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Effects of pomegranate (*Punica granatum*) juice and peel extract on biochemical parameters in streptozotocin induced diabetic rats

Yashas R Kumar, HD Narayanaswamy, Suguna Rao, ML Satyanarayana, Prakash Nadoor and D Rathnamma

Abstract
Medicinal plants have a vast potential in the treatment of various ailments due to the presence of therapeutically important phytochemicals. The present study was undertaken to evaluate the efficacy of antihyperglycemic effect of pomegranate juice and peel extract individually and in combination in STZ induced diabetes in rats for a period of 45 days. The study included seven treatment groups comprising of ten albino Wistar rats each. The various groups in this study included normal control (Group I (NC)), diabetic control (Group II (DC), diabetic rats treated with metformin at the rate of 500 mg/kg bwt (Group III (MF)), diabetic rats treated with pomegranate fresh juice at (100% dosage) Group IV(PJ)), diabetic rats treated with pomegranate peel extracts (at 100% dosage) (Group V (PPE)), diabetic rats treated with pomegranate fresh juice (1mL) + pomegranate peel extracts (100mg) at 50 per-cent dosage and metformin (half dose) Group-VI (PJ+PPE+MF 50%), diabetic rats treated with pomegranate fresh juice (0.5mL) + pomegranate peel extracts extract (50mg) at 50 per-cent dosage and metformin (half dose) Group-VII (PJ+PPE @ 50% +MF 50%). The results showed significant ($P \leq 0.05$) increase in mean (± SE) serum glucose, triglyceride, cholesterol, ALT, AST levels in Group II diabetic control rats. All the treatment groups treated with pomegranate juice or pomegranate peel extract (Group IV to VII) individually or in combination showed a significant improvement in all the parameters tested compared to the diabetic control rats. It was observed that the pomegranate peel extract treated rats yielded better improvement in the tested parameters compared to the pomegranate juice treated group. In addition the groups treated with combination of pomegranate juice, peel extract and metformin at half dose revealed a better improvement compared to individual treatment groups. From the results this could be inferred that pomegranate juice and pomegranate peel extract have antihyperglycemic, hepatoprotective and hypolipidemic actions and peel extract is better in alleviating the effects of STZ induced changes in rats.

Keywords: Pomegranate fresh juice (PJ), pomegranate peel extract (PPE), metformin (MF) antihyperglycemic, hepatoprotective, hypolipidemic

Introduction
Many metabolic disturbances accompany diabetes mellitus including hyperglycemia, hyperlipidemia, relative or absolute deficiency of insulin, and increased oxidative stress. These abnormalities represent the backbone in the pathogenesis of diabetic complications. (American Diabetes Association, 2016) [2]. Pomegranate (*Punica granatum* L.) is a member of the family of *Punicaceae*, one of the most ancient edible fruits, widely grown in Mediterranean regions including Iran, Egypt, Iraq and India, but sparsely cultivated in USA, China, Japan and Russia **(**Matthaeus and Ozcan, 2016) [18]. The Pomegranate fruit has valuable compounds in different parts of the fruit such as peel, seeds and arils (Zhang *et al.,* 2010) [33].
An important product obtained from pomegranate fruit is the juice that can be obtained from arils or from whole fruit. The edible part of pomegranate fruit represents 52% of total fruit weight, comprising 78% juice and 22% seeds. (Amri *et al.,* 2017) [3]. Both pomegranate juice and pomegranate peel extract (PPE) are rich in bioactive compounds such as polyphenols, anthocyanidins, tannic acid, gallic acid, and ellagic acid that exert antioxidant activities and prevent oxidative stress (Faghihimani *et al.,* 2017) [7]. The aim of the present study was to determine the effect of pomegranate (*Punica granatum*) juice and peel extract on biochemical parameters individually and in combination with metformin in STZ induced diabetes in Wistar rats.

Materials and methods

Experimental animals

Male albino Wistar rats weighing 180-200 g were used for the present study. They were maintained under standard laboratory conditions and offered *ad libitum* quantity of standard commercial rat feed and clean drinking water. The animals were kept for acclimatization after procurement in an experimental lab animal house for 2 weeks. The study was carried out with prior approval from Institutional Animal Ethics Committee (IAEC) with IAEC No (VCH/IAEC/2019/128). The experiment was carried out for a period of 45 days.

