kg) and honey(1ml/100g). All treatments were administered orally, once daily for 28 days and animals were weighed weekly throughout the study. Blood was obtained from 5 rats in each group weekly post treatments through retro-orbital plexus, FBG was assessed using glucometer(Acucheck) and serum from remaining blood sample was used for assessing total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), spectrophotometrically using Agape Biochemical kits. Low density lipoprotein (LDL), Very low density lipoprotein (VLDL), atherogenic index and HDL/LDL ratio were mathematically calculated. Histology of the liver was done using H&E stain. The FBG was significantly reduced in all treatment groups (3, 4 and 5) compared with Groups 1 and 2 (the controls). Group 5 animals showed significant reduction in TC, TG, LDL, VLDL and significant increase in HDL level by day 28 of treatment compared with controls. Histopathological results showed severe diffuse fatty infiltration of the hepatocytes and extensive periportal vacuolar degeneration of hepatocytes at day 28 in hyperlipidemic group while group 5 showed very mild diffuse periportal cellular infiltration of the hepatic parenchyma with no visible lesion. It was concluded that the combination of both metformin and honey treatments significantly reduced blood glucose level, serum total cholesterol, triglycerides, low density lipoprotein and fatty infiltration of hepatic parenchyma and increase high density lipoprotein level which was not achieved with administration of either metformin or honey alone in hyperlipidemic rats.

INTRODUCTION

Cardiovascular disease remains the chief cause of death in most countries of the world, and atherosclerosis, the principal cause of myocardial infarction, accounts for the majority of these deaths (Yokozawa *et al*, 2006). Hyperlipidemia is characterized by elevated cholesterol and fatty substances in the blood, and this is one important factor associated with atherosclerosis, a major risk factor for Coronary heart disease (Lecerf & de Lorgeril 2011). Hyperlipidemia is therefore a cause of concern for health professionals, being one of the major risk factors for the development of cardiovascular diseases (Gomes *et al*, 1998; Gerhardt and Gallo, 2008). Hyperlipidemia induces vascular functional changes that may leads to local ischemia and vascular remodeling (Bentley *et al*, 2002). Clinical trials show that lowering lipid level reduces the morbidity and mortality associated with

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cardiovascular complication, thus regulating the serum cholesterol level helps to prevent atherosclerosis (Amundsen *et al*, 2002).

Metformin is an oral anti diabetic drug belonging to the class of biguanide. It is a drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people characterized by hypercholesterolemia-inducing insulin resistance (Lord *et al*, 2003). Elevated levels of cholesterol and free fatty acid commonly seen in hyperlipidemic patients usually result into the development of insulin resistance (Clore, 1991). Insulin resistance with the ensuing hyperinsulinemia is associated with hypertriglyceridemia and low serum high-density lipoprotein (HDL) cholesterol concentrations.

The fact that antioxidants have several preventive effects against different diseases, such as cancer, coronary diseases, inflammatory disorders, neurological degeneration, and aging, led to the search for food rich in antioxidants. Available evidence indicates that individuals with chronic or degenerative diseases are more susceptible to oxidative stress and damage because they have elevated levels of oxidants and/or reduced antioxidants (Kandebach, 2009, Ienco *et al,* 2011).

Honey, a natural product formed from flower nectar by honeybees, has been reported to be beneficial in management of altered lipid states (Nemoseck *et al*, 2011). Honey is a commonly consumed product in most households (Bogdanov, 2008). Administration of honey alone or in combination with conventional therapy could therefore produce better therapeutic benefits in the management of chronic heart diseases, atherosclerosis and hyperlipidemia which are commonly associated with oxidative stress. In view of the dearth of information on the combined use of metformin and honey in hyperlipidemic state, this study was designed to evaluate the combined effect of metformin and honey treatments on lipid metabolism in diet-induced hyperlipidemic rats.

