Received: 12 May 2023, Accepted: 25 May 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 6, 990-996

Effects of Oral Administration of Atorvastatin or Fenofibrate on Hyperlipidemia Induced by Betamethasone Dipropionate Injection in Rabbits

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Abstract

Betamethasone, a fluorinated and synthetic steroid, is a commonly used glucocorticoid. To our knowledge, no available studies exist concerning the hyperlipidemic effect of betamethasone dipropionate (BDP) in rabbits. Therefore, the current study was conducted to highlight the effects of intramuscular injection of BDP on lipid profile in rabbits, investigate the possible mechanism underlying the produced effects and evaluate the possible antihyperlipidemic effect of atorvastatin (ATR) and fenofibrate (FFB). For this purpose, twenty male New Zealand rabbits were classified into control, BDP (0.5 ml/kg B.wt/ IM/day/single dose), BDP+ATR; rabbits were IM injected with BDP, then they were orally given ATR (1.9 mg./kg. B.wt./ once/ day/ month) and group IV (BDP+FFB); rabbits were IM injected with BDP then they were orally given FFB (7.5 mg/kg B.wt/ once/ day/ month). The obtained result revealed that single IM injection of BDP produced a significant elevation in triglycerides, total cholesterol, LDL level with a significant decline in HDL in comparison to control group on the 3rd,7th,14th, 21st, 30th day of the experiment. On the 30th day of the experiment there was an increase in the ALT, AST, MDA, VCAM-1 as well as a significant decrease in TAC. Furthermore, BDP induced a significant increase in HMG-COA reductase gene expression and a significant decrease in lipoprotein lipase gene expression. Oral administration of ATR or FFB concurrently with BDP for a month succeeded in reducing the hyperlipidemia induced by BDP in rabbits.

KEYWORDS
Hyperlipidemia, Cardiovascular diseases, Betamethasone dipropionate, Triglycerides, Cholesterol, low-density lipoproteins.

INTRODUCTION

Dyslipidemia is any disorder in lipid level within the body. Hyperlipidemia is various genetic and acquired disorders that describe elevated lipid levels within the body, and it is the most common form of dyslipidemia (Hill and Bordoni 2021). By 2023, In major nations, the quantity of patients suffering with dyslipidemia is predicted to increase each year till reach 78 million. (Bian *et al*., 2019). Hyperlipidemia mainly occurs due to improper fat metabolism in the body accompanied by high triglycerides (TG), high plasma cholesterol, high, low density lipoprotein levels in blood. There are many metabolic diseases associated with hyperlipidemia such as fatty liver, type 2 diabetes, hypertension and atherosclerosis (Wang *et al*., 2017). Hyperlipidemia also represents the main cause that lead to atherosclerosis with cardiovascular complication and certain endothelial dysfunction are caused by sustained and prolonged hyperlipidemia (Johnston *et al*., 2017; Hewage *et al*., 2018). Hyperlipidemia can be classified into two main types primary one which is generally inherited due to genetic anomalies and secondary one which developed as a result of predisposing factors like obesity, chronic renal failure, drugs (B-blockers), alcoholism, hypothyroidism and thyroid dysfunction. (Nouh *et al*., 2019; Naser *et al*., 2021).

Although glucocorticoids are vital for maintaining lipid homeostasis, too much of them can raise free fatty acids blood levels and cause lipid buildup in the skeletal muscle and hepatic tissue, both of which are linked to resistance of insulin (Dourakis *et al*., 2002; Petersen and Shulman 2006; Samuel *et al*., 2010). Glucocorticoids stimulate adipocyte differentiation in human adipose tissue, increasing resistance of insulin and obesity (Hauner *et al*., 1987; Hauner *et al*., 1989; Gathercole *et al*., 2011). Betamethasone, fluorinated and synthetic steroids, that is a commonly used glucocorticoid that has little adverse effects (Sun and Chu, 2017). Betamethasone helps in the systemic treatment of ophthalmic inflammation, collagen diseases, rheumatic diseases, dermatologic diseases, allergic states, allergic and insufficiency of adrenal cortex and pulmonary diseases. Betamethasone dipropionate (BDP), a dipropionate pro drug, is converted by esterase enzymes to the active metabolite betamethasone 17-monopropionate (B17P), as well as the minor active metabolite betamethasone (BOH, CAS 378-44-). (Wurthwein,1992).

statins)3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors) are the main lipid-lowering drug utilized to prevent coronary heart disease either secondary or even primary. Atorvastatin (ATR) competitively hinder HMG-CoA reductase. Statins can decrease cholesterol synthesis in the hepatic tissue by inhibiting the conversion of HMG-CoA to mevalonate. Also, atorvastatin can increase the number of low density lipoproteins

ISSN: 2090-6277/2090-6269/

(LDL) receptors on the surface of hepatic cells (McIver and Siddique, 2022). For the controlling and treatment of mixed dyslipidemia, primary hypercholesterolemia or hypertriglyceridemia, the FDA has approved the fibric acid derivative fenofibrate (FFB). Fibrates like FFB activate PPAR-alpha, which upregulates lipoprotein lipase, induces the synthesis of high-density lipoprotein (HDL), and lowers the liver's production of apolipoprotein C. Fibrates ultimately improve plasma catabolism and the clearance of triglyceride-rich particles. Fibrates also increase acyl CoA synthetase-mediated fatty acid oxidation, which further suppresses the synthesis of TG. Very-low-density lipoprotein (VLDL) levels and plasma triglyceride are ultimately decreased (McKeage and Keating 2011; Sidhu and Tripp 2022).

To the best of the authors' knowledge, no available studies exist concerning the hyperlipidemic effect of betamethasone dipropionate in rabbits. Therefore, the objective of this current study was to highlight on the effects of intramuscular injection of BDP on lipid profile in rabbits, to investigate the possible mechanism underlying the produced effects and to study the possible antihyperlipidemic effect of lipid-lowering medications with different mechanisms such as atorvastatin and fenofibrate.

MATERIALS AND METHODS

Drugs

Betamethasone dipropionate (Diprofos®) was supplied from Schering Plough, Sedico, Egypt. Atorvastatin (Ator®, 40 mg) was purchased from Epico Co, Egypt. Fenofibrate (Lipanthyl®, Supra 160 mg tablet) was supplied from Abbott, Mina Pharm Co, Egypt. Human dose of Betamethasone dipropionate (1 to 5 ampoule daily (2 to 10 ml), Atorvastatin (40 mg daily for one month) and Fenofibrate (160 mg daily for one month) was converted to a rabbit dose (0.50 ml/kg B.wt, 1.9 mg./.kg B.wt and 7.5 mg./.kg B.wt), respectively according to (Paget and Barnes 1964).

Animals and experimental design

Twenty male New Zealand rabbits weighing, 1 to 1.5 kg, were purchased from Lab Animal Unit at Faculty of Veterinary Medicine, Zagazig University, Egypt. The rabbits were housed under standard conditions (25 degree, forty to sixty percent relative humidity, twelve h light and twelve h dark cycle) and provided standard rabbit diet from local market and tap water applied to rabbits ad libitum. The rabbits were acclimated for two weeks before experimentation. The experiment and rabbit management approved by ZU–IACUC ethical committee of scientific research (ZU– IACUC/2/F/31/2021).

Weighted rabbits were randomly divided into four equal groups (n=5) two weeks after acclimatization as follows:
Group I (control group): Each rabbit in this group received distilled water for a month.
Group II (BDP): Rabbits were injected intramuscularly with a single BDP dose (0.5 ml/kg B.wt).
Group III (BDP+ATR): Rabbits were injected intramuscularly with a single BDP dose, then ATR were given (1.9 mg/kg B.wt) dissolved in distilled water once daily for a month.
Group IV (BDP+FFB): Rabbits were injected intramuscularly with a single BDP dose then they were orally given FFB (7.5 mg/kg B.wt) dissolved in distilled water for once daily a month.

Sampling

Blood samples

Blood samples were drawn from ear vein of each rabbit on 0, 3rd, 7th, 14th, 21st and 30th day of the experiment. Blood was collected from the animal then transferred into a uncontaminated clean centrifuge tube without EDTA then kept at room temperature for ten minutes to form a clot for 10 then serum was collected. serum samples which were prepared on 0, 3rd, 7th, 14th, 21st and 30th day of the experiment were used for lipogram profile determination. To measure other biochemical parameters in serum, total antioxidant capacity (TAC), malondialdehyde (MDA), Cluster of Differentiation 63 (CD-63), vascular cellular adhesion molecule-1 (VCAM-1) and Homeostatic model assessment for insulin resistance (HOMA IR), amount of the serum which prepared on 30th day of the experiment was kept at –20°C.

Tissue sampling

After collection of the blood, animals were euthanized by decapitation. Instantly after decapitation aortas, livers and kidneys were removed, washed with cold normal saline, dried with filter paper and processed for the following analyses. Portions of liver samples were quickly frozen at -80°C for the evaluation of lipoprotein lipase (LPL) and Hydroxymethylglutaryl-coenzyme A reductase (HMG CO reductase) gene expression. For histopathological examination, aorta, kidney and the other portion of liver samples were stored in buffered neutral formalin 10 % concentration.

Analysis of serum fractions

Serum total cholesterol (TC) level (Meiattini *et al*., 1978), serum triglycerides (TG) level (Fossati and Prencipe 1982) and serum high density lipoprotein cholesterol (HDL-C) level (Grove, 1979) were determined using diagnostic kits provided from Spinreact, Co., Spain. Serum low density lipoprotein cholesterol (LDL) level was determined according to (Friedewald *et al*., 1972) from the following formula:
LDL-C (mg/dl) = TC – [HDL-C + TG/5], where TG did not exceed 400 mg/dl.

Serum VCAM-1 was measured using ELISA kit (RayBiotech

Table 1. Primer sequences for the quantitative RT-PCR for the analyzed genes in the hepatic tissue.

Target gene	Primers sequences	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Reference
				Secondary denaturation	Annealing (Optics on)	Extension	
B-actin	CAACACAGTGCTGTCTGGTGG ATCGTACTCCTGCTTGCTGAT						Abdul-Careem Hunter *et al*. (2008)
HMG-CoA reductase	CAGGATGCAGCACAGAATGT CTTTGCATGCTCCTTGAACA	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	55˚C 30 sec.	72˚C 30 sec.	Wu Sarna *et al*. (2013)
Lipoprotein lipase	GCATCTGGGAAG↓GAACTAGGG TGAACCACAAGCATAGCCCA						Davis *et al*. (1991)

Life, Parkway Lane, Peachtree Corners, GA, United States) (Grabmaier *et al*., 2016). According to (Ohkawa *et al*., 1979; Koracevic *et al*., 2001), serum MDA and TAC were estimated, respectively. Serum level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to (Nsiah *et al*., 2011). Serum glucose level was determined according to (Wang *et al*., 2010). Serum insulin level was determined according to (Wadood *et al*., 2007). HOMA IR was determined by equation HOMA-IR = (insulin × glucose) / 405 according to (Majid *et al*., 2017).

Gene expression assay

Using the QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH), RNA may be extracted from liver tissue samples by adding two hundred µl of the sample to six hundred µl RLT buffer that contain ten µl β-mercaptoethanol per one ml, keeping at room temperature for ten min. Following the instructions in the Purification of Total RNA technique of the QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH), one volume of 70% ethanol was added to the cleared lysate. Then, the expression of LPL and HMG CO gene was evaluated by qRT-PCR using primers obtained from Metabion (Germany) (Table 1). The CT of each sample was compared with that of the positive control group according to the "ΔΔCt" method stated by (Yuan *et al*., 2006) for estimation of the relative fold change in the expression of gene on the RNA of the different samples.

Histopathological examination

After the experiment, rabbits were decapitated and necropsied then the standard protocol is applied (King *et al*., 2016). Then, representative tissue samples from the Aortas, kidneys, and livers of five rats from each group were randomly chosen. (Ruehl-Fehlert *et al*., 2003) and quickly fixed in buffered neutral formalin 10% strength for seventy-two hours. After the previous step, the tissue samples were carefully cleaned in distilled water, ascending series of ethanol used for dehydration and the HistoChoice® is used as clearing agent (Sigma-Aldrich). Then, the samples were prepared for paraffin impregnation and embedding, cut into 5 µm thick sections, and stained with hematoxylin and eosin (H&E) (Suvarna *et al*., 2018).

Statistical analysis

For each group, the data are provided as the mean ± SE. By using one-way analysis of variance (ANOVA) followed by Duncan's multiple range post hoc test for pairwise comparisons, the variation between groups was statistically analyzed. Differences were considered significant at $p<0.05$.

RESULTS

Effects of oral administration of atorvastatin or fenofibrate on the lipogram of betamethasone dipropionate injected rabbits.

On zero day of the experiment, serum total cholesterol level revealed non-significant changes between the different groups. On the 3rd, 7th, 14th, 21st, and 30th day of the experiment a significantc($p<0.05$) elevation in total cholesterol level was noticed within BDP-group in comparison to control group. A significant decline ($p<0.05$) in total cholesterol level was recorded within BDP+ ATR treated group as well as BDP+ FFB treated group when compared to BDP only injected group. BDP-injected rabbits treated with ATR revealed a significant reduction of total cholesterol level when compared with those treated with FFB (Table 2).

On zero day of the experiment, serum total triglyceride level revealed non-significant changes between the different groups. On the 3rd, 7th, 14th, 21st, and 30th day of the experiment, there was a significant ($p<0.05$) elevation in total triglyceride level was noticed in BDP-group when compared to untreated group. On the 3rd day of experiment, A significant ($p<0.05$) elevation in total triglyceride level was documented in BDP+ ATR treated group as well as BDP+ FFB treated group when compared to BDP only injected group. On the 7th, 14th, 21st, and 30th of the experiment, a significant ($p<0.05$) reduction in total triglyceride level was documented within BDP+ ATR treated group as well as BDP+ FFB treated group when compared to BDP only injected group. On the 3rd, 7th, 14th, 21st, and 30th day of the experiment BDP-injected rabbits treated with FFB revealed a significant reduction of total triglyceride level when compared with those treated with ATR (Table 3).

On zero day of the experiment, serum HDL level revealed non-significant changes between the different groups. On the 7th

Table 2. Effects of oral administration of atorvastatin or fenofibrate on cholesterol level in betamethasone dipropionate injected rabbits.

Groups	Cholesterol (mg/dl)					
	Days of Experiment					
	Zero	3rd	7th	14th	21st	30th
Control	143±0.63^{a}	144.26±0.93^{d}	143.33±0.63^{c}	143.16±1.09^{c}	143.33±0.63^{c}	143.70±0.81^{c}
BDP	140.26±0.66^{a}	234.93±2.94^{a}	274.76±2.91^{a}	393.66±2.31^{a}	299.13±1.95^{a}	290.66±2.33^{a}
BDP+ATR	140.83±0.23^{a}	152.40±1.40^{c}	112.46±1.64^{d}	110.33±1.45^{d}	105.56±2.01^{d}	97.86±1.44^{d}
BDP +FFB	143.60±0.78^{a}	171.20±1.32^{b}	177.33±1.45^{b}	170.90±2.42^{b}	156.40±0.83^{b}	148.66±2.02^{b}

BDP: Betamethasone dipropionate; ATR: atorvastatin; FFB: fenofibrate

Table 3. Effects of oral administration of atorvastatin or fenofibrate on triglycerides level in betamethasone dipropionate injected rabbits.

Groups	Triglycerides (mg/dl)					
	Days of Experiment					
	Zero	3rd	7th	14th	21st	30th
Control	62.90±0.26^{a}	62.36±0.58^{d}	61.36±0.84^{d}	62.76±0.39^{d}	61.80±0.85^{d}	61.83±0.78^{d}
BDP	62.1±1^{a}	110.33±1.45^{c}	181±3.78^{a}	433.46±2.65^{a}	344±4.16^{a}	324.56±2.43^{a}
BDP+ATR	61.36±0.84^{a}	197.66±1.45^{a}	145.20±2.88^{b}	139.13±1.50^{b}	126.80±2.31^{b}	109.33±2.33^{b}
BDP +FFB	62.36±0.58^{a}	192.50±1.44^{b}	108.43±1.26^{c}	105.26±2.2^{c}	103.16±2.53^{c}	80.53±0.92^{c}

BDP: Betamethasone dipropionate; ATR: atorvastatin; FFB: fenofibrate

and 30th days of the experiment. There was a significant (p<0.05) reduction in HDL level within BDP-group when compared to control group. On the 3rd day of experiment, a significant (p<0.05) elevation in HDL level was recorded in BDP+ ATR treated group as well as BDP+ FFB treated group when compared to BDP only injected group. On the 7th and 30th days of the experiment, a significant (p<0.05) increase in HDL level was recorded in BDP+ ATR treated group when compared to BDP only injected group. BDP+ FFB treated group revealed a significant reduction (on the 3rd day of the experiment) and a significant increase (on the 30th day of the experiment) in HDL level when compared to BDP only injected group. On the 3rd, 7th, 14th and 30th days of the experiment BDP-injected rabbits treated with FFB revealed a significant reduction of HDL level when compared with those treated with ATR (Table 4).

On zero day of the experiment, serum LDL level revealed non-significant changes between the different groups. On the 3rd, 7th, 14th, 21st, and 30th days of the experiment. a significant (p<0.05) increase in LDL level was noticed in BDP-group when compared to control group. On the 3rd, 7th, 14th, 21st, and 30th days, a significant (p<0.05) decline in LDL level was recorded in BDP+ ATR treated group when compared to BDP only injected group. BDP+ FFB treated group revealed a significant elevation (on the 3rd day of the experiment) and a significant reduction (on the 7th, 14th, 21st, and 30th days of the experiment) in LDL level when compared to BDP only injected group. BDP-injected rabbits treated with ATR revealed a significant reduction of LDL level when compared with those treated with FFB (Table 5).

Effects of oral administration of atorvastatin or fenofibrate on the serum AST, ALT, TAC, MDA, VCAM-1 levels of betamethasone dipropionate injected rabbits.

A significant (p<0.05) increase in ALT and AST were seen in BDP group in comparison to control group. A significant (p<0.05) decline in ALT and AST was observed in BDP+ATR as well as BDP+FFB treated group when compared to BDP only injected group. BDP-injected rabbits treated with FFB revealed a significant reduction of AST and ALT levels when compared with those treated with ATR (Table 6).

A significant (p<0.05) decrease in serum TAC and a significant (p<0.05) increase in serum MDA level were recorded in BDP treated group when in comparison to control group. A significant (p<0.05) increase in serum TAC level was documented in BDP+ATR treated group as well as BDP+ FFB treated group when compared to BDP treated group. A significant (p<0.05) decrease in serum MDA level in BDP+ATR treated group as well as BDP+ FFB treated group compared to BDP only treated group. BDP-injected rabbits treated with FFB revealed a significant reduction of MDA levels when compared with those treated with ATR (Table 6).

A significant (p<0.05) elevation in VCAM-1 level was recorded in BDP treated group when compared to control group. A significant (p<0.05) decrease in the level of VCAM-1 was recorded in BDP+ATR treated group as well as BDP+ FFB treated group compared to BDP treated group. BDP-injected rabbits treated with ATR revealed a significant reduction of MDA levels when compared with those treated with FFB (Table 6).

Table 4. Effects of oral administration of atorvastatin or fenofibrate on HDL level in betamethasone dipropionate injected rabbits.

Groups	HDL (mg/dl)					
	Days of Experiment					
	Zero	3rd	7th	14th	21st	30th
Control	48.36±0.31^{a}	43.66±0.21^{a}	48.03±0.57^{a}	48.16±0.49^{a}	47.96±0.63^{a,b}	47.76±0.39^{b}
BDP	47.60±0.25^{a}	46.20±0.60^{a,b}	43.03±0.54^{b}	47.53±0.33^{a,b}	45.50±0.78^{b}	33.23±0.63^{c}
BDP+ATR	48.36±0.31^{a}	43.66±0.21^{b}	47±0.57^{a}	48.60±0.30^{a}	47.33±0.33^{a,b}	50.06±0.54^{a}
BDP +FFB	47.16±0.57^{a}	37.43±1.66^{c}	43.43±0.33^{b}	46.80±0.30^{b}	46±0.57^{a,b}	47.40±0.36^{b}

BDP: Betamethasone dipropionate; ATR: atorvastatin; FFB: fenofibrate

Table 5. Effects of oral administration of atorvastatin or fenofibrate on LDL level in betamethasone dipropionate injected rabbits.

Groups	LDL (mg/dl)					
	Days of Experiment					
	Zero	3rd	7th	14th	21st	30th
Control	82.76±1.26^{a}	81.29±1.01^{c}	83.07±0.92^{c}	80.14±0.83^{c}	83.07±0.92^{c}	83.07±0.92^{b}
BDP	80.40±1.70^{a}	99.70±2.96^{b}	208.40±2.35^{a}	294.55±2.24^{a}	294.55±2.24^{a}	325.46±3.01^{a}
BDP+ATR	81.26±1.01^{a}	56.93±1.03^{d}	37.49±2.28^{d}	39.50±0.82^{d}	41.05±0.85^{d}	33±1.33^{c}
BDP +FFB	83.06±0.92^{a}	104.26±2.49^{a}	102.40±1.21^{b}	104.26±0.87^{b}	94.10±1.78^{b}	83.46±1.68^{b}

BDP: Betamethasone dipropionate; ATR: atorvastatin; FFB: fenofibrate

Table 6. Effects of oral administration of atorvastatin or fenofibrate on serum AST, ALT, TAC, MDA, VCAM-1 and CD-63 levels in betamethasone dipropionate injected rabbits.

Groups	Parameters				
	AST(U/l)	ALT(U/l)	TAC (Um/l)	MDA (nmol/ml)	VCAM (ng/ml)
Control	71.66± 0.95^{b}	53± 1.32^{d}	6.94±0.50^{a}	1.91±0. 1^{b}	210±1.20^{c}
BDP	106.4±1.7^{a}	120.6±1.5^{a}	1.17±0. 3^{c}	6.24±0.4^{a}	380.6±1^{a}
BDP+ATR	56.3±3.4^{c}	95.8±1.5^{b}	3.58±0.1^{b}	1.176±0. 1^{d}	108.4±0.5^{d}
BDP +FFB	54 ±1.8^{d}	93±1.51^{c}	3.852±0.1^{b}	1.556±0. 1^{c}	255.2±2.4^{b}

BDP: Betamethasone dipropionate; ATR: atorvastatin; FFB: fenofibrate

Effects of oral administration of atorvastatin or fenofibrate on glucose, insulin and HOMA IR of betamethasone dipropionate injected rabbits.

A significant ($p<0.05$) increase in glucose level, insulin level and HOMA IR was present in BDP group compared to control group. A significant decline within glucose level, insulin level and HOMA IR was recorded in BDP+ATR treated group as well as BDP+ FFB treated group compared to BDP only treated group. BDP-injected rabbits treated with FFB revealed a significant reduction of insulin level and HOMA IR when compared with those treated with ATR (Table 7).

Table 7. Effects of oral administration of atorvastatin or fenofibrate on glucose, insulin and HOMA IR in betamethasone dipropionate injected rabbits.

Groups	Parameters		
	Glucose(mg/dl)	Insulin (ng/ml)	Homa IR
Control	77±1.5^{c}	4.94±0.82^{b}	0.94± 0.08^{b}
BDP	287±8.4^{a}	13.65±1.8^{a}	9.7±1.5^{d}
BDP+ATR	131±8.2^{b}	3.876± 0.17^{c}	1.2±0.08^{a}
BDP +FFB	133.6±7.2^{b}	2.62±0.1^{d}	0.8 ±0.07^{c}

BDP: Betamethasone dipropionate; ATR: atorvastatin; FFB: fenofibrate

Effects of oral administration of atorvastatin or fenofibrate on HMG-CoA reductase and lipoprotein lipase gene expression of betamethasone dipropionate injected rabbits.

Intramuscular injection of a single dose of BDP induced a significant increase in HMG-CoA reductase gene expression (1.82 ± 0.08) in comparison to control group (1.00 ± 0.00). A significant decline in HMG-CoA reductase gene expression were recorded in BDP+ATR treated group (0.04 ± 0.00) and BDP+ FFB treated group (0.60 ± 0.06) compared to BDP only treated group respectively. BDP-injected rabbits treated with ATR revealed a significant reduction of HMG-CoA reductase gene expression when compared with those treated with FFB.

Intramuscular injection of a single dose of BDP induced a significant decline in lipoprotein lipase gene expression (0.05 ± 0.00) in comparison to control group (1.00 ± 0.00). Non-significant change in lipoprotein lipase gene expression in were recorded in BDP+ATR treated group (1.00 ± 0.00) but a significant increase in BDP+ FFB treated group (1.98 ± 0.01) compared to BDP only treated group (Fig 1).

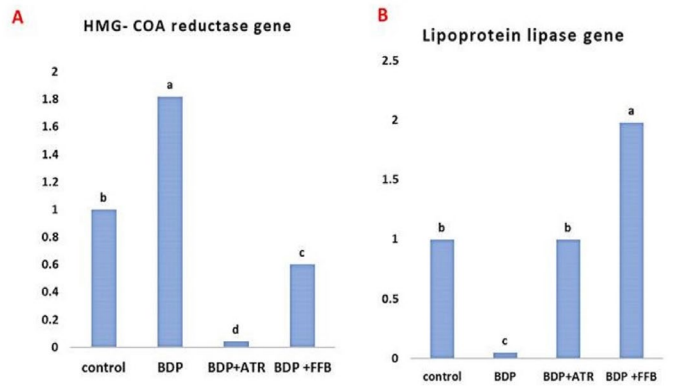

Fig. 1. Fig. 1. Effects of oral administration of atorvastatin or fenofibrate on HMG-CoA reductase and lipoprotein lipase gene expression of betamethasone dipropionate injected rabbits

Histopathological findings

Hepatic tissue section of the control rabbit showed normal histological picture (Fig. 2A). The hepatic tissue section of the BDP treated rabbit showed heterophilic infiltration, notable cytoplasmic vacuolations and single-cell necrosis (Fig. 2D). The hepatic tissue section of the BDP +ATR treated rabbit showed mild cytoplasmic vacuolations of the hepatocytes, focal minute mononuclear cell aggregate (Fig. 2G). The hepatic tissue section of the BDP+FFB treated rabbit showed mild cytoplasmic vacuolations of the hepatocytes, portal infiltration with few mononuclear cells, hemosiderosis, and cholangiocyte hypertrophy (Fig. 2J).

Renal tissue section of the control rabbit showed normal histological picture (Fig. 2B). The Renal tissue section of the BDP treated rabbit showed glomerular necrosis, interstitial mononuclear cell infiltration, and tubular vacuolations (Fig. 2E). The Renal tissue section of the BDP +ATR treated rabbit showed tubular vacuolation and attenuation (Fig 2H). The Renal tissue section of the BDP+FFB treated rabbit showed tubular dilatation with flattened epithelial lining and hyaline casts, besides, interstitial mononuclear cell infiltration (Fig. 2K).

Aortic tissue section of the control rabbit showed normal histological picture (Fig. 2C). The aortic tissue section of the BDP treated rabbit showed mucoid degeneration (Fig. 2F). The aortic tissue section of the BDP+ATR treated rabbit showing endothelial hypertrophy (Fig. 2I). The aortic tissue section of the BDP+FFB treated rabbit showed subendothelial vacuolations (Fig. 2L).

DISCUSSION

The obtained result revealed that single intramuscular injection of BDP (0.5ml) produced a signficant increase in total cholesterol, TG, LDL level but signficant decrease in HDL when compared to control group on the 3rd,7th,14th, 21st, 30th days of the experiment. This mean that the incidence of hyperlipdemia in this method occure on the 3rd day of the experiment and remain high for about 30 day. It was found also that on the thirty-day of the experiment there was an increase in liver enzyme ALT and AST. This mean that the liver affected and there was decrease in TAC and increase in MDA which mean that BDP increase oxidative stress. There was signficant increase in VCAM-1, it is an endothelial marker that indicate development of atherosclerosis and this confirmed by histopathology but less than atherosclerosis that developped in high fat diet induced hyperlipdemia (Elseweidy *et al.*, 2020). It was found that injection of BDP produce signficant increase in HMG-COA reductase gene expression the enzyme that responsible for cholesterol biosynthesis.This mean that BDP increase cholesterol level in the blood through activation HMG-COA reductase (the key enzyme in cholesterol biosynthesis). But produce significant decrease in lipoprotein lipase gene expression this enzyme responsible for degradation of triglyceride circulating in blood. This mean that BDP increase triglyceride through inhibtion of lipoprotein lipase increasing the circulating triglycerides. When we use the perviously mentioned method for induction of hyperlipidemia then use the usual treatment of hyperlipidemia atorvastatin, one of HMG- COA reductase inhbitors. Our result revealed that injection BDP and with oral adminstration of atorvastatin(1.9 mg/kg B.wt) for one month. BDP+ATR group showed a signficant decrease in all lipogram except HDL there was a signficant increase when compared to BDP treated group. In comparison to the result obtained by solanki and bhatt that found that atorvastatin also decrease all lipid profil parameters and also treat developped atherscleross when used followed high fat diet treated group (Solanki and Bhatt 2010; Sharma and Choudhary 2014).

On the 30th day of the experiment there was a signficant decrease in liver enzymes (ALT and AST) in BDP +ATR group when compared to BDP group. This mean that atorvastatin has beneficial effect on liver and a signficant elevation appeared in TAC when compared to BDP group but a signficant decrease in MDA when compared to BDP group which mean that atorvastatin correct ox-

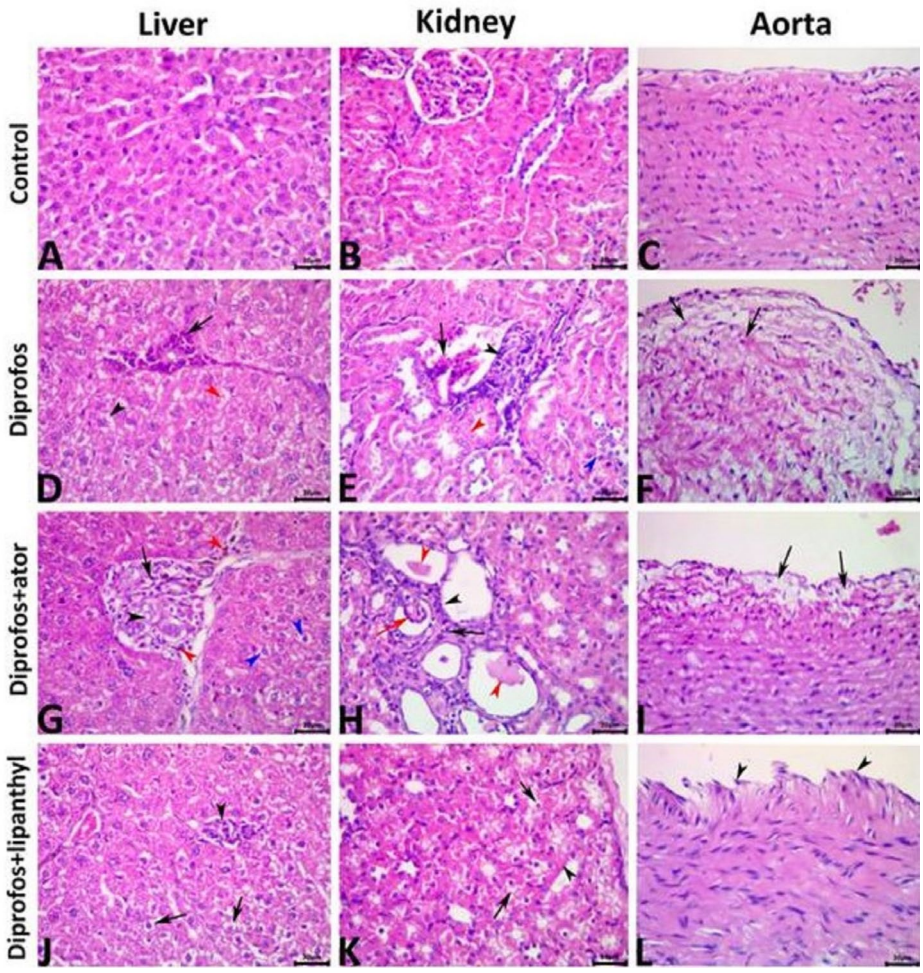

Fig. 2. Representative photomicrograph of the H&E- stained hepatic tissue section of the control rabbit showing normal histological picture (Fig 2A). The hepatic tissue section of the BDP treated rabbit showing heterophilic infiltration, notable cytoplasmic vacuolations and single-cell necrosis (Fig 2D). The hepatic tissue section of the BDP +ATR treated rabbit showing mild cytoplasmic vacuolations of the hepatocytes, focal minute mononuclear cell aggregate (Fig 2G). The hepatic tissue section of the BDP+FFB treated rabbit showing mild cytoplasmic vacuolations of the hepatocytes, portal infiltration with few mononuclear cells, hemosiderosis, and choanocytes hypertrophy (Fig 2J). Representative photomicrograph of the H&E- stained renal tissue section of the control rabbit showing normal histological picture (Fig 2B). Renal tissue section of the BDP treated rabbit showing glomerular necrosis, interstitial mononuclear cell infiltration, and tubular vacuolations (Fig 2E). The Renal tissue section of the BDP +ATR treated rabbit showing tubular vacuolation and attenuation (Fig 2H). The Renal tissue section of the BDP+FFB treated rabbit showing tubular dilatation with flattened epithelial lining and hyaline casts, besides, interstitial mononuclear cell infiltration (Fig 2K). Representative photomicrograph of the H&E- stained aortic tissue section of the control rabbit showing normal histological picture (Fig 2C). Aortic tissue section of the BDP treated rabbit showing mucoid degeneration (Fig 2F). Aortic tissue section of the BDP+ATR treated rabbit showing endothelial hypertrophy (Fig 2I). Aortic tissue section of the BDP+FFB treated rabbit showing subendothelial vacuolations (Fig 2L).

idative stress occurred by BDP. There was a signficant decrease in VCAM-1 when compared to BDP which indicate that atorvastatin aids in treatment of atherosclerosis. There was a signficant decrease in glucose level ,insulin and HOMA IR in BDP+ATR group when compared to BDP treated group that indicate atorvastatin decrease glucose level, insulin and HOMA-IR. The obtained result revealed also that there was a significant decrease in HMG-COA reductase gene expression in BDP +ATR group compared to BDP group as atorvastatin is one of HMG -CO A reductase inhbitors, but a significant increase in LPL gene expression in a contrast to BDP group which mean that atorvastatin increase degredation of triglycerides that increased by BDP.

Result from this study revealed also that injection of BDP with oral adminstration of fenofibrate(7.5 mg/kg B.wt) for one month. BDP+FFB group demonstrated a significant decline in lipogram from the 3rd day of experiment till the end of experiment when compared to BDP group exept HDL that is not signficantly affected during the experiment. On the 30th day of the experiment BDP+FFB group showed a significant decline in liver enzymes either ALT and AST compared to BDP group this mean that fenofibrate has benfecial effect on liver , a signficant increase in TAC and a signficant decrease in MDABDP+FFB group compared to BDP group this mean that fenofibrate decrease oxadative stress, a signficant decrease also in VCAM -1 this mean that fenofibrate decrease the atherosclerosis and a signficant decrease in glucose, insulin level and HOMA-IR in BDP+FFB group in comparison to BDP group. BDP +FFB group showed a signficant decrease in HMG CO-A reductase gene expression but a signficant decrease in LPL gene expression when compared to BDP group this result agreed with the exact mechanism of action of fenofibrate in lowering lipogram by activation of LPL (Rosenson, 2008). Rosenson decalared also that fenofibrates is a medication for hypertriglyceridemia and mixed dyslipidemia, as well as hypercholesterolemia, lipid disorders frequent in people at increased cardiovascular disease risk (Rosenson, 2008), Fibrates play an essential role in regulating LDL size and subtypes as well as TG metabolism. In order to control dyslipidemia, fibrates target atherogenic dyslipidemia by raising plasma HDL-C concentrations and lowering TGs and small dense LDL (sdLDL) particles. Fibrates reduce TG by increasing lipoprotein lipase synthesis. Additionally, fibrates have positive effects on oxidative stress. (Aasum *et al*., 2008;Katsiki *et al*., 2013).

CONCLUSION

The obtained result revealed that single IM injection of BDP produces a significant elevation in total cholesterol, triglycerides, LDL level with a significant decline in HDL. Also, results in an increase in the ALT, AST, MDA, VCAM-1 as well as a significant decrease in TAC on the 30th day of the experiment. Furthermore,

BDP induces a significant increase in HMG-COA reductase gene expression and a significant decrease in lipoprotein lipase gene expression. Oral administration of ATR or FFB concurrently with BDP for a month succeeded in reducing the hyperlipidemia induced by BDP in rabbits.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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