Experimental design

Group I	Normal control: Normal rats administered orally with saline
Group II	Diabetic control: Rats administered intra-peritonially with streptozotocin (STZ) at 45mg/ kg body weight.
Group III	Diabetic rats treated with metformin (full dose) at 500 mg/ kg body weight orally
Group IV	Diabetic rats treated with *pomegranate fresh juice* at (1mL/day).
Group V	Diabetic rats treated with *pomegranate peel* extracts (100mg /day).
Group VI	Diabetic rats treated with *pomegranate fresh juice* (1mL) + *pomegranate peel* extracts (100mg) and metformin (half dose)
Group VII	Diabetic rats treated with *pomegranate fresh juice* (0.5mL) + *pomegranate peel* extracts (50mg) at 50 per-cent dosage and metformin (half dose)

Drugs and chemicals

Streptozotocin (STZ)

Streptozotocin, was procured from Sigma Chemicals, St. Louis, USA (No. SO130). The working injectable STZ solution was made in freshly prepared 0.1 M citrate buffer with pH 4.5 and stored at 4-8 °C.

Administration of STZ solution

The STZ solution was administered by intraperitoneal route at the rate of 45 mg/kg bw, to all the rats of Groups II, III, IV, V, VI, VII

Metformin

Metformin (500 mg) an oral hypoglycemic drug, purchased from a local chemist shop was powdered and suspended in distilled water (20 ml) to make a concentration of 100 mg/ml. The solution was prepared every day and administered orally at a dose rate of 500 mg/kg body weight (full dose) for treatment group-III and 250mg/ kg body weight (half dose) for treatment groups VI and VII.

Plant extracts

Pomegranate juice (PJ)

The fruits of fresh pomegranate were washed and manually peeled, without separating the seeds. Pomegranate juice was obtained using a commercial blender and filtrated with a Buchner funnel to remove water insoluble materials.

Preparation of pomegranate peel extract solution (PPE)

Peel of fresh pomegranate fruit was removed, shade dried for 20 days, and then put in a freeze dryer for complete dryness. Dried peels were crushed, and an amount of 500g of dried plant material was mixed with hydro-alcoholic solution prepared with water: methanol (40:60) containing two litres of distilled water and three litres of methanol (absolute) for maceration in a closed conical flask at room temperature. The flasks were then mixed by agitation by keeping in a rotary shaker and allowed to stand for one week. It is then filtered using Buchners funnel and Whatman No.1 filter paper. The resultant filtrate was taken and methanol was evaporated by using a rotary flask evaporator (Rotavapor, BUCHI, Switzerland) at room temperature not exceeding 45°C. Crude PPE was obtained and kept at -80°C and the extract was further subjected to lyophilization.

Administration of the treatment solution

Throughout the period of experiment, the pomegranate juice, pomegranate peel extract and metformin were administered orally to their respective groups by using clean gavaging/rat feeding needle attached to an appropriate disposable syringe during morning hours daily for a period of 45 days.

Experimental induction of diabetes

The animals were fasted overnight and diabetes was induced in Group II to VII by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (45 mg/kg bw) in 0.1 M cold citrate buffer with a pH 4.5. Control **(**Group I) animals received citrate buffer alone.

Confirmation of diabetes

The blood glucose levels were estimated 72 hours post STZ injection and the rats with blood glucose levels above 200 mg/dL were considered as diabetic. After confirmation of diabetic state, all the groups received their respective treatments daily for 45 days.

Clinical observations

Rats of all the groups were observed for feed and water intake, general behaviour, alertness, urine output, diarrhoea and any other clinical symptoms manifested.

Collection of serum sample

Blood was drawn from the retro-orbital plexus of the rats under light ether anaesthesia at different time intervals such as 3rd 15th, 30th and 45th day post STZ injection of the study. First few drops of blood were collected in ethylene diamine tetra acetic acid (EDTA) for haematological analysis, and about 2 ml of blood from each animal of all groups was collected in clot accelerator tubes, allowed to clot for 30 min and then centrifuged at 3000 rpm for 10 min. The separated serum was collected into 2 ml Eppendorff tube and subjected for glucose estimation immediately after collection and the remaining serum samples were stored at -20^{o}C for further analysis.

Sacrifice of animals

The effects of treatments given to different groups were studied by sacrificing two rats from each group under xylazine and ketamine anaesthesia on 3rd, 15th and 30th day and remaining rats on 45th day of the experiment. Sacrificed animals were subjected for detailed post mortem examination and gross changes, if any were recorded. A piece of liver was collected in ice cold normal saline for estimation of antioxidant enzymes. Representative tissue samples from pancreas, liver, kidney, lungs, heart, intestine and spleen were collected in 10% neutral buffered formalin (NBF) for histopathology and immunohistochemistry.

Haematological evaluation
On 3rd, 15th, 30th and 45th day, the blood collected in EDTA from all the treatment groups were subjected for estimation of hemoglobin, TRC, TLC and platelet count using the automated hematology analyzer (BC-2800 vet, Mindray).

Biochemical analysis
The serum samples collected at various intervals were subjected for biochemical estimation of serum concentration of glucose, cholesterol, triglycerides, ALT and AST using semi-automatic biochemical analyser with commercial biochemical kits (Erba diagnostics, Mumbai) as per the procedure described by Tietz (1976) [32].