Materials and Methods

A total of eighty five male Wister rats were used in the study. Hyperlipidemia was induced in sixty-five (65) rats (80-100g) with continuous high fat diet for 30days (Sheyla *et al,* 2005; Vikram and Arindum, 2011; Nyangono *et al*, 2012) with free access to water while 20 normal rats were fed normal rat chow and watered ad libitum. The high-fat diet was composed based on the method of Blank *et al* (1963), adapted by Sheyla *et al,* (2005) and *Dhaval et al*, (2011) with little modification. The composition of this diet is shown in Table 1. Proximate analysis of the experimental diet shows it contained Gross Energy of 3,967kcal/kg, crude protein of 6.47g%, 16.79g% of fat, 3.83g% crude fibre and ash 7.03g%. After 30days of feeding the animals with high fat diet, five rats each from control and hyperlipidemic groups were randomly selected and sacrificed for assessment of Fasting Blood Glucose and Hyperlipidemic state.

Fifteen normal rats, fed normal rat chow with free access to water served as control. In addition, it was daily given 0.3ml distilled water orally. The remaining hyperlipidemic rats (60) were classified into group 2 (hyperlipidemia control), group 3 - (hyperlipidemia treated with metformin (100mg/kg b.w) (Young *et al*, 2008), Group 4 (hyperlipidemia treated with honey (1ml/100g b.w) (Asiyah *et al*, 2011). Group 5 (hyperlipidemia treated with both metformin (100mg/kg b.w) and honey (1ml/100g b.w). Feeding with high fat diet was continued throughout the experiment.

All treatments were administered orally using orogastric tube once daily for 28 days. The weights of all animals were monitored weekly throughout the experiment. Blood samples were obtained weekly, post treatments through the retro-orbital sinus. The FBG was assessed using glucometer and remaining blood was allowed to coagulate. The serum obtained was centrifuged at 3000 rpm for 15mins with a bench top centrifuge to obtain clear serum and used for assessing total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) were estimated spectrophotometrically using Agape Biochemical kits (Young 2001, Akpanabiatu *et al*, 2005). Low density lipoprotein (LDL), Very low density lipoprotein (VLDL), atherogenic index and HDL/LDL ï Cholesterol ratio were mathematically calculated (Friedewald, 1972). Animals were sacrificed by cervical dislocation to harvest the livers for histological examinations using H&E staining techniques (Banchroft *et al*, 1996).

Statistical analysis:

Data obtained were expressed as Mean ± SEM and analyzed using ANOVA. Statistical level of

Table 1: Composition of experimental diet

INGREDIENT	Kg
Maize	40.00
Groundnut cake	13.60
Cornstarch	14.00
Glucose	21.00
Palm Kernel Oil	10.00
Bone meal	1.00
Lysine	0.10
Methionine	0.10
Salt	0.20

significance was chosen at $p < 0.05$ and $p< 0.01$ (Steel and Torrie, 1980).

Results

The composition of this diet is shown in Table 1. Proximate analysis of the experimental diet shows it contained Gross Energy of 3,967kcal/kg, crude protein of 6.47g%, 16.79g% of fat, 3.83g% crude fibre and ash 7.03g%. Animals were fed for 30days to induce hyperlipidemia and their weights monitored weekly (Sheyla *et al,* 2005; Vikram and Arindum, 2011; Nyangono *et al*, 2012).

