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ORIGINAL RESEARCH ARTICLE

Preventive Effect of Syringic acid on Hepatic Marker Enzymes and Lipid Profile against Acetaminophen-Induced Hepatotoxicity Rats

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ABSTRACT

Syringic acid is a phenolic that exists in nature and is the major component of some traditional medicinal herbs. We investigated the hepatoprotective and antihyperlipidaemic potential of syringic acid against acetaminophen (APAP) (a single intraperitoneal injection 750 mg/kg BW) induced hepatotoxicity in male albino Wistar rats. APAP rats inhibited increased liver marker enzyme activities aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase levels and lipid profile. Rats when treated with syringic acid at different concentrations (25, 50 and 100 mg/kg BW, p.o.) caused a significant decreased serum marker enzyme activities. It also decreased the levels of very low density lipoprotein cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C) while high density lipoprotein cholesterol (HDL-C) phospholipids significantly increased. It also decreased the levels of total cholesterol, triglycerides, phospholipids and free fatty acids in the plasma and tissues of liver and kidney. The activity of syringic acid (50 mg/kg) is comparable with silymarin, a known hepatoprotective drug. Syringic acid, thus exhibits hepatoprotective and antihyperlipidemic activity.

Keywords: Acetaminophen, Anti-hyperlipidemia, Hepatic marker enzymes, Syringic acid.

INTRODUCTION

Acetaminophen (APAP) is one of the most frequently used safe analgesic and antipyretic drugs that are safe when ingested at therapeutic levels however, an overdose causes hepatotoxicity However, it cause centrilobular hepatic necrosis, renal failure and even death in humans and experimental animals when taken in overdoses or in moderate doses in combination with other drugs or alcohol [1]. The liver is critical in the normal metabolism of energy substrates especially lipid metabolism. Any clinical defect or toxic drug that alters the normal balance can have a profound impact on lipid metabolism [2].

Syringic acid (4-hydroxy-3, 5-dimethoxybenzoic acid) (**Fig.1**) is a phenolic compound derived from edible plants and fruits is used as a sedative and local anesthetic, with antitussive and expectorant effect. It is widely used as a medicine for bronchitis [3]. Recently syringic acid has been demonstrated to show strong antioxidant, antiproliferative [4-5], anti-endotoxic [6], anti-cancer activity [7] and hepatoprotective activity [8]. In this study, we evaluated the effect of syringic acid on hepatic marker enzymes and lipid profiles against APAP induced hepatotoxicity in albino Wistar rats. Silymarin is a standardised extract of the flavonolignans silybinin, silydianin and silychris tin, and other minor compounds from the seeds of *Silybum marianum* (L.) Gaertn. Silymarin is able to provide hepatoprotection against poisoning by ethanol, galactosamine, thioacetamid e, halothane, acetaminophen and carbon tetrachlor ide [9] hence, it was used a reference drug in this study.

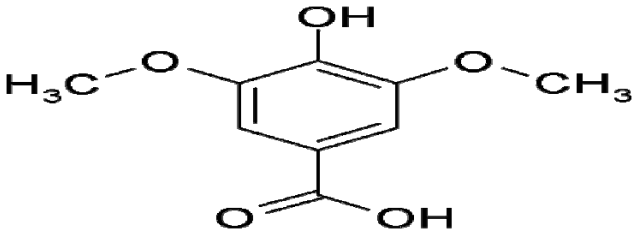

Figure.1 Chemical Structure of syringic acid ($C_9H_{10}O_5$)

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MATERIALS AND METHODS

Animals

Adult male albino rats of Wistar strain weighing about 140–160 g were procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University and maintained in an air-conditioned room (25 ± 2ºC) with a 12 h light/12 h dark cycle. Feed and water were provided *ad libitum.* All the experimental studies were conducted in the Department of Biochemistry, Faculty of Science, Annamalai University, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH 1985); the experimental study was approved by the Ethical Committee of Rajah Muthiah Medical College and Hospital (Reg No.160/1999/CPCSEA, Pro. No.595), Annamalainagar.