Statistical analysis
Statistical analysis was performed using the statistical software Graph pad prism, version 8.0.1 for windows. Mean values and standard error were calculated and all values were expressed as mean (± SE). The data were analysed by two way analysis of variance (ANOVA) for all parameters.

Results and Discussion
Treatment of rats with streptozotocin is an established model for Type I or insulin-dependent diabetes. All the rats of Group-II, III, IV, V, VI and VII became diabetic and showed hyperglycaemia with an increase in mean serum glucose levels ranging from 457.33 ± 2.39 to 485.58 ± 1.15 mg/dL by 72 hours after STZ administration.
Streptozotocin (STZ) is a naturally occurring compound produced by the bacterium *Streptomyces achromogenes* that exhibits broad spectrum antibacterial properties. It is assumed that the toxicity of streptozotocin is dependent upon the DNA alkylating activity of its methylnitrosourea moiety, especially at the O_6 position of guanine and the transfer of the methyl group from streptozotocin to the DNA molecule that causes damage, which along a defined chain of events, results in the fragmentation of the DNA. The DNA damage induces activation of poly ADP-ribosylation which leads to depletion of cellular NAD+ and ATP. The depletion of the cellular energy stores ultimately results in β-cell necrosis. (Sweetman 2002; Lenzen 2008) [27, 14].

Group-I (Normal control)
In the present investigation, all animals in the normal control group remained healthy at different intervals of the study. All the values of various parameters analysed were within the normal range and indicated their healthy status.

Group-II (Diabetic control)
In the diabetic control animals (Group-II), analysis of several parameters analysed indicated hyperglycaemic effects and a significant increase (P≤0.05) in serum glucose, cholesterol, triglyceride, ALT and AST levels was observed. A hyperglycemic state in diabetes could be attributed to the action of STZ, which is transported into pancreatic β-cells by GLUT-2 that causes β-cell toxicity and destruction of cells of langherhans, resulting in insulin deficiency which in turn causes loss of glucose homeostasis. Lack of insulin and elevations of the counter-regulatory hormones lead to activation of enzymes (hormone sensitive lipase) that stimulate lipolysis and enhanced release of free fatty acids from adipose tissue. Hyperlipidemia is a known complication of diabetes mellitus and coexists with hyperglycemia and is characterized by increased levels of cholesterol, triglycerides and phospholipids and also changes in lipoproteins. A rise in ALT and AST activity is almost always due to hepatocellular damage with leakage of enzymes from cytosol and organelles of hepatocytes caused by STZ. The present findings were in the same line as with those reported by (Pillion *et al.*, 1988 ; Rao *et al*.,1989; Li *et al*., 2001; Akbarzadeh *et al*., 2007) [22, 15, 1] (Table 1-5; Fig.1-5)

Group III (Diabetic rats treated with metformin at 500mg/ kg bwt.)
Metformin is an insulin-sensitizing agent with potent antihyperglycemic properties. Its efficacy in reducing hyperglycemia in type 2 diabetes mellitus is similar to that of sulfonylureas, thiazolidinediones, and insulin. Antihyperglycemic properties of metformin are mainly attributed to suppression of hepatic glucose production, especially hepatic gluconeogenesis, and increased peripheral tissue insulin sensitivity (Kirpichnikov *et al.*, 2002) [12].
In the present study, there was significant progressive improvement (P≤0.05) in several parameters analysed in comparison with those of diabetic control rats such as serum glucose values, cholesterol, triglyceride, ALT and AST levels in Group III rats. (Table 1-5; Fig. 1-5).
The serum glucose levels in metformin treated rats at a dose of 500 mg/kg bwt showed a significant (P≤0.05) improvement from 15th day onwards compared to diabetic control group (Table.1-5). The decrease in serum glucose values was in accordance with those of previous findings. (Cheng *et al*., 2006; Pushparaj *et al*.., 2005; Poumaghi *et al*., 2012 ; Kamboj *et al*.,2013; Nna *et al*., 2018) [21]. This could be due to metformin induced AMP activation of protein kinase (AMPK), a liver enzyme that plays an important role in insulin signalling along with fat and glucose metabolism. This activated AMPK in turn suppresses hepatic gluconeogenesis. Metformin further recruits more insulin receptors and thus improves insulin resistance (Lord *et al.*,1983). Further, serum cholesterol, triglyceride, ALT, AST levels were observed to be significantly reduced (P≤ 0.05) from 15th to 45th day in the metformin treated rats compared to diabetic control rats in the present study, the findings were similar to studies conducted by Verma *et al.*, (1994) [30] ; Maghrani *et al.*, (2004) [17]; Islam *et al*., (2009) [11]; Narsolahi *et al*., (2012) [20]; Geerling *et al*., (2014) [9] and Roehrs *et al*., (2014) [24]. It was demonstrated that metformin does not affect hepatic VLDL-TG production, but selectively promotes VLDL-TG clearance by BAT (brown adipose tissue), an effect associated with elevated components of intracellular lipolytic and mitochondrial fatty acid oxidation in highly active metabolic tissue (Hassanzadeh-Taheri *et al*., 2018; Nna *et al*.,2018; Yazdi *et al*., 2019; Rezai *et al*., 2020) [10, 21, 29, 26].