Table 2: Effects of metformin and honey on body weight (g) in diet-induced hyperlipidemic rats

Body weight (grams)	Treatment days post hyperlipidemia	Group 1 (Control)	Group 2 (Metformin)	Group 3 (Honey)	Group 4 (Metformin+Honey)
	0	69.30±3.80	69.30±3.80	69.30±3.80	69.30±3.80
	7	70.80±6.03	69.20±1.49	74.80±8.09	61.20±9.44
	14	73.20±2.52	66.60±2.84	66.00±7.64	52.40±5.97*
	28	73.80±5.54	66.20±4.62	70.80±5.83	52.40±2.84*

*Significantly different from control, $p< 0.05$

Table 3: Effects of metformin and honey on fasting blood glucose in diet-induced hyperlipidemic rats

Fasting blood glucose (mg/dl)	Treatment days post hyperlipidemia	Group 1 (Control)	Group 2 (Metformin)	Group 3 (Honey)	Group 4 (Metformin + Honey)
	0	117.33±6.02	117.33±6.02	117.33±6.02	117.33±6.02
	7	122.40±4.79	103.60±9.59	104.00±6.63	84.60±3.26*
	14	118.20±5.98	100.00±3.56*	93.60±6.56*	78.60±6.92**
	28	120.20±4.84	97.20±1.53*	92.80±6.44*	71.00±1.70**

Significantly different from control, *$p< 0.05$; **$p<0.0$

Table 4: Effects of metformin and honey on lipid profiles in diet-induced hyperlipidemic rats

Parameter	Treatment days post hyperlipidemia	Group 1 (Control)	Group 2 (Metformin)	Group 3 (Honey)	Group 4 (Metformin + Honey)
Total cholesterol (mg/dl)	0	171.28±11.10	171.28±11.10	171.28±11.10	171.28±11.10
	7	157.62±15.70	132.40±7.03	169.02±11.80	134.87±12.91
	14	163.18±21.30	136.49±12.60	148.35±13.60	130.47±11.89
	28	183.13±16.40	112.62±9.99**	131.39±15.60	111.99±17.40*
Triglycerides (mg/dl))	0	117.90±17.20	117.90±17.20	117.90±17.20	117.90±17.20
	7	142.30± 9.45	121.88±6.28	135.69±12.70	109.23±12.75
	14	189.45±14.7	97.99±4.29*	110.53±12.78*	92.53± 6.38**
	28	194.02±22.1	83.68±7.54*	79.41±4.07*	66.11±1.61**
HDL-CHOL (mg/dl	0	33.03±4.40	33.03±4.40	33.03±4.40	33.03±4.40
	7	26.04±3.48	33.28±2.61	28.41±4.38	33.87±3.35
	14	24.59±2.47	34.94±4.55	34.28±3.68	47.25±3.99*
	28	20.34±2.80	38.45±2.67**	37.26±5.19*	56.35±6.67**
LDL-CHOL (mg/dl)	0	114.67±9.49	114.67±9.49	114.67±9.49	114.67±9.49
	7	103.11±19.42	74.74±8.79	113.47±14.45	79.15±15.09
	14	100.69±24.55	82.15±16.93	92.96±11.84	64.71±14.58*
	28	123.98±19.59	57.43± 8.87*	78.25±16.44	42.42±17.92*
VLDL-CHOL (mg/dl)	0	23.58±3.43	23.58±3.43	23.58±3.43	23.58±3.43
	7	28.47±1.89	24.38±1.26	27.14±2.54	21.85±2.55
	14	37.89±2.95	19.39±0.89*	22.11±2.56*	18.51±1.28*
	28	38.80±4.42	16.74±1.51*	15.88±0.81*	13.22±0.32**

