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ORIGINAL RESEARCH ARTICLE

Therapeutic Effects of Vanillic Acid on Acetaminophen-Induced Hepatotoxicity in Rats.

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ABSTRACT

Acetaminophen is a widely used analgesic and antipyretic drug. An overdose can cause life-threatening hepatotoxicity and nephrotoxicity, in humans and experimental animals. In this study the potential protective role of vanillic acid one of the major phenolic derivatives from edible plants and fruits was evaluated against the acetaminophen (APAP) induced toxicity in rats. Toxicity was induced in adult male albino rats, weighing 140–160 g, by an intraperitoneal injection of APAP (750 mg/kg body weight) for 7 days. Rats were treated with vanillic acid (100 mg/kg body weight) by oral administration. APAP intoxicated rats showed significantly increase in the levels of renal function markers such as urea, uric acid and creatinine in serum. Further, increased levels of total cholesterol, triglycerides, free fatty acids (FFA), LDL (low density lipoproteins), VLDL (very low density lipoproteins) and decreased HDL (high density lipoproteins) in plasma and tissues such as liver and kidney were observed in APAP intoxicated rats when compared to control. These parameters were reversed after the treatment with vanillic acid. Histopathological findings of vanillic acid against APAP rat's kidney confirmed the biochemical findings of this study. In conclusion, vanillic acid administration significantly decreased the level of renal function markers and showed beneficial effects on lipid profile in APAP rats.

Key Words: Acetaminophen, Vanillic acid, Lipid profile, Renal markers.

INTRODUCTION

Acetaminophen (APAP), also known as paracetamol, is most widely used in the world as an analgesic and antipyretic drug that is safe at therapeutic dosages.[1] APAP is known to cause hepatic necrosis and renal failure in both humans and animals when administered in overdoses.[2] Renal damage and acute renal failure can occur even in the absence of liver injury. Renal insufficiency occurs in approximately 1–2% of the patients with an overdose of APAP.[3]

The liver plays an important role in regulation of plasma lipoprotein metabolism[4]. The liver injury of different etiologies is often accompanied by secondary lipoproteinemia, which may lead to the development of atherosclerosis, particularly when associated with hypercholesterolemia characterized by an increase in low density lipoprotein (LDL) cholesterol [5] and decrease in high density lipoprotein (HDL) cholesterol. [6] Numerous medicinal plants and their formulations are used for liver disorders in ethno-medical practices and in traditional systems of medicine. However a satisfactory remedy for serious liver diseases is not still available, so search for effective hepatoprotective drugs are continued. Biological compounds with antioxidant properties contribute to the protection of cells and tissues against deleterious effects of ROS and other free radicals. Vanillic acid (4-hydroxyl-3-methoxy benzoic acid) (Figure 1) is a phenolic derivative from edible plants and fruits known to posses antimicrobial and antifilarial activities.[7] It is a major chemical constituent of vanilla, a neutraceutical plant. Derivatives of vanillic acid are used in Europe as an analeptic medicine. Vanillic acid has been reported to inhibit the mutagenesis induced by chemical and physical mutagens in various models.[8] It also showed chemopreventive effect in chemical carcinogenesis models in the rat.[9] Vanillic acid has been reported to have potential as an agent for treatment of sickle cell anemia. More recently,

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reports showed that vanillic acid displayed inhibited DNA-dependent protein kinase and enhanced sensitivity of cancer cells to cisplatin.[10] However, no study has been carried out on the effects of vanillic acid on renal function markers and lipid metabolism in APAP intoxicated rats. Hence, the aim of the present work was to evaluate the influence of vanillic acid administration on lipid profile (total cholesterol, triglycerides, low density lipoprotein, high density lipoprotein and very low density lipoprotein levels) and renal function markers (urea, uric acid and creatinine) in normal and APAP treated rats.