Group-IV (Diabetic rats treated with pomegranate fresh juice (1mL/day) and Group V (Diabetic rats treated with pomegranate peel extract (100mg/day)
The STZ diabetic rats were treated with pomegranate juice (Group-IV) and pomegranate peel extract (Group-V) for a period of 45 days. In the present study significant (P ≤ 0.05) improvement was observed in the values of serum glucose, cholesterol, triglyceride, ALT and AST levels from 15th to 45th day of the study compared to the diabetic control animals (Table 1-5; Fig. 1-5) in both the groups and did not vary between the groups.
The hypoglycemic response of pomegrenate could be attributed to its bioactive compounds. Pomegranate contains

many bioactive compounds, primarily including phenolic acids such as gallic acid, caffeic acid, chlorogenic acid, ferulic acid, and coumaric acids. It also contains non-phenolic acids, citric acid, succinic acid, malic acid, oxalic acid, and ascorbic acid. (Krueger DA., 2012) [13]. It has been suggested that polyphenols may affect glucose levels by improving the sensitivity of insulin receptors with increased activity of the peroxisome proliferator-activated gamma receptor (PPAR-c) resulting in improved insulin receptor sensitivity and further decreasing glucose absorption by inhibiting digestive enzymes, modulating the expression of glucose transporter GLUT4, increasing glucose uptake by peripheral tissues, inhibition of gluconeogenesis, and increasing insulin secretion (Virgen-Carrillo *et al.*, 2020) [31].

Pomegranate reduces the cellular uptake of oxidized LDL and inhibits cholesterol synthesis resulting in reduction in macrophages cholesterol accumulation and foam cell formation and attenuation of atherosclerosis development (Fuhrman 2005) [8]. One of the possible actions of pomegranate may be due to its inhibition of endogenous synthesis of lipids. The fruit appears to confer health benefits due to its anti-oxidant activity (Bhandari 2012) [5].

Administration of pomegranate mainly reduces inflammatory symptoms and histological injuries, diminishing MPO activity, TNF- α production and reducing COX-2 and iNOS expression through the inhibition of MAPKs and NF- κB. Antioxidant supplements may have a role in preventing or treating hepatic lesion in diabetic rats. The present findings were in accordance with those of Banihani *et al.*, (2013) [4]; Faddladdeen and Ojaimi.,(2019) [6]; Ramzy., M *et al.*, (2019) [25]; Mabrouk gabr., (2017) [16] and Xu *et al*., (2020) [28]. However, it was observed that the pomegranate peel extract treated rats yielded better improvement in the tested parameters compared to the pomegranate juice treated group numerically. (Table 1-5; Fig. 1-5).

Group VI: Diabetic rats treated with *pomegranate fresh juice* (1mL) + *pomegranate peel* extracts (100mg) and metformin half dose (50%) and Group-VII: Diabetic rats treated with *pomegranate juice* (0.5mL) + *pomegranate peel* extracts (50mg) at 50 per-cent dosage and metformin (half dose).

It was observed that there was a significant ($P \leq 0.05$) reduction from 15th to 45th day in the serum mean glucose values and improvement in serum cholesterol, triglyceride, ALT and AST levels in both the groups. The groups treated with combination of pomegranate juice, peel extract and metformin both at half dose revealed better results compared to individual treatment groups (Groups IV and V).

The perusal of literature did not reveal any reports on the effect of combination of pomegranate juice and pomegranate peel extract and metformin, However, in combined treatment groups the improvement observed could be attributed to the presence of high levels of antioxidants in pomegranate juice and peel extract such as polyphenols and flavonoids and also inclusion of metformin which antagonizes the damaging effects of pro-oxidants and reduces oxidative stress which could boost quenching of free radicals inside the cells causing decreased lipid peroxidation, as well as protect liver tissue from oxidative stress damage. The mean serum glucose, cholesterol, triglyceride, ALT and AST values showed no-significant difference between the combined treatment groups VI and VII throughout the study period of 45 days. However, there was a dose dependent effect with group VI (PJ+PPE+MF 50%) better than group VII (PJ+PPE@ 50% +MF 50%) showing improvement in various parameters numerically. However, the improvements observed in diabetic rats due to combined treatment cannot be attributed to any of the constituents individually in the present study.

Conclusion

The present study has shown that:

- Metformin significantly alleviated the effects of STZ in diabetic rats. However, the improvement was not on par with normal control animals.
- Pomegranate peel extract treated rats yielded better improvement in the tested parameters compared to the pomegranate juice treated group
- Groups treated with combination of pomegranate juice, peel extract and metformin both at half dose revealed a better result compared to individual treatment groups.

Table 1: The mean (± SE) serum glucose (mg/dL) values of different treatment groups at different intervals of time

Groups	Mean (±SE) serum glucose (mg/dL)			
	Days post-treatment			
	3rd day	15th day	30th day	45th day
Group-I (NC)	106.57 ± 4.11 [a]	109.34 ± 2.86 [a]	108.97 ± 4.27 [a]	104.67 ± 4.10 [a]
Group-II (DC)	485.58 ± 1.15 [b]	491.98 ± 1.82 [b]	496.33 ±1.98 [b]	417.33 ± 4.46 [b]
Group-III (MF)	473.00 ± 1.50 [b]	385.65 ± 1.25 [c]	264.33 ± 2.97 [c]	184.33 ± 7.62 [c]
Group-IV (PJ)	481.67 ± 1.40 [b]	423.00 ± 2.59 [c]	377.00 ± 3.35 [c]	271.00 ± 4.02 [c]
Group-V (PPE)	469.00 ± 4.09 [b]	442.00 ± 1.07 [c]	320.00 ± 1.27 [c]	220.00 ± 8.08 [c]
Group-VI (PJ+PPE +MF 50%)	499.00 ± 1.74 [b]	416.66 ± 1.41 [c]	313.33 ± 1.69 [c]	202.67 ± 4.91 [c]
Group-VII (PJ+PPE @50% dosage +MF 50%)	457.33 ± 2.39 [b]	421.00 ± 1.15 [c]	359.00 ± 2.69 [c]	211.00 ± 3.79 [c]

All values are mean (± SE). Mean values with different superscript differ significantly. Values are statistically significant at $P \leq 0.05$

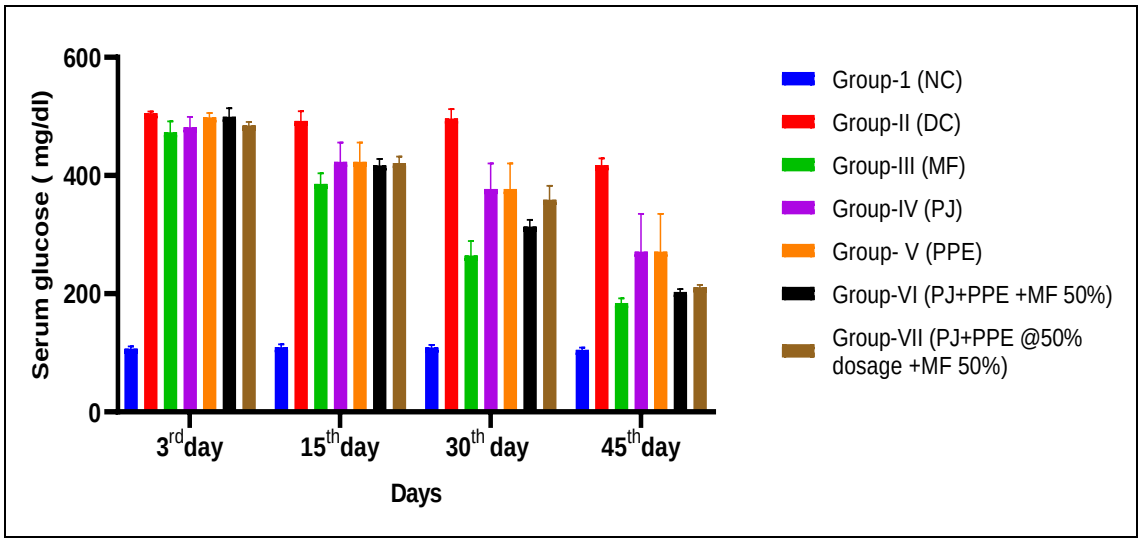

Fig 1: The mean (± SE) serum glucose (mg/dL) values of different treatment groups at different intervals of time.

Table 2: The mean (± SE) serum cholesterol (mg/dL) values of different treatment groups at different intervals of time

Groups	Mean (±SE) serum cholesterol (mg/dL)			
	Days post-treatment			
	3rd day	15th day	30th day	45th day
Group-I (NC)	71.00 ± 1.52 a	77.66 ± 0.33 a	79.33 ± 0.33 a	81.33 ± 0.88 a
Group-II (DC)	108.67 ± 0.88 b	115.67 ± 2.33 b	130.33 ± 0.88 b	147.67 ± 2.40 b
Group-III (MF)	107.00 ± 4.35 b	99.33 ± 1.45 c	96.00 ± 3.46 c	90.00 ± 9.59 c
Group-IV (PJ)	109.00 ± 5.56 b	106.00 ± 2.08 c	101.00 ± 3.60 c	100.00 ± 3.21 c
Group-V (PPE)	108.00 ± 3.05 b	106.00 ± 2.51 c	100.00 ± 2.64 c	95.00 ± 2.51 c
Group-VI (PJ+PPE +MF 50%)	105.93 ± 0.72 b	102.00 ± 2.51 c	98.00 ± 3.05 c	93.00 ± 2.87 c
Group-VII (PJ+PPE @50% dosage +MF 50%)	110.00 ± 4.04 b	105.00 ± 2.08 c	99.00 ± 2.51 c	93.00 ± 2.08 c

