

DOES ASPIRIN PRETREATMENT PREVENT DIABETES IN RATS?

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Summary

Aspirin is irreversibly acetylates cyclooxygenase, thus inactivating this enzyme and blocking prostaglandin synthesis, is rapidly deacetylated by esterases in the body, yielding salicylate, which has anti-inflammatory, antipyretic, and analgesic effects and anti thrombotic, antioxidant and anti diabetic effects. Diabetes was induced in neonatal rats by streptozotocin (STZ). Rat pups (neonates) were divided into three groups consisting of six each. It was found that only by 8 weeks of animals and thereafter n5 STZ rats showed mild hyperglycemia. The first group served as control group, second group was treated only with streptozotocin (90mg/kg, *i.p*) and third group was treated with aspirin (1mg/kg/day, *p.o*) for one week (0-7days) before streptozotocin. On day 8th, blood samples were collected and estimated fasting serum glucose levels (10 weeks), total antioxidant status and lipid profiles for two weeks (8th and 10th). Aspirin treated groups showed no increase in blood glucose levels following STZ treatment when studied for 10 weeks. Total antioxidant status was decreased in STZ treated group and increased in aspirin treated group similarly there was a significant influence on lipid profile following aspirin protection. The present study indicates that aspirin pretreatment seems to protect pancreas from damage caused by STZ and maintains good lipid profile in diabetic rats and increases insulin sensitivity. It also brings about improvement of antioxidant status in diabetes mellitus.

KEYWORDS: Aspirin, type 2 diabetes, streptozotocin, neonatal rats.

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Introduction

Diabetes mellitus is a metabolic disorder with characteristics of hyperglycemia and insufficiency of secretion or action of endogenous insulin [1-2]. Chronic hyperglycemia causes many of the major complications of diabetes, including nephropathy, retinopathy, neuropathy, and macro vascular and micro vascular damage [3-4]. Increased oxidative stress, defined as a persistent imbalance between the production of highly reactive molecular species (chiefly oxygen and nitrogen) and antioxidant defenses, is a widely accepted participant in the development and progression of diabetes and its complications [5-6]. Hyperglycemia was also found to promote lipid peroxidation of low-density lipoprotein (LDL) by a superoxide-dependent pathway to generate free radicals [7]. Free radicals can be generated in glucose oxidation, which is believed to be the main source of free radicals, which are not degraded by catalase or glutathione peroxidase, and in the presence of transitional metals, can lead to production of extremely reactive hydroxyl radicals [8]. Aspirin irreversibly acetylates cyclooxygenase, thus inactivating this enzyme and blocking prostaglandin synthesis. Aspirin is rapidly deacetylated by esterases in the body, yielding salicylate, which has anti-inflammatory, antipyretic, analgesic effect [9] and anti thrombotic [10], antioxidant [11] and antidiabetic [12], drug has new approach in type 2 diabetes. Salicylates inhibit serine/ threonine caused insulin resistance and IKK- β activity and restore insulin sensitivity, both *in-vitro* and *in vivo*. Salicylates alter the phosphorylation patterns of IRS proteins, resulting in the decrease serine phosphorylation, increased tyrosine phosphorylation and improved insulin action [13]. Aspirin, which has antioxidant, antidyslipidemic and anti diabetic properties may play a greater role in the prevention of cardiovascular diseases. We made an attempt to investigate whether aspirin plays a role in preventing non-insulin diabetes mellitus.

Methods

Materials:

Aspirin pure substance was a kind gift from Natco Pharma Ltd., Hyderabad, India. Diphenyl picryl hydrazyl (DPPH) and streptozotocin were purchased from Sigma, St. Louis, USA. Glucose and lipid profile kits were purchased from Excel diagnostics Ltd., Hyderabad. Methanol (analytical grade) purchased from E. Merck Ltd., Mumbai, India.

Animals:

Male rat pups, weighing between 10-20g were obtained from Mahaveer Enterprises, Hyderabad. The selected animals were housed in acrylic cages in standard environmental conditions (20-25°C), fed with standard rodent diet and water *ad libitum*. The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use. The experiment was planned after getting the approval from the Institutional Animal Ethical Committee.