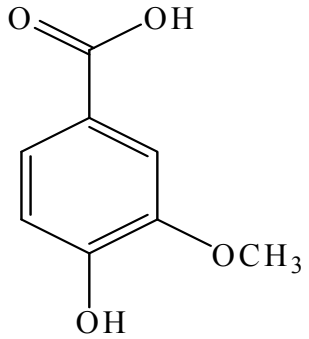

Fig.1 Structure of vanillic acid ($C_8H_8O_4$)

MATERIALS AND METHODS

Animals

Male albino Wister rats, 6-7 weeks old (weighing 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 a air conditioned room (25 ± 2° C) with the 12h light/12h 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 Rajah Muthiah Medical College and Hospital (Reg No. 160/1999/CPCSEA, Pro. No.594), Annamalainagar, Tamil Nadu.

Chemicals

Acetaminophen (APAP) and vanillic 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 design

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 first day. The rats were randomly divided into five groups of six animals each as given below. Vanillic acid and silymarin were administered orally once in a day in the morning for 7 days. The compound was suspended in 0.9% saline vehicle solution and fed by intubation.

Group I: Control rats received 0.9% saline only
Group II: Control rats + vanillic acid (100 mg/kg BW)
Group III: APAP rats (750 mg/kg BW)
Group IV: APAP + vanillic acid (100 mg/kg BW)
Group V: APAP + silymarin (25 mg/kg BW)

The experimental duration was 7 days. On the 8th day the rats were sacrificed by cervical dislocation. Blood was collected in a dry test tube and allowed to coagulate at ambient temperature for 40 min. Serum was separated by centrifugation at 2000 rpm for 10 min. The blood, collected in a heparinized centrifuge tube was centrifuged at 2000 rpm for 10 min and the plasma was separated by aspiration. Liver and kidney tissues (250 mg) were sliced into pieces and homogenised in appropriate buffer in cold condition (pH 7.0) to give 20% homogenate (w/v). The homogenate was centrifuged at 1000 rpm for 10 min at 0 °C in cold centrifuge. The supernatant was separated and used for various biochemical estimations.

Assessment of renal function markers

Urea and uric acid was estimated by Fawcett and Scott[11] and Caraway.[12] Creatinine by the method of Tietz[13] using Jaffe[14] color reaction.

Assessment of lipid profile in plasma, liver and kidney

Plasma and tissue lipids were extracted by the methods of Folch *et al.*,[15] Plasma and tissue total cholesterol, triglycerides and free fatty acids by the methods of Allain *et al*., [16] McGowan *et al*., [17], Falholt *et al.,*[18], and Zilversmit and Davis[19], respectively. Plasma high density lipoprotein-C was estimated by the method of Izzo *et al*.,.[20] Low density lipoprotein-C, very low density lipoprotein-C were calculated by Friedwald's formula. [21]

Statistical analysis

Data were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using a statistical software package (SPSS for Windows, V. 13.0,

Chicago, USA). Results are presented as means ± S.D. *P*-values < 0.05 were considered statistically significant.

RESULTS

Table 1 shows the levels of renal function markers such as urea, uric acid and creatinine in serum of normal and APAP rats. Our results showed that the levels of urea, uric acid and creatinine were significantly increased in APAP-intoxicated rats and administration of vanillic acid and silymarin brought back these renal marker levels towards normal.

Table 1. Effect of vanillic acid on the level of renal function markers in the serum of APAP-hepatotoxic and control rats

Groups	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Control	23.45 ± 2.54^{a}	1.4 ± 0.08^{a}	0.81 ± 0.04^{a}
Control + vanillic acid (100 mg/kg BW)	$24.38 \pm 2.35^{a,c}$	1.69 ± 0.09^{b}	0.87 ± 0.05^{a}
APAP rats (750 mg/kg BW)	45.91 ± 4.4^{b}	2.12 ± 0.21^{c}	1.7 ± 0.30^{b}
APAP + vanillic acid (100 mg/kg BW)	27.63 ± 3.79^{c}	$1.57 \pm 0.14^{a,b}$	0.93 ± 0.07^{a}
APAP + silymarin (25 mg/kg BW)	$25.92 \pm 2.31^{a,c}$	1.5 ± 0.08^{a}	0.88 ± 0.04^{a}

Values are given as means ± SD for six rats in each group.
Values not sharing a common superscript differ significantly at p < 0.05. (DMRT).