All values are mean (± SE). Mean values with different superscript differ significantly. Values are statistically significant at P ≤ 0.05

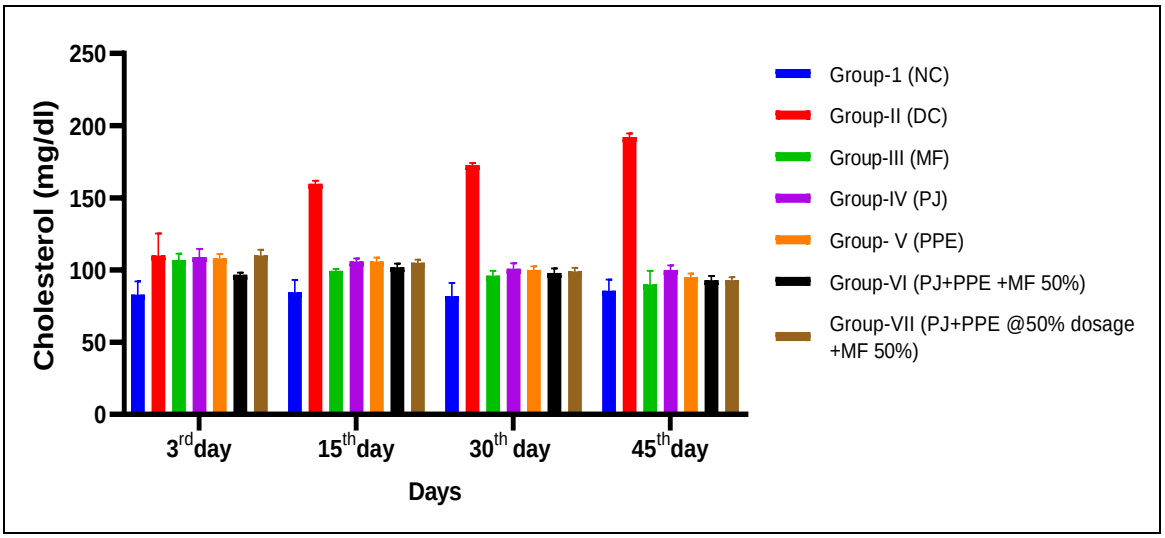

Fig 2: The mean (± SE) serum cholesterol (mg/dL) values of different treatment groups at different intervals of time.

Table 3: The mean (± SE) serum triglyceride (mg/dL) values of different treatment groups at different intervals of time.

Groups	Mean (±SE) serum triglyceride (mg/dL)			
	Days post-treatment			
	3rd day	15th day	30th day	45th day
Group-I (NC)	85.30 ± 1.40 a	92.73 ± 2.94 a	92.39 ± 1.54 a	92.28 ± 4.93 a
Group-II (DC)	166.30 ± 5.08 b	178.72 ± 1.43 bc	190.06 ± 2.88 b	213.00 ± 2.08 b
Group-III (MF)	121.25 ± 4.71 b	132.32 ± 1.90 cd	117.63 ± 3.88 c	109.89 ± 2.25 a
Group-IV (PJ)	135.53 ± 8.19 b	139.37 ± 5.22 bd	123.16 ± 3.96 cd	114.05 ± 2.09 a
Group-V (PPE)	131.85 ± 3.03 b	138.77 ± 4.68 cd	119.65 ± 0.84 c	112.85 ± 5.54 a
Group-VI (PJ+PPE +MF 50%)	130.25 ± 1.08 b	133.20 ± 1.17 cd	113.15 ± 5.15 cd	108.68 ± 3.24 a
Group-VII (PJ+PPE @50% dosage +MF 50%)	132.53 ± 2.04 b	136.66 ± 6.85 cd	120.29 ± 4.26 c	110.61 ± 2.20 a

All values are mean (± SE). Mean values with different superscript differ significantly. Values are statistically significant at P ≤ 0.05

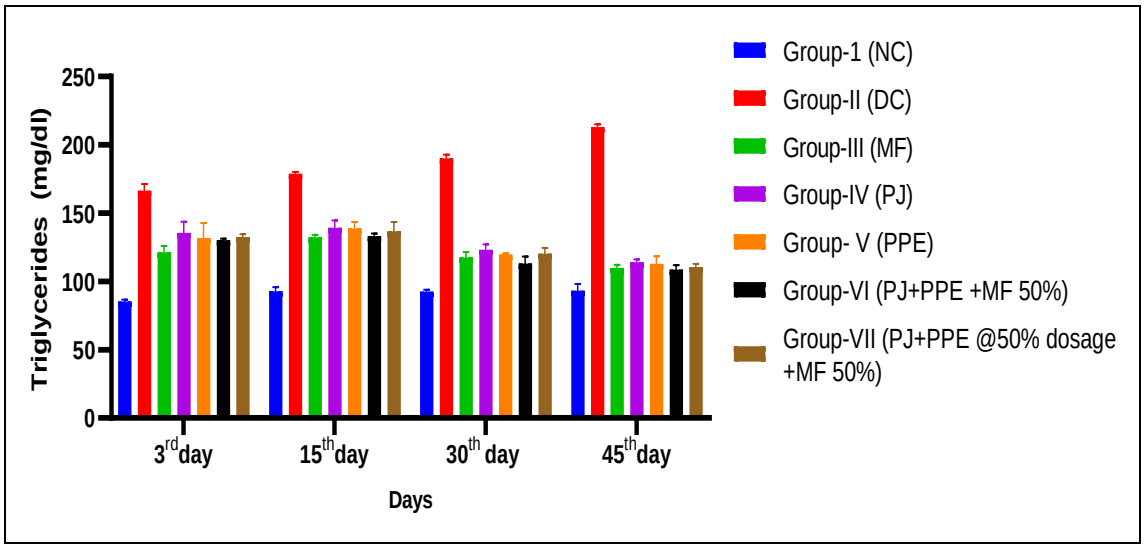

Fig 3: The mean (± SE) serum triglyceride (mg/dL) values of different treatment groups at different intervals of time.

Table 4: The mean (± SE) Serum Alanine aminotransferase ALT (IU/L) values of different treatment groups at different intervals of time.

Groups	Mean (±SE) ALT (IU/L)			
	Days post-treatment			
	3rd day	15th day	30th day	45th day
Group-I (NC)	68.75 ± 0.89 a	69.90 ± 0.92 a	70.44 ± 0.66 a	71.27 ± 1.64 a
Group-II (DC)	133.33 ± 1.12 b	155.57 ± 1.94 b	184.55 ± 2.54 b	212.55 ± 1.25 b
Group-III (MF)	127.53 ± 2.13 b	119.10 ± 0.95 c	106.81 ± 1.66 c	96.57 ± 1.42 c
Group-IV (PJ)	129.88 ± 0.90 b	124.01 ± 0.99 c	117.30 ± 1.47 c	109.21 ± 1.04 d
Group-V (PPE)	130.61 ± 0.60 b	122.37 ± 1.20 c	111.07 ± 0.97 cd	100.25 ± 0.55 c
Group-VI (PJ+PPE +MF 50%)	128.75 ± 0.89 b	118.21 ± 1.48 c	107.24 ± 0.90 c	98.14 ± 1.07 c
Group-VII (PJ+PPE @50% dosage +MF 50%)	129.44 ± 0.67 b	121.20 ± 0.62 c	110.82 ± 1.83 c	102.41 ± 1.22 c

All values are mean (± SE). Mean values with different superscript differ significantly. Values are statistically significant at P ≤ 0.05