Study design:

Rat pups (neonates) were divided into three groups consisting of six. Blood sample was drawn by making small incision on the tail vein from all the animals. The first group did not receive any medication served as control group. Second group treated only with streptozotocin (90mg/kg, *i.p*) and third group treated with aspirin (1mg/kg/day, *p.o*) for one week (0-7days) before streptozotocin. On day 8th, blood sample were collected from all the animals and estimated fasting blood sugar levels using with ACU-CHEK glucometer for 1-7 weeks and glucose-oxidase-peroxidase (GOD-POD) method [14] for two weeks (8th & 10th). Total anti oxidant status was estimated using DPPH method [15] and lipid profiles using cholesterol oxidase-peroxidase (CHOD / POD) method [16] for two weeks (8th & 10th).

Statistical analysis:

All variables were expressed as means \pm SD. Group differences of continuous variables were compared using ANOVA followed by Newman Keuls *posthoc* test. For all analyses, a P value $<$ 0.05 was considered to be statistically significant. All analyses were performed using INSTAT 1.12 (Graph-Pad Software, Inc., San Diego, CA).

Results

In the present study, results showed that increased blood glucose levels were increased in streptozotocin induced group. Control and aspirin treated groups showed equal blood glucose levels and demonstrated significant effects at all time points, except at 1st, 8th and 10th weeks. The blood glucose levels of streptozotocin induced, control and aspirin treated groups serum levels were represented in table 1 (1-10 weeks). The present study reveals that, the total antioxidant status is decreased in streptozotocin induced group and increased aspirin treated group, it shows very protective action of aspirin pretreatment in diabetes and given statistically very significant ($p < 0.0001$) effects for two weeks. Lipid profiles i.e. total cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol levels were increased in streptozotocin induced group (except HDL- cholesterol) and remained low in aspirin treated group (except HDL-cholesterol). Upon statistical analysis (ANOVA) among different groups; control, streptozotocin treated and streptozotocin + aspirin for two weeks (8th and 10th) treated had a statistically significant effect on for lipid profile, except HDL-cholesterol for two weeks. The total antioxidant status and lipid profiles of streptozotocin induced, control and aspirin treated group were shown in figure 1, 2 & 3 respectively (8th & 10th weeks).

Table: 1 Blood glucose levels in n5-Stz (in 10 weeks results)

	I	II	III	IV	V	VI	VII	VIII	X
Weeks/ Groups	RANDOM	RANDOM	RANDOM	FBS	FBS	FBS	FBS	FBS	FBS
Control	105 \pm 13.6	126.1 \pm 4.5	125.5 \pm 7.9	113.8 \pm 13.8	108.5 \pm 12.3	99.5 \pm 9.6	107.1 \pm 13	71.1 \pm 53	76.7 \pm 56
n5 Stz	128.8 \pm 10.5	165.5 \pm 21.4	171.5 \pm 20.6	146.5 \pm 17.2	148.1 \pm 16	143.5 \pm 12.1	155.5 \pm 21	167 \pm 64	164.2 \pm 54.2
Stz+aspirin	106.8 \pm 24	136.6 \pm 9.3	145.8 \pm 4.6	109.1 \pm 26	98.5 \pm 13.8	105.6 \pm 16.6	106 \pm 18	82.2 \pm 55	85.9 \pm 36
p-value	0.0515	0.0005	$p < 0.0001$	0.0096	$p < 0.0001$	$p < .0001$	0.0002	0.1	0.1
Significance	Ns	***	***	**	***	***	***	Ns	Ns