Table 2 and 3 shows the levels of lipid profile in plasma and tissues such as liver and kidney in control and APAP rats. An increased level of total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol, triglycerides, free fatty acids and decreased level of high density lipoprotein cholesterol were observed in APAP-intoxicated rats. Administration of vanillic acid significantly prevented the increase in lipid profile level which was brought to near normal. The effect of vanillic acid was comparable with that of standard drug silymarin.

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

Groups	Plasma (mg/dL)					
	Total cholesterol	HDL-C	LDL-C	VLDL-C	Triglycerides	Free Fatty acids
Control	70.86 ± 5.21^{a}	48.82 ± 3.94^{a}	15.19 ± 1.38^{a}	11.84 ± 1.00^{a}	59.21 ± 5.10^{a}	52.21 ± 5.20^{a}
Control + vanillic acid (100 mg/kg BW)	72.91 ± 6.65^{a}	49.11 ± 3.45^{a}	16.14 ± 1.55^{a}	12.39 ± 1.08^{a}	61.98 ± 5.41^{a}	55.73 ± 4.32^{a}
APAP rats (750 mg/kg BW)	128.02 ± 8.71^{b}	24.34 ± 1.84^{b}	82.19 ± 5.03^{b}	21.48 ± 1.84^{b}	107.43 ± 9.21^{b}	116.23 ± 11.56^{b}
APAP + vanillic acid (100 mg/kg BW)	92.12 ± 6.62^{c}	38.66 ± 3.20^{c}	27.19 ± 1.80^{c}	14.29 ± 1.13^{c}	71.43 ± 5.67^{c}	76.32 ± 5.51^{c}
APAP + silymarin (25 mg/kg BW)	82.88 ± 8.64^{d}	46.02 ± 3.45^{a}	24.34 ± 2.01^{c}	12.62 ± 0.70^{a}	63.11 ± 3.52^{a}	66.43 ± 5.17^{d}

Values are given as means ± SD for six rats in each group.
Values not sharing a common superscript differ significantly at p < 0.05. (DMRT).

Table 3. Effect of vanillic acid on lipid profile in the tissues (liver and kidney) of acetaminophen-hepatotoxic and control rats

Groups	Liver (mg/g of tissue)			Kidney (mg/g of tissue)		
	Total cholesterol	Triglycerides	Free Fatty acids	Total cholesterol	Triglycerides	Free Fatty acids
Control	3.4 ± 0.2^{a}	3.71 ± 0.3^{a}	7.62 ± 0.4^{a}	3.63 ± 0.2^{a}	3.71 ± 0.3^{a}	$3.82 \pm 0.3^{a,d}$
Control + vanillic acid (100 mg/kg BW)	3.3 ± 0.2^{a}	3.41 ± 0.1^{a}	7.34 ± 0.5^{a}	3.4 ± 0.2^{a}	4.33 ± 0.3^{a}	3.44 ± 0.2^{a}
APAP rats (750 mg/kg BW)	7.5 ± 0.5^{b}	7.82 ± 0.3^{b}	13.22 ± 0.3^{b}	8.12 ± 0.5^{b}	7.30 ± 0.3^{b}	8.2 ± 0.6^{b}
APAP + vanillic acid (100 mg/kg BW)	4.29 ± 0.3^{c}	4.84 ± 0.42^{c}	8.68 ± 0.5^{c}	4.56 ± 0.4^{c}	5.41 ± 0.3^{c}	5.3 ± 0.3^{c}
APAP + silymarin (25 mg/kg BW)	3.51 ± 0.2^{a}	4.52 ± 0.3^{c}	7.82 ± 0.3^{a}	4.21 ± 0.3^{c}	4.64 ± 0.2^{a}	4.22 ± 0.2^{d}

Values are given as means ± SD for six rats in each group.
Values not sharing a common superscript differ significantly at p < 0.05. (DMRT).

HISTOPATHOLOGY

Fig 2 histopathology of kidney (a) Normal rats showing normal glomeruli and normal tubules (20X) (b) Normal rats + vanillic acid showing normal glomeruli and tubules (20X) (c) APAP toxic rats showing cloudy swelling of tubules and glomerulosclerosis (20X) (d) APAP rats treated with vanillic acid showing mild inflammation parenchymal cells and normal glomeruli and tubules (20X), (e) APAP rats + silymarin treatment showing normal glomeruli and normal tubules.

