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EC PHARMACOLOGY AND TOXICOLOGY

Research Article

Evaluation of Sinapic Acid on STZ Induced Hepatotoxicity in Diabetic Wistar Rats

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Received: March 24, 2022; **Published:** April 29, 2022

Abstract

Currently, diabetes is common chronic and life-threatening disease of civilization. Diabetic complications are related to organ toxicity like cardiotoxicity, nephrotoxicity, neurotoxicity, liver toxicity etc. Hepatotoxicity includes liver cirrhosis, fatty liver, hepatic failure. Sinapic acid is phenolic acid proven with anti-inflammatory, antioxidant, antibacterial and neuroprotective in diabetes induced neuropathy. The present work was aimed to evaluate hepatoprotective activity of sinapic acid against STZ-induced hepatotoxicity in diabetic Wistar rats. Animals were divided into six groups (n = 6). Diabetes was induced by single dose of STZ injection 55 mg/kg i.p. After confirmation of hyperglycemia Sinapic acid was administered orally (5, 10 and 20 mg/kg) for 4 weeks. Oxidative stress (NO, MDA), antioxidant enzymes (SOD, GSH), biochemical parameters (SGPT, SGOT, TP, bilirubin) and histopathology of liver were examined. The result indicates, Sinapic acid have decreased oxidative stress, SGPT, SGOT, bilirubin levels and increased antioxidant enzymes and total protein. Morphological changes of liver tissue were normalized by Sinapic acid. Thus, Sinapic acid is hepatoprotective in diabetes induced hepatotoxicity.

Keywords: *Sinapic Acid; STZ; Diabetic Liver Toxicity; Antioxidant; Oxidative Stress; Biochemical Parameters; Histopathology*

Introduction

Diabetes Mellitus is endocrine metabolic disorder which is mainly characterized by high blood glucose level and low insulin secretion [1]. Diabetes is associated with liver toxicity and includes many liver disorders like increasing liver enzyme, fatty liver disease, cirrhosis, hepatocellular carcinoma, and acute liver failure etc [2]. Free radicals play essential role in tissue damage [3]. This free radical leads to oxidative stress related tissue damage. In hepatotoxicity elevated level of reactive oxygen species decrease the action of antioxidants and results into hepatic damage [4]. Streptozotocin (STZ), an anticancer agent, is used to induce diabetes in experimental animals and study diabetic complications. It shows cytotoxic and carcinogenic effects on liver and pancreas. STZ has destructive effect on pancreatic beta cell causing hyperglycemia. Hyperglycemia induced oxidative stress is a key contributor in various diabetic complications [5,6].

The phytochemicals viz. phenolic acids are found in plants and used in treatment of liver diseases, renal diseases, cardiac disorders, neuronal disorders and many others. They are also used in other complications like diabetes and cancer. These plant phytochemicals are less harmful than synthetic drugs and shows various biological activities viz. antioxidant, anti-inflammatory, antiapoptotic etc [6,7].

Sinapic acid (4-hydroxy-3,5-dimethoxycinnamic acid) is hydroxycinnamic acid belongs to phenylpropanoid family [8]. Other common hydroxycinnamic acids are ferulic, caffeic and coumaric acid [9]. Sinapic acid mainly found in citrus fruits like lemon, orange, mango, avocado, barriers include strawberries, cranberries and its concentration is varies in every fruit [10]. Sinapic acid and its derivatives also found in various vegetables like cabbage, turnip, leaf mustard, white onion, red onion, garlic etc [11]. Wheat, rice, spices, oil seeds and cereals are another sources of sinapic acid [10]. Sinapic acid is considered as a antioxidant, antimicrobial, anti-anxiety, anti-inflammatory, anticancer, antihyperglycemic [11]. Sinapic acid is proven for its neuroprotective activity in diabetic neuropathy [6]. Derivatives of Sinapic acid are characterised as acetylcholinesterase inhibitor such as syringaldehyde, sinapine, 4-vinylsyringol [12]. Sinapic acid treatment is used in retinopathy, nephropathy, cardiopathy, neuropathy [13].