Values are mean \pm SD (n = 6). *** $P < 0.0001$

Ns - Not significant

** $P < 0.001$

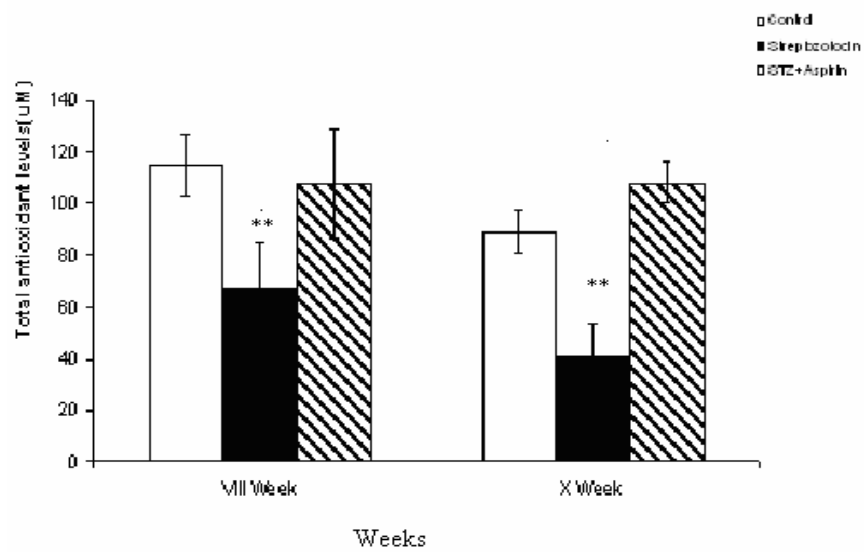


Figure: 1

Values are mean \pm SD (n =6).

** $P < 0.001$

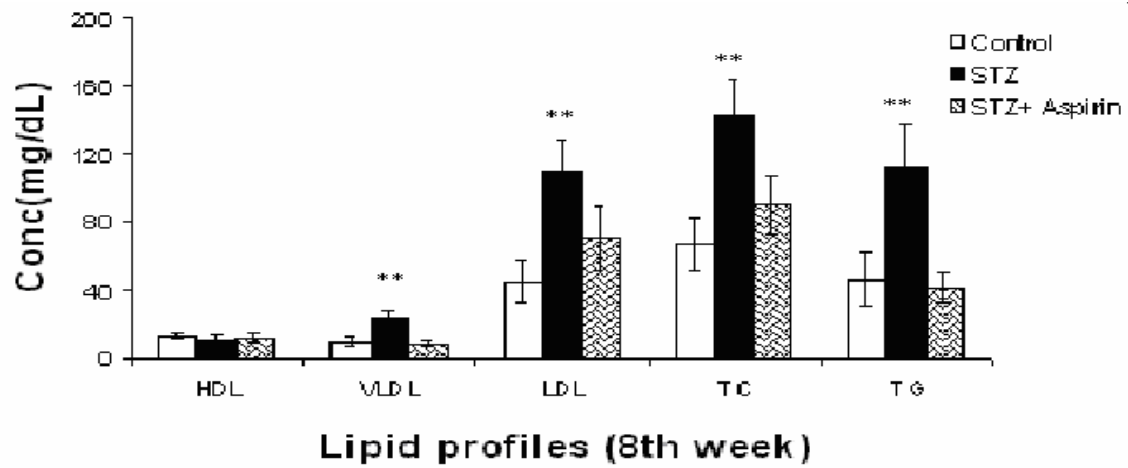


Figure: 2

Values are mean \pm SD (n =6).