Significantly different from control, $^{*}p< 0.05$; $^{**}p<0.01$

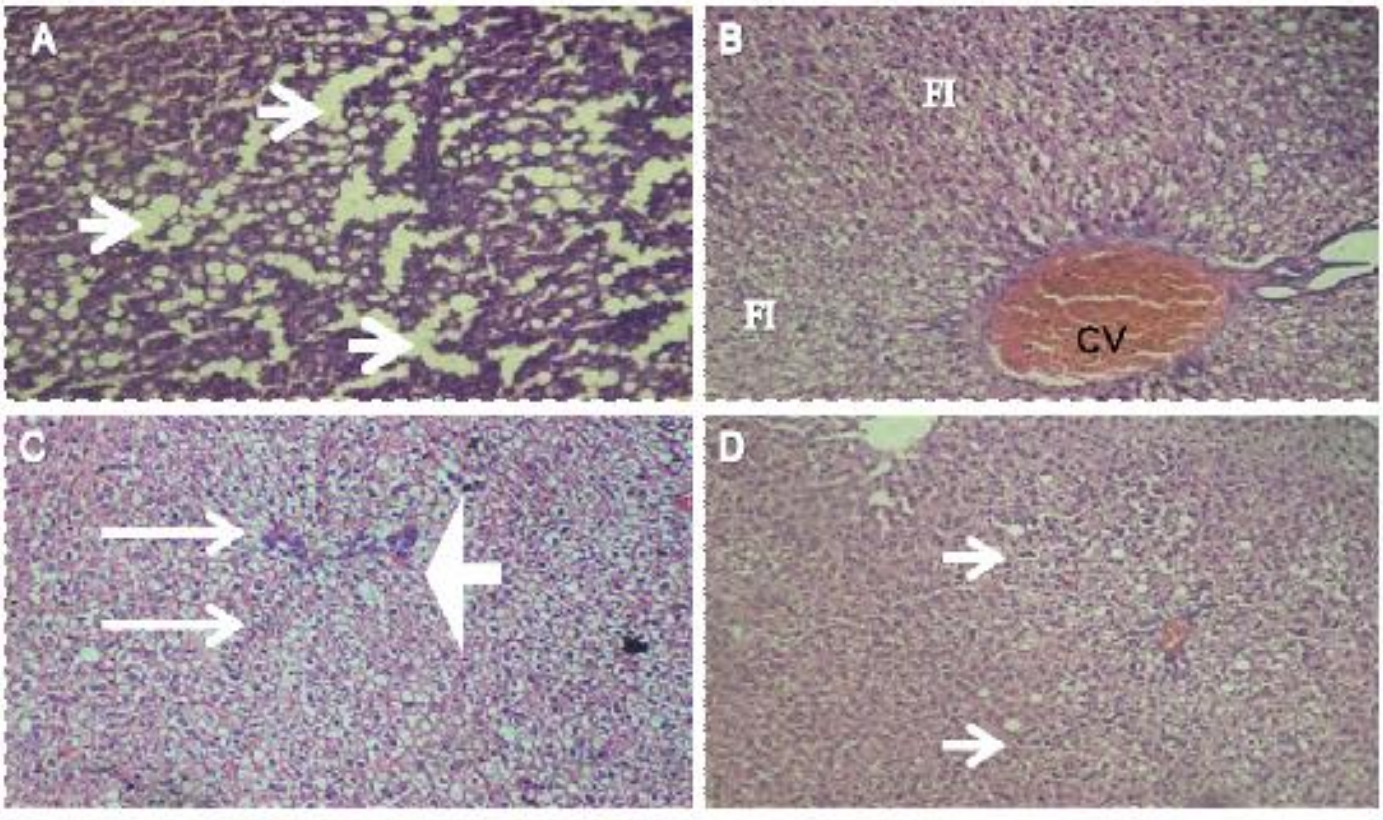


Fig. 1: Histopathological results of diet-induced hyperlipidemic rats

1A. Photomicrograph of the Liver histology (H & E Staining) of hyperlipidemic rats after being fed with high calorie diet for 30 days, showing severe portal congestion and diffuse fatty infiltration of the hepatic parenchyma (white arrows) (x 200).

1B. Photomicrograph of the Liver histology (H & E Staining) of rat after 14days administration of 100mg/kg body weight of Metformin, showing diffuse mild fatty infiltration (**FI**) of the hepatic parenchyma, the congested Central Vein (**CV**) is also visible (x100)

1C. Photomicrograph of the Liver histology (Hematoxylin & Eosin Staining) of Honey treated hyperlipidemic rat at 14th day of 1ml/100g body weight of honey administration, showing severe diffused fatty infiltration of the hepatic parenchyma and vacuolar degeneration (white arrows). The Portal Canal (thick white arrow) is also visible (x100).