Oxidative stress induced by hyperglycemia is responsible for diabetic complications, so natural phytochemicals with potent antioxidant activity can be evaluated in the treatment [6]. No literature was found indicating protective activity of sinapic acid in diabetic hepatotoxicity. Thus, present study was designed for (a) to evaluate effect of STZ and sinapic acid treatment on oxidative stress i.e. nitric oxide (NO), lipid peroxidation (MDA) and antioxidant enzymes i.e. superoxide dismutase (SOD), reduced glutathione (GSH) (b) to evaluate hepatic biochemical parameters viz. SGPT, SGOT, total protein and bilirubin (c) to investigate histological changes in liver tissue [14].

Materials and Methods

Animal housing

The experimental protocol was approved by the Institutional Animal Committee (MGV/PC/CPCSEA/XXXVIII/01/2021-22/01) and the experiment was carried out according to the guidelines of Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental design

The rats were fasted overnight. Diabetes was induced by a single dose of STZ 55 mg/kg i.p. injection. STZ was prepared in 0.1M sodium citrate buffer. The animals which showed blood glucose level above 200 mg/dl after 72 hrs of STZ administration were considered as diabetic rats and selected for further experimental study [6]. These rats were divided into six groups (n = 6) as follows:

- Group 1 (Normal): Non-diabetics rats received a normal diet and served as control.
- Group 2 (Diabetic control): Diabetic control rats received STZ 55 mg/kg, i.p.
- Group 3 (STZ + SP1): Received STZ + Sinapic acid 5 mg/kg, p.o.
- Group 4 (STZ + SP 2): Received STZ + Sinapic acid 10 mg/kg, p.o.
- Group 5 (STZ + SP 3): Received STZ + Sinapic acid 20 mg/kg, p.o.
- Group 6 (STZ + Standard): Received STZ + Silymarin 100 mg/kg, p.o.

Antioxidant and oxidative study

Superoxide dismutase (SOD): To 0.05 ml of liver tissue supernatant, 2.0 ml of carbonate buffer and 0.5 ml of EDTA solution was added. By addition of 0.5 ml of epinephrine reaction was started. The change in optical density was measured at 480 nm for 5 minutes in every 1 minute [15].

Reduced glutathione (GSH): Glutathione concentration was measured by using Sedlak and Lindsay method. To 1 ml of 10% TCA, 1.0 ml of liver tissue homogenate was added and mixture was centrifuged. 1ml of supernatant was added with 0.5 ml of DTNB (Ellmans reagent) and 3 ml of phosphate buffer (pH 8.0). The colour developed was measured at 412 nm [15].

Lipid peroxidation (MDA)

Malondialdehyde is a marker of lipid peroxidation. To 0.1 ml of liver tissue homogenate 2 ml of TBA-TCA-HCL reagent (1:1 ratio) were added and placed in water bath for 15 minutes at 37°C, cooled and centrifuged at room temperature for 10 minutes at 1000 rpm. The absorbance of clear supernatant was measured at 535 nm [15].

Nitric oxide (NO)

The nitrite level in tissue sample was measured spectrophotometrically. To liver tissue homogenate Griss reagent was added (1:1 ratio) [freshly prepared 2% sulfanilamide in 5% HCl and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride]. 1 ml of distilled water was added and stirred for 5 minutes and the reading was taken at 550 nm using a spectrophotometer [15].

Biochemical study

Blood glucose level was checked after 72 hrs of STZ injection by using glucometer (Dr. Morepain) and for this blood was collected from tail vein under light anaesthesia.

After 4 weeks of treatment, biochemical parameters viz. Alanine aminotransferase (ALT/SGPT), Aspartate aminotransferase (AST/SGOT), Total protein (TP), Direct, indirect and total bilirubin were evaluated by using biochemical kits (Autospan kit) and for this blood was collected from retro orbital plexus by using fine capillary under light anaesthesia.