Chemicals

Acetaminophen (APAP) and syringic acid were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All other chemicals used in this study were of analytical grade obtained from E. Merck and HIMEDIA (Mumbai, India).

Experimental induction of hepatitis

Hepatotoxicity was induced in animals by an intraperitoneal injection of acetaminophen (750 mg/kg body weight) in a freshly prepared physiological saline solution kept in warm boiling water bath and used after cooling at 37°C, as a single dose on the 1st day [10].

Experimental design

The animals were randomly divided into seven groups of six animals each. Syringic acid and silymarin were administered orally once in a day in the morning for 6 days. The syringic acid was suspended in 0.9% saline vehicle solution and fed by intubation. Group I Normal control rats received 0.9% saline for 7 days, Group II served as normal rats treated with syringic acid (100mg/kg BW, p.o.) in 0.9% saline for 7 days, Group III served a hepatotoxic rats (APAP 750 mg/kg BW, i.p.), Group IV, V, VI served as hepatotoxic rats treated with (25, 50 and 100 mg/kg BW, p.o.) of syringic acid and Group VII served as hepatotoxic rats treated with silymarin (25 mg/kg BW, p.o.).

On 8th day morning the animals were sacrificed by cervical dislocation. The blood was collected in clean dry test tubes and allowed to coagulate at ambient temperature for 30 min. Serum was separated by centrifugation at 2000 rpm for 10 min. The blood, collected in a heparinised centrifuge tube, was centrifuged at 2000 rpm for 10 min and the plasma separated by aspiration was used for estimations. The liver and kidney were immediately removed and washed in ice-cold saline to remove the blood. The tissues were sliced and homogenized in 0.1 MTris–HCl buffers (pH 7.0). The homogenates were centrifuged at 1000 rpm for 10 min at 0 °C in a cold centrifuge. The supernatants were separated and used for biochemical estimations

Biochemical estimations

Assessment of hepatic function markers

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated using the method of Reitman and Frankel [11], Alkaline phospha-tase and gamma-glutamyl transpeptidase (GGT) were estimated using the method of Kind and King [12] and Rosalki and Rau [13], respectively.

Assessment of lipid profile in plasma, liver and kidney

Plasma and tissue lipids were extracted by the methods of Folch et al [14]. Plasma and tissue total cholesterol, triglycerides, free fatty acids, and phospholipids were estimated by the methods of Allain et al [15], McGowan et al [16], Falholt et al [17], and Zilversmit and Davis [18], respectively. Plasma high density lipoprotein-C was estimated by the method of Izzo et al [19]. Low density lipoprotein-C, very low density lipoprotein-C were calculated by Friedwald's formula [20] were estimated.

Statistical analysis

Statistical analyses were performed by one-way analysis of variance (ANOVA) and groups were compared by Duncan's multiple range test (DMRT) using SPSS Software Package v.10.0. Results were expressed as mean ± S.D. for six rats in each group. A value of $p \leq 0.05$ was considered to be statistically significant.

RESULT

(**Table 1**) shows the levels of serum hepatic markers in normal and experimental rats. Intraperitoneal administration of APAP caused abnormal liver function in all rats. Activities of serum hepatic enzymes such as AST, ALT, ALP, GGT were significantly increased ($p<0.05$) in APAP treated rats. Administration of syringic acid (50 mg/kg) with APAP significantly decreased ($p<0.05$) the activities of serum hepatic markers when compared to other two doses (25 and 100 mg/kg) of syringic acid.