1D. Photomicrograph of the Liver Histology (Hematoxylin & Eosin Staining) of rat treated with Metformin in adjunct with Honey for 14days, showing mild portal congestion and periportal cellular infiltration by mononuclear cells (white arrows) (x100).

Table 5: Effects of metformin and honey on lipid indices in diet- induced hyperlipidemic rats

Parameters	Treatment days post hyperlipidemia	Group 1 (Control)	Group 2 (Metformin)	Group 3 (Honey)	Group 4 (Metformin + Honey)
Atherogenic index	0	27.47±4.05	27.47±4.05	27.47±4.05	27.47±4.05
	7	33.05±1.80	26.74±1.29	31.81±2.56	24.39±2.73
	14	42.22±1.93	22.15±1.12*	24.93±2.91*	19.98±0.99*
	28	45.66±4.24	18.21±1.47*	18.27±0.99*	14.09±0.55**
HDL/LDL-ratio	0	0.31±0.05	0.31±0.05	0.31±0.05	0.31±0.05
	7	0.34±0.14	0.49±0.09	0.29±0.08	0.57±0.19
	14	0.33±0.10	0.66±0.32	0.39±0.07	1.02±0.34
	28	0.20±0.07	0.73±0.09*	0.63±0.20	2.87±0.40*

Significantly different from control, $^{*}p< 0.05$; $^{**}p<0.01$

In table 2, hyperlipidemic control group showed significant ($p< 0.05$) increase in body weight compared to normal control, while hyperlipidemic Metformin and Honey treated group showed significant ($p< 0.05$) decrease in body weight between the 14^{th} and the 28^{th} days of treatment, compared to hyperlipidemic control and normal.

Figure 1 shows significant ($p<0.05$) decrease in fasting blood glucose levels in Metformin alone and Honey alone treated groups, while there was significant decrease (<0.01) in the Metformin and Honey combined treated group.

Figure 2 shows significant decrease ($P<0.01$) in serum total cholesterol in Group 3 and ($P<0.05$) decrease in group 5 compared to group 2, the hyperlipidemic untreated. Figure 3 shows significant ($P<0.05$) decrease in serum triglycerides in groups 3&4 and ($P<0.01$) decrease in group 5 when compared to group 2, the hyperlipidemic untreated, but similar to group1, the normal control.