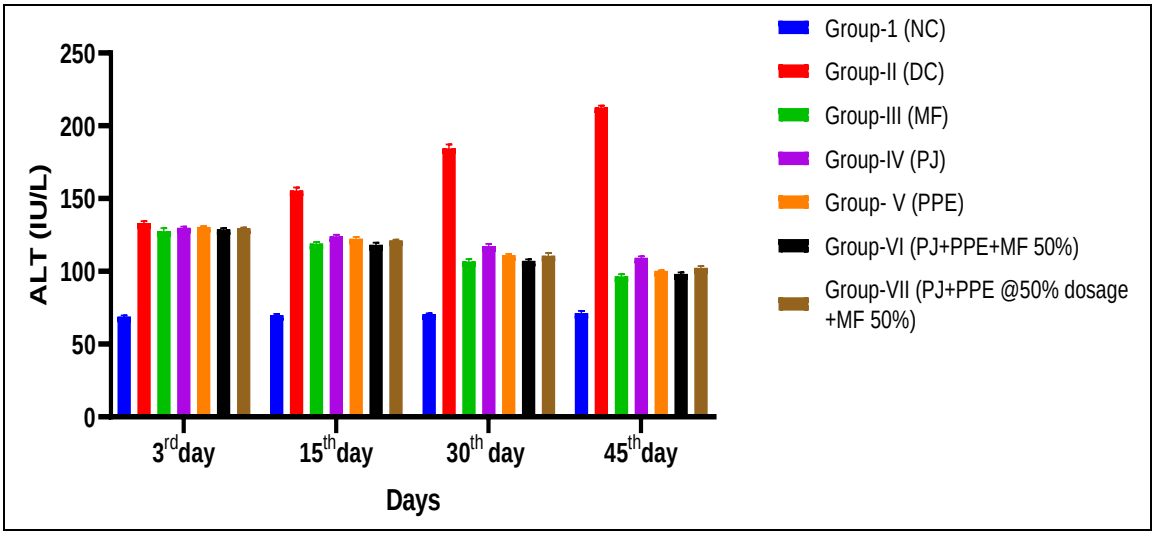

Fig 4: The mean (± SE) Serum Alanine aminotransferase ALT (IU/L) values of different treatment groups at different intervals of time.

Table 5: The mean (± SE) Serum Aspartate aminotransferase AST (IU/L) values of different treatment groups at different intervals of time.

Groups	Mean (±SE) AST (IU/L)			
	Days post-treatment			
	3rd day	15th day	30th day	45th day
Group-I (NC)	87.60 ± 1.25 a	88.54 ± 1.26a	89.16 ± 2.70a	89.97 ± 2.27a
Group-II (DC)	188.66 ± 1.25 b	279.51 ± 5.52b	291.88 ± 1.76b	297.66 ± 1.69b
Group-III (MF)	184.66 ± 0.33 b	203.14 ± 0.54c	180.76 ± 1.40c	161.82 ± 2.52c
Group-IV (PJ)	185.00 ± 1.52 b	223.89 ± 8.21cd	207.10 ± 1.17d	197.50 ± 1.13d
Group-V (PPE)	184.66 ± 1.74 b	225.12 ± 1.55 cd	200.09 ± 0.61de	189.14 ± 1.59de
Group-VI (PJ+PPE +MF 50%)	186.33 ± 0.33 b	210.98 ± 3.86cd	196.35 ± 0.32e	176.00 ± 2.64ce
Group-VII (PJ+PPE @50% dosage +MF 50%)	184.00 ± 2.00 b	215.39 ± 3.72cd	199.76 ± 1.99 de	181.85 ± 2.61de

All values are mean (± SE). Mean values with different superscript differ significantly. Values are statistically significant at P ≤ 0.05

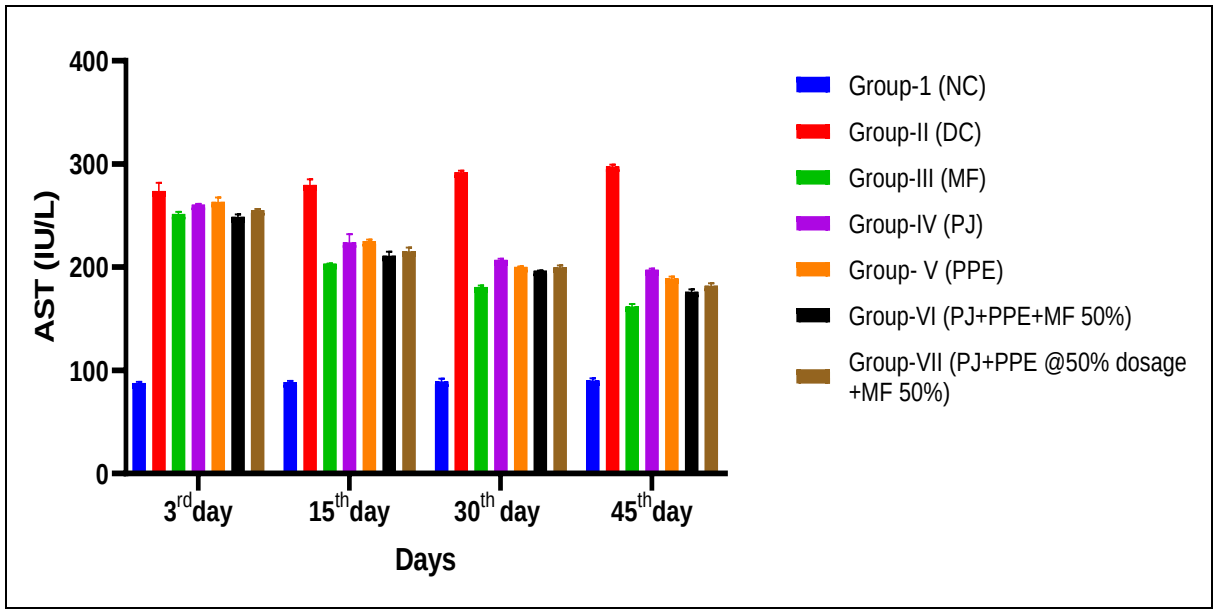

Fig 5: The mean (± SE) Serum Aspartate aminotransferase AST (IU/L) values of different treatment groups at different intervals of time

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