Table 1. Effect of syringic acid on hepatic marker enzymes in the serum of APAP induced hepatotoxic and control rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)
Control	70.08 ± 3.51[a]	34.78 ± 2.12[a]	80.56 ± 4.89[af]	2.48 ± 0.19[a]
Control + syringic acid (100 mg/kg BW)	73.04 ± 3.63[af]	30.97 ± 2.35[a]	78.87 ± 6.84[a]	2.18 ± 0.15[a]
APAP (750 mg/kg BW)	125.49 ± 10.89[b]	69.13 ± 4.83[b]	130.48 ± 12.26[b]	5.43 ± 0.41[b]
APAP + syringic acid (25 mg/kg BW)	108.17 ± 8.55[c]	58.18 ± 351[c]	118.87 ± 10.37[c]	4.49 ± 0.44[c]
APAP + syringic acid (50 mg/kg BW)	82.61 ± 3.72[d]	40.45 ± 3.24[d]	92.39 ± 6.91[d]	3.08 ± 0.47[d]
APAP + syringic acid (100 mg/kg BW)	93.63 ± 7.7.95[e]	49.38 ± 3.37[e]	108.85 ± 9.24[e]	3.68 ± 0.32[c]
APAP + silymarin (25 mg/kg BW)	79.90 ± 7.06[fd]	38.97 ± 3.24[d]	89.88 ± 4.34[fd]	2.69 ± 0.16[fd]

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a, b, c, d and f) differ significantly with each other ($P < 0.05$, DMRT).

(**Table 2**) show the effect of syringic acid on the plasma lipid profile in the control and APAP - hepatotoxic rats. The APAP induced rats had elevated levels of plasma total cholesterol (TC), triglycerides (TG), phospholipids (PL), free fatty acids (FFA), LDL-C and VLDL-C and decreased level of HDL-C when compared with control rats. APAPhepatotoxic rats treated with syringic acid and silymarin improved these lipid profiles toward normal levels.

Table 2. Effect of syringic acid on lipid profile in the plasma of APAP induced hepatotoxic and control rats

Groups	Plasma (mg/dL)						
	Total cholesterol	HDL-C	LDL-C	VLDL-C	Triglycerides	Phospholipids	Free fatty acids
Control	75.80 ± 6.13[a]	50.39 ±	14.11 ±	11.42 ±	57.12 ±	105.47 ±	50.73 ±
Control + Syringic acid (100 mg/kg BW)	72.35 ± 5.63[a]	51.93 ± 4.41[a]	20.42 ± 1.21	10.67 ± 0.69[a]	53.37 ± 3.48[a]	102.11 ± 8.73[a]	52.95 ± 3.46[ab]
APAP (750 mg/kg BW)	122.63 ± 11.92[b]	33.19 ± 2.57[c]	71.88 ± 7.65[c]	25.00 ± 2.01[b]	125.01 ± 10.08[b]	175.74 ± 12.98[b]	115.73 ± 10.39[c]
APAP + Syringic acid (25 mg/kg BW)	110.54 ± 9.45[c]	35.75 ± 2.24[c]	54.37 ± 4.86[d]	22.98 ± 2.00[c]	114.91 ± 10.03[c]	145.84 ± 7.41[c]	95.45 ± 7.02[d]
APAP + Syringic acid (50 mg/kg BW)	86.13 ± 5.94[d]	46.38 ± 3.77[d]	26.62 ± 0.98[e]	14.13 ± 1.17[d]	70.66 ± 5.89[d]	116.63 ± 8.00[d]	62.50 ± 4.62[e]
APAP + Syringic acid (100 mg/kg BW)	99.05 ± 7.87[e]	39.10 ± 3.11[c]	39.73 ± 3.33[f]	19.94 ± 1.42[e]	99.71 ± 7.12[e]	131.58 ± 14.33[e]	88.65 ± 3.42[f]
APAP + Silymarin (25 mg/kg BW)	83.46 ± 6.89[fd]	48.61 ± 2.16[da]	20.45 ± 3.41[b]	13.40 ± 1.30[d]	67.01 ± 6.50[d]	110.17 ± 10.46[ad]	59.02 ± 2.53[be]

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a, b, c, d and f) differ significantly with each other ($P < 0.05$, DMRT).