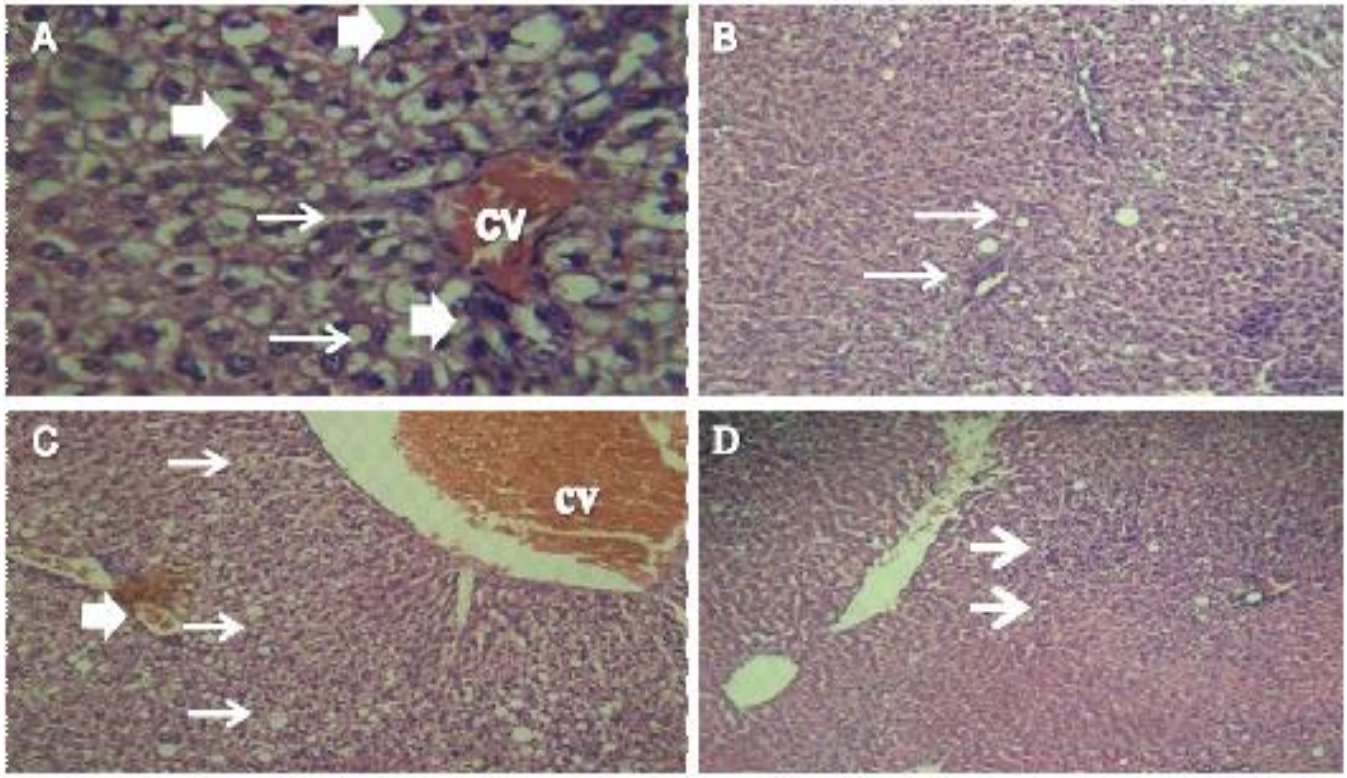
Figure 4 shows significant increase ($P<0.01$) in serum HDL-Cholesterol in groups 3&5 when compared to group 2. While group 4 showed significant ($P<0.05$) increase in serum Hdl when compared to group 2, more than the normal control group.

Figure 5 shows significant ($P<0.01$) reduction in VLDL-C in group 5 compared to group 2, and this value is comparable to normal.

Table 3 shows significant ($P<0.05$) decrease in LDL-Cholesterol in groups 3&5 compared to group 2, and these values are comparable to the normal group.

Table 4 shows significant ($P<0.01$) reduction in Atherogenic index in group 5 compared to group 2.

Table 5 shows significant ($P<0.05$) increase in HDL/LDL- ratio in groups 3&5, compared to group 1&2.



2A. Photomicrograph of the Liver Histology (H & E Staining) of rat in the negative control group, without any form of treatment after 28days, showing very severe diffuse fatty infiltration of the hepatocytes (thick white arrows) and extensive periportal vacuolar degeneration of hepatocytes (white arrows). Severely congested Central vein is also visible (CV) (x 400).

2B. Photomicrograph of the Liver Histology (H & E Staining) of rat treated with 100mg/kg body weight Metformin for 28days, showing mild portal congestion (white arrows) with no visible lesion (x 100).

2C. Photomicrograph of the Liver Histology (H & E Staining) of rat treated with 1ml/100g body weight Honey for 28days, showing mild diffused periportal fatty infiltration (white arrows) of the hepatic parenchyma and severe congestion of the Centre vein (CV) .The portal canal (thick white arrow) is also visible (x 100).

2D. Photomicrograph of the Liver Histology (H & E Staining) of rat treated with 100mg/kg body weight of Metformin in adjunct with 1ml/100g of Honey for 28days, showing very mild diffuse periportal cellular infiltration (white arrows) with no visible lesion (x 100).