** $P < 0.001$

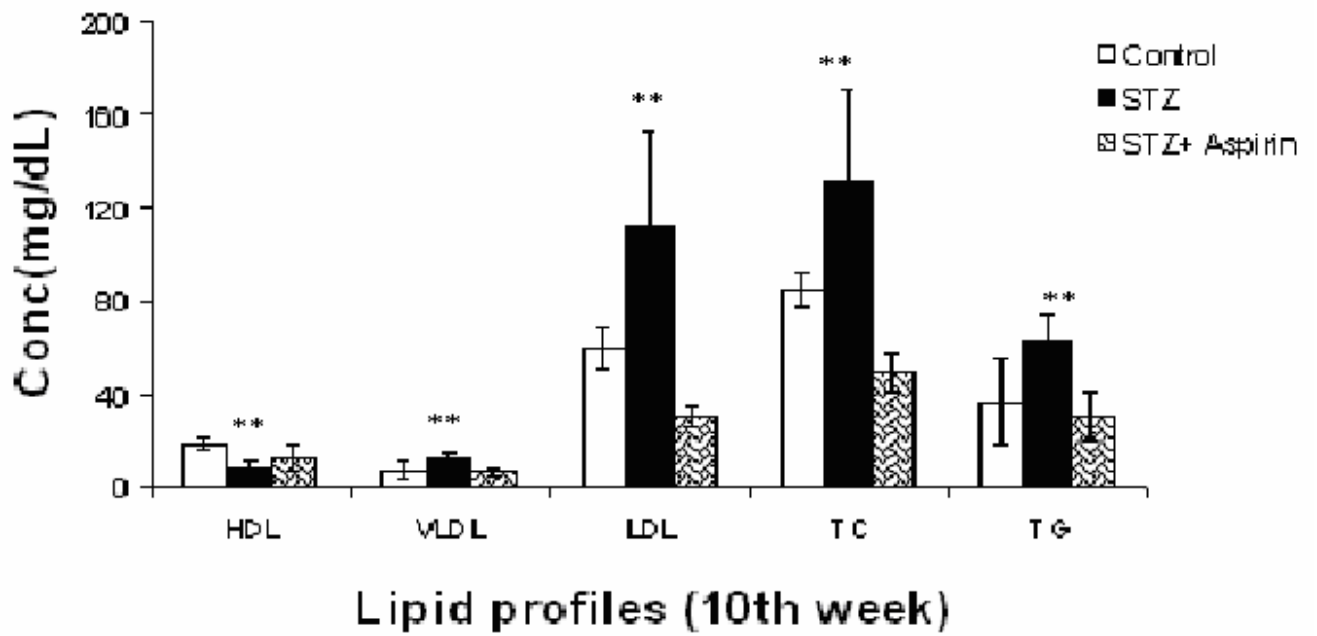


Figure: 3

Values are mean \pm SD (n =6).

** $P < 0.001$

Discussion

Salicylates inhibit IKK-B activity (I κ B kinase as stress kinase) and restore insulin sensitivity, both *in vitro* and *in vivo* [12]. Recently reported treatment of nine type 2 diabetic patients for two weeks with high dosages of aspirin (7g/day) resulted in reduced hepatic glucose production and fasting hyperglycemia and increased insulin sensitivity [17]. A previous study reported that aspirin stimulates insulin as well as glucagons secretion and increases glucose tolerance in normal and diabetic subjects [18]. Another reported that acetyl salicylic acid (ASA) alleviates glucose intolerance in maturity onset diabetics by a direct enhancement of insulin secretion[19]. In our present ten weeks study, blood glucose levels were significantly decreased in aspirin treated diabetic rats (except 1st, 8th & 10th weeks).

The protective effects of antioxidants on oxidative stress-induced insulin resistance could relate to their ability to preserve the intracellular redox balance (neutralizing ROS) or, analogous to pharmacological agents (e.g., salicylates), to block the activation of stress-sensitive kinases [12]. Salicylate has a well-known antioxidant property. Hydroxyl free radicals, which are generated *via* the iron-catalyzed Haber-Weiss reaction[11], or alternatively, *via* nitric oxide-related mechanisms [20], react with salicylate and generate 2, 3 and 2, 5-dihydroxybenzoic acids (DHBAs). The formation of DHBAs after systemic administration of salicylate is used as an index of hydroxyl radical generation in many tissues [21]. The present study results revealed that aspirin produced significant increase of total antioxidant status in diabetic rats' 8th & 10th weeks. In all lipoprotein levels were decreased and HDL-cholesterol level was increased in aspirin treated rats [22-24]. In our present study aspirin reduced the TG (triglycerides) levels as a result of decreased hepatic synthesis of VLDL (very low density lipoprotein) and decreased total cholesterol levels (TC) through LDL-cholesterol and there was a trend toward increased HDL-cholesterol. Finally aspirin might be improved the lipid profiles (TC, TG, LDL & VLDL except HDL-cholesterol) in diabetic rats.

Conclusion

The present study results indicate that aspirin pretreatment seems to protect pancreas from damage caused by STZ (streptozotocin) and maintains good lipid profile in diabetic rats and increases insulin sensitivity. It also brings about improvement of antioxidant status in diabetes mellitus.

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