Histopathological examination

The liver tissue was fixed in 10% formalin solution and sent to laboratory for histopathological examination. Slides were observed in the light microscope under 10X and 40X magnification power.

Biostatistical analysis

All presented data were measured as mean ± SEM and evaluated by one-way ANOVA followed by multiple comparison Dunnett's test.

Result

Oxidative and antioxidant parameters

STZ injection induces hyperglycemia and animals showing blood glucose level > 200 mg/dl are further selected in study. This sustained hyperglycemia causes imbalance between oxidative stress and antioxidants. Oxidative stress markers NO and MDA were found to be increased in control group than normal. Sinapic acid and standard drug treatment have reduced these biomarkers significantly ($p < 0.001$). SP1 have reduced MDA but statistically non-significant. SOD and GSH antioxidant enzymes were decreased significantly. Sinapic acid treatment have increased SOD and GSH but statistically non significant (Table 1 and figure 1).

Treatment	SOD	GSH	NO	MDA
Normal	1.177 ± 0.367	1.018 ± 0.351	0.473 ± 0.158	0.228 ± 0.095
Control	0.462 ± 0.139	0.158 ± 0.042	1.977 ± 0.215	1.485 ± 0.256
STZ + SP1	0.882 ± 0.294$^{\#}$	0.377 ± 0.138$^{\#}$	1.057 ± 0.194***	1.173 ± 0.210$^{\#}$
STZ + SP2	1.002 ± 0.321$^{\#}$	0.450 ± 0.104$^{\#}$	0.905 ± 0.215***	0.645 ± 0.204**
STZ + SP3	1.051 ± 0.320$^{\#}$	0.792 ± 0.165$^{\#}$	0.642 ± 0.079***	0.469 ± 0.161***
STZ + Std	1.336 ± 0.293$^{\#}$	0.980 ± 0.307*	0.614 ± 0.119***	0.221 ± 0.050***

Table 1: *Effect of Sinapic acid on hepatic oxidative and antioxidant parameters of rat.*

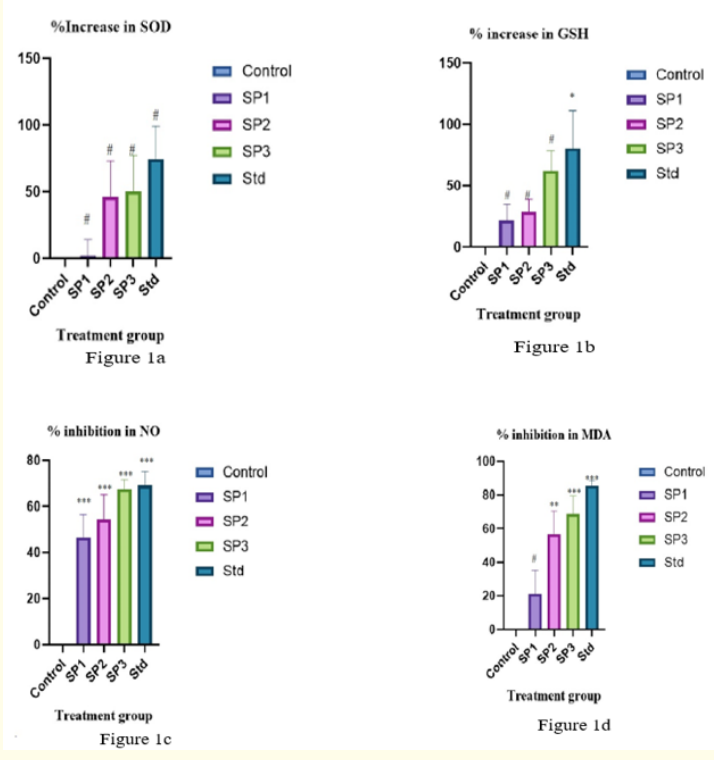

Figure 1: *Changes in oxidative stress biomarkers NO (Figure 1c), MDA (Figure 1d) and antioxidant enzymes SOD (Figure 1a), GSH (Figure 1b). #: Represents non significant changes as compare to control and *: Indicates significant changes. *: Indicates P < 0.05, **: Indicates P < 0.01, ***: Indicates P < 0.001.*

Biochemical parameters

Glutamic-oxalacetic transaminase (SGOT) and glutamic-pyruvic transaminase (SGPT) and bilirubin were measured as biomarkers of liver dysfunctioning and toxicity. In diabetic control group level of SGPT and SGOT increases due to hepatic toxicity and after treatment of sinapic acid it was significantly decreased that shows protective effect. Total protein level were decreased in diabetic control group and improved significantly ($p < 0.001$) with sinapic acid treatment as shown in table 2. Direct, indirect and total bilirubin was significantly increased in control group and decreased significantly ($p<0.001$) with sinapic acid treatment (Table 2 and figure 2 and 3).

Treatment	SGPT	SGOT	TP	Direct Bilirubin	Indirect Bilirubin	Total Bilirubin
Normal	23.51 ± 4.44	70.84 ± 2.95	7.07 ± 0.15	9.43 ± 0.86	5.56 ± 0.65	14.98 ± 1.24
Control	77.30 ± 14.42	98.98 ± 3.56	5.98 ± 0.16	156.26 ± 8.00	41.64 ± 6.85	198.9 ± 10.71
STZ+SP1	66.52 ± 5.56$^{\#}$	70.37 ± 8.26$^{\#}$	7.20 ± 0.22*	104 ± 23.751$^{\#}$	12.51 ± 4.46***	116.63 ± 27.16**
STZ+SP2	45.01 ± 12.78$^{\#}$	60.02 ± 8.83$^{\#}$	7.99 ± 0.41***	66.82 ± 21.62***	14.56 ± 4.85***	81.38 ± 26.17***
STZ+SP3	44.79 ± 9.85$^{\#}$	34.62 ± 10.25*	9.45 ± 0.13***	24.44 ± 2.83***	5.76 ± 0.96***	30.2 ± 1.89***
STZ+ STD	24.15 ± 6.69**	76.22 ± 30.03$^{\#}$	8.92 ± 0.32***	28.26 ± 6.04***	8.39 ± 1.78***	36.66 ± 6.46***

Table 2: *Effect of Sinapic acid on hepatic biochemical parameters of rat.*

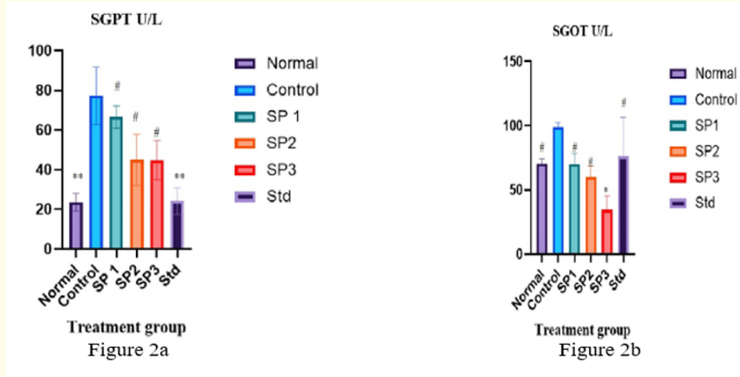

Figure 2: *Changes in SGPT (Figure 2a) and SGOT (Figure 2b) in normal, control and treated groups. # represents non significant changes as compare to control and *indicates significant changes. *: Indicates P < 0.05, **: Indicates P < 0.01, ***: Indicates P < 0.001.*

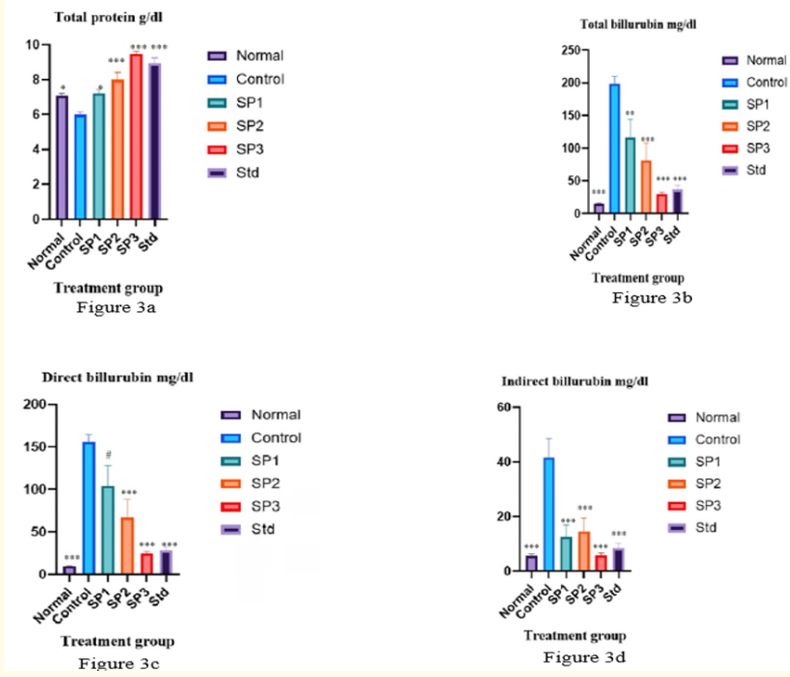

Figure 3: *Changes in Total protein (Figure 3a), total bilirubin (Figure 3b), direct (Figure 3c) and indirect (Figure 3d) bilirubin in normal, control and treated groups. #: Represents non significant changes as compare to control and *: Indicates significant changes. *: Indicates P < 0.05, **: Indicates P < 0.01, ***: Indicates P < 0.001.*

Histopathological examination

The histological aspect of liver cells section of normal, diabetic control and treated groups are shown below. Cellular degeneration, excessive vacuolization, infiltration of leukocytic cell due to accumulation of leukocyte was observed in diabetic hepatocytes. The diabetic groups were treated with sinapic acid and silymarin shown protective effect on liver cells. The damage by STZ was found to be normalised after treatment as shown in figure 4.

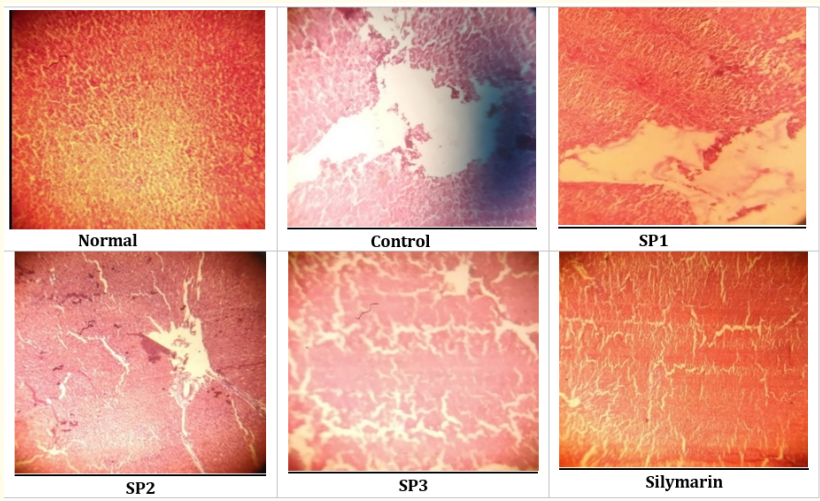

Figure 4: *Histopathological changes in liver tissue section of normal, control and treated groups.*

Discussion

Streptozotocin (STZ) is alkylating agent belongs to class nitrosourea. In humans it is used to treat tumors like lymphoma and sarcomas. STZ was the well-known hepatotoxin and causes hepatocellular carcinoma in rats. STZ has definite toxic effect on pancreatic beta cells [24]. Single dose of STZ (45 to 60 mg/kg, i.p.) shows hyperglycaemic effect in rats and induces diabetes. STZ induced diabetic complication is widely used preclinical model [6].

In STZ induced diabetic rats, hyperglycemia further induces oxidative stress resulting into various complications. So, agent that has potent antioxidant activity can be preferred in the treatment. As natural medicines are associated with many adverse effects, natural phytoconstituents are safe and efficacious alternatives [6]. Many phenolic acids are known to avert drug induced acute liver failure attributed to their antioxidant effects. Hence, the study of phenolic acid is more focused as safe and effective drug treatment for various metabolic syndrome [24].

Sinapic acid ameliorates hyperglycemia dose dependently. Therefore, sinapic acid is known as antihyperglycemic agent in diabetic rats [6]. Sinapic acid (4-hydroxy-3,5-dimethoxycinnamic acid) is found in plant including citrus fruits, vegetables, oil seed, cereals, rapeseeds, wheat, rice etc. Sinapic acid is identified as potent antioxidant; it has greater antioxidant property as compared to other polyphenolic acid [16]. Hence the present work was undertaken to evaluate effect of sinapic acid in diabetic hepatotoxicity.

Silymarin used as a standard drug which belongs to family Aster (Asteraceae or Compositae). Silymarin shows antioxidant, immunomodulatory, antiviral, anti-proliferative and anti-fibrotic effect. It acts on DNA and RNA. It nourishes the uniformity of hepatic cell

membrane and hinders the toxic matter. It is phenolic in nature hence shows better antioxidant property and affects on reactive oxygen species. In hepatic cell silymarin elevate the protein synthesis by restoring RNA polymerase activity [24]. In the present study, treatment of 100 mg/kg p.o. dose of silymarin showed significant protective effect on diabetic liver toxicity caused by STZ.

Unsaturated lipid oxidation process which is surrounded by cell membrane is known as lipid peroxidation. Malondialdehyde (MDA) is a biproduct of lipid peroxidation. In this process various oxidants harms the cell membrane and enhanced the toxicity. Administration of STZ inhibits the detoxifying effect on liver. Malondialdehyde has chemotactic effect elaborate this injury. Therefore, hepatocytic injury is outcome of lipid peroxidation in diabetic rats. It is indicated by increase level of MDA in STZ induced diabetic rat [17]. Nitric oxide level has significantly increased in STZ induced diabetic rats and acts as an indicator of increased oxidative stress [18]. High concentration of GSH and SOD in liver tissue provides a strong tissue defence against oxidative stress and lowers the hepatotoxic effect that is occurring due to STZ [3]. Oxidative stress markers NO and MDA were found to be increased in control group than normal. Sinapic acid and standard drug treatment have reduced these biomarkers significantly ($p < 0.001$). SP1 have reduced MDA but statistically non-significant. SOD and GSH antioxidant enzymes were decreased significantly. Sinapic acid treatment have increased SOD and GSH but statistically non-significant.

In biochemical parameters, level of SGPT, SGOT and bilirubin in injured liver tissue has been increased; whereas level of total protein has been decreased [19]. Treatment of sinapic acid repairs all these disturbed levels. Serum protein concentration was determined by biuret method [20]. STZ causes significant decrease in level of total protein, albumin and globulin [21].

Cellular degeneration, excessive vacuolization, infiltration of leukocytic cell due to accumulation of leukocyte was observed in diabetic hepatocytes. The diabetic groups were treated with sinapic acid and silymarin shown protective effect on liver cells. The damage by STZ was found to be normalised after treatment. Administration of 10 mg/kg and 20 mg/kg sinapic acid have restored histological change in liver cell. It improved vacuolization of cell due to cellular degeneration [1].

Conclusion

The present study indicates protective effect of sinapic acid in treatment of diabetic hepatotoxicity. This protective effect is attributed to its antioxidant, anti-inflammatory and cytoprotective action. Reversal of total protein, SGPT, SGOT and bilirubin changes indicates hepatoprotection by sinapic acid supported by histopathological study. Thus, sinapic acid can be a promising agent in clinical treatment of diabetic hepatotoxicity.

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