Plate 1 shows the photomicrographs of the liver histology on day7 post-treatments. Severe portal congestion and diffuse fatty infiltration of hepatic parenchyma were observed in hyperlipidemic untreated group, compared to control which has normal hepatocyte architecture, while Metformin and Honey combined treated group shows mild diffuse fatty infiltration of the hepatic parenchyma.

Plate 2 shows the photomicrographs of the liver histology on day14 post-treatments. Severe periportal vacuolar degeneration was seen in the hyperlipidemic untreated rat, whereas mild fatty infiltration and periportal cellular infiltration by mononuclear cells were seen in the Metformin and Metformin with Honey combined treated group.

Plate 3 shows the photomicrographs of the liver histology on day28 post-treatments. The fatty infiltration of the hepatocytes seen in the hyperlipidemic untreated rat was ameliorated in hyperlipidemic rats treated individually with metformin

and honey and almost completely ameliorated with combined metformin and honey at day 28 post-treatments.

Discussion

Increased body weight observed in the hyperlipidemic group could be attributed to increased fat deposition from the fat-rich diet (Zulet and Martinez, 1995, Woo and Henry, 1996, Ramachandran *et al*, 2003). Reduced body weight observed in the treated groups compared with controls showed that treatment of hyperlipidemic rats with metformin or honey and combination of both reduced weight gain. These observations revealed that the treatment controlled excessive weight gain in dyslipidemia and are in agreement with the use of various supplements for weight control (Millgate and Robert, 1995, Cho *et al*, 2005).

The elevated level of blood glucose observed in the hyperlipidemic untreated (negative control) may be due to increased insulin resistance which could have developed as a result of increased ingestion of fatty food substances by the experimental rats. Previous reports have shown that elevated levels of cholesterol and free fatty acid commonly seen in hyperlipidemic patients usually result in development of insulin resistance (Clore, 1991, Wellen *et al*, 2005). One of the mechanisms by which hyperlipidemia results into insulin resistance may be through the action of the saturated fatty acids that impairs the transduction of the insulin signaling pathway hence affecting the metabolism of glucose by insulin, resulting largely in increased resistance (Wellen *et al*, 2005).

The hypoglycemic activities of metformin could be a result of its ability to reduce free fatty acid oxidation. Increased fatty acid oxidation inhibits key enzymes of the glycolytic pathway by accumulation of by-products of free fatty acid oxidation (Hundal *et al* 1992). Results indicate that combination of metformin with honey further reduced the elevated blood glucose level not achieved with administration of either metformin or honey alone.

Studies have been done to evaluate the hypercholesterolemic effect of high cholesterol and high-fat diet in experimental animals (Edijala *et al,* 2005; Fki *et al*, 2005; Hartvigsen *et al,* 2007; Gopichanchinta *et al,* 2009; Nyangono *et al,* 2012). The increased blood levels of total cholesterol, Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C) and triglyceride-carrying lipoproteins as well as lowered levels of High Density Lipoprotein Cholesterol (HDLC) has been identified in development of hyperlipidemia (Ross, 1999). This study showed that adjunct use of metformin with honey lowers the risks of Coronary Heart Disease (CHD) development by reducing the elevated levels of serum total cholesterol, triglycerides and LDL ï Cholesterol level while it increases HDL-Cholesterol level. The positive effect of the combined therapy was confirmed by the increased atherogenic index (Hawkins and Ounpuu, 2004). Histopathological studies of the liver confirmed the biochemical results obtained (Figures 2,3&4).

In conclusion, this study showed that combination of metformin and honey treatments reduce elevated blood glucose level, serum total cholesterol, triglycerides, Low Density Lipoprotein and fatty infiltration of hepatic parenchyma, while it increased the High Density Lipoprotein level not achieved with administration of either metformin or honey alone in hyperlipidemic rats.

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