(**Tables 3**) and 4 show the effect of syringic acid on the lipid profiles of liver and kidney tissues in control and acetaminophen rats. Significantly ($p<0.05$) elevation of tissues of total cholesterol (TC), triglycerides (TG), and free fatty acids (FFA) and tissues reduction of phospholipids (PL) were observed in APAP- rats. Oral administration of syringic acid and silymarin significantly ($p<0.05$) decreased TC, TG, FFA and increased PL levels.

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Table 3. Effect of syringic acid on lipid profile in the liver of APAP induced hepatotoxic and control rats

Groups	Liver (mg/g of tissue)			
	Total cholesterol	Triglycerides	Phospholipids	Free fatty acids
Control	3.69 ± 0.30[ad]	3.55 ± 0.28[a]	19.82 ± 0.78[a]	7.74 ± 0.51[a]
Control + Syringic acid (100 mg/kg BW)	3.36 ± 0.27[a]	3.34 ± 0.20[a]	19.28 ± 1.44[a]	7.42 ± 0.42[a]
APAP (750 mg/kg BW)	7.10 ± 0.45[b]	6.86 ± 0.56[b]	8.71 ± 0.55[b]	13.84 ± 0.81[b]
APAP + Syringic acid (25 mg/kg BW)	6.04 ± 0.33[c]	5.64 ± 0.57[c]	11.55 ± 0.99[c]	12.18 ± 0.86[c]
APAP + Syringic acid (50 mg/kg BW)	3.92 ± 0.18[d]	4.09 ± 0.25[d]	16.88 ± 1.04[d]	9.06 ± 0.54[d]
APAP + Syringic acid (100 mg/kg BW)	5.05 ± 0.42[e]	5.11 ± 0.17[e]	14.48 ± 1.03[e]	10.96 ± 0.67[e]
APAP + Silymarin (25 mg/kg BW)	3.72 ± 0.30[ad]	4.00 ± 0.20[d]	16.88 ± 1.04d	8.22 ± 0.43[f]

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a, b, c, d and f) differ significantly with each other ($P < 0.05$, DMRT).

Table 4. Effect of syringic acid on lipid profile in the kidney of APAP induced hepatotoxic and control rats

Groups	Kidney (mg/g of tissue)			
	Total cholesterol	Triglycerides	Phospholipids	Free fatty acids
Control	3.51 ± 0.30[a]	4.80 ± 0.21[ad]	15.82 ± 1.15[a]	4.12 ± 0.37[af]
Control + Syringic acid (100 mg/kg BW)	3.25 ± 0.28[a]	4.51 ± 0.11[a]	15.20 ± 1.29[ab]	3.92 ± 0.29[a]
APAP (750 mg/kg BW)	6.04 ± 0.29[b]	7.10 ± 0.43[b]	8.17 ± 0.55[b]	8.08 ± 6.72[b]
APAP + Syringic acid (25 mg/kg BW)	5.22 ± 0.39[c]	6.04 ± 0.39[c]	9.42 ± 0.80[d]	6.72 ± 0.52[c]
APAP + Syringic acid (50 mg/kg BW)	4.12 ± 0.43[d]	4.92 ± 0.30[d]	13.86 ± 0.75[e]	4.36 ± 0.33[af]
APAP + Syringic acid (100 mg/kg BW)	4.59 ± 0.28[e]	5.41 ± 0.38[e]	11.82 ± 0.91[f]	6.20 ± 0.19[d]
APAP + Silymarin (25 mg/kg BW)	4.09 ± 0.30[d]	4.77 ± 0.20[ad]	14.13 ± 1.10[eb]	4.20 ± 0.25[af]

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a, b, c, d and f) differ significantly with each other ($P < 0.05$, DMRT).

DISCUSSION

APAP is a widely used analgesic and antipyretic drug, known to cause hepatotoxicity in experimental animal and human at high doses. It is mainly metabolized in liver to excreatable glucuronide and sulphate conjugate. However, hepatotoxicity of toxic metabolites, when a part of APAP is activated by hepatic cytochrome P_{450} to a highly reactive metabolite *N*-acetyl-*p*-benzoquinoneimine, which is normally conjugated with GSH and excreted in the urine as, conjugates. Over dose of APAP leads to mitochondrial dysfunction followed by acute hepatic necrosis damage to the sctctural integrity of liver is reflected by an increase in level of serum transaminonases these are cytoplasmic in

location and are released into circulation after cellular damage [21]. Present study revealed a significant rise in the level of serum marker enzymes viz, AST, ALT, ALP and GGT level on exposure to APAP, indicating considerable hepaocellular injury. The observed decrease in the activities of these enzymes shows that syringic acid, to some extent, preserves the structural integrity of the liver from the toxic effect of APAP.

Liver injury causes the accumulation of abnormal amounts of fats, predominantly triglycerides in the parenchymal cells. Triglycerides accumulation can be thought of as resulting from an imbalance between the rate of synthesis and the rate of release of triglycerides by the parenchymal cells into the systemic circulation [22]. The elevated plasma triglycerides levels observed might have been partially due to lipoprotein lipase. Modest hypertriglyceridemia occurs in association with alcohol, virus and drug induced hepatitis [23]. The mechanism of this process may involve reduction of lipolytic enzymes, namely, hepatic triglyceride lipase and lipoprotein lipase [22]. The reduction of these enzymes may lead to decreased removal of triglycerides from plasma and the accumulation of triglycerides in tissues. We observed significantly reduced levels of lipids in plasma and tissues of syringic acid treated rats, thus showing the beneficial effect of syringic acid against APAP-toxicity.

The serum level of LDL-C was significantly elevated in APAP animals. The phenolic compounds have been shown to form phenoxyl radicals in the presence of peroxidases. APAP drug with a tyrosine-like monophenolic structure to form phenoxyl radicals and to act as an LDL-C prooxidant [24]. The elevated level of LDL-C was significantly reduced in syringic acid treated rats may be due to the antioxidant property of syringic acid which is capable of inhibiting the LDL-C peroxidation. It has been reported that hypolipidemic drugs with antioxidant properties, may prevent LDL-C peroxidation and retard the accumulation [25]. The depleted levels of serum HDL-C in the APAP rats may be due to hypertriglyceridemia induced by reactive metabolite formed during biotransformation. Oxidative stress is one of the major pathways, which involves either oxygen derived species or N-acetyl-p-benzoquinone imine as free radical to initiate lipid peroxidation [26]. The HDL-C is a free radical scavenger and prevents peroxidation of beta lipoproteins [27]. Decreased HDL-C may be due to diminished lecithin cholesterol acyl tranferase (LCAT) activity and may also contribute to the increased cholesterol level. An increase of LDL-c and VLDL may also cause a greater decrease of HDL-C as there is a reciprocal relationship between the concentration of VLDL-C and HDL-C. In our study, the levels of LDL-C and VLDL-C were found to be increased and HDL-C decreased in APAP-treated rats. Administration of syringic acid or silymarin significantly decreased the levels of VLDL-C and LDL-C and increased HDL-C.

In conclusion, our findings demonstrate that syringic acid has hepatoprotective and antihyperli pidaemic effect, which is evidenced by the decreased levels of total cholesterol, triglycerides, free fatty acids, phospholipids, low density lipoprotein-C, very low density lipoprot ein-C and elevated levels of HDL-C in the plasma and tissues of APAP-hepatotoxicity in rats. These properties are comparable to the standard drug silymarin. The 50 mg dose showed promising hepatoprotective and antihyperlipidaem ic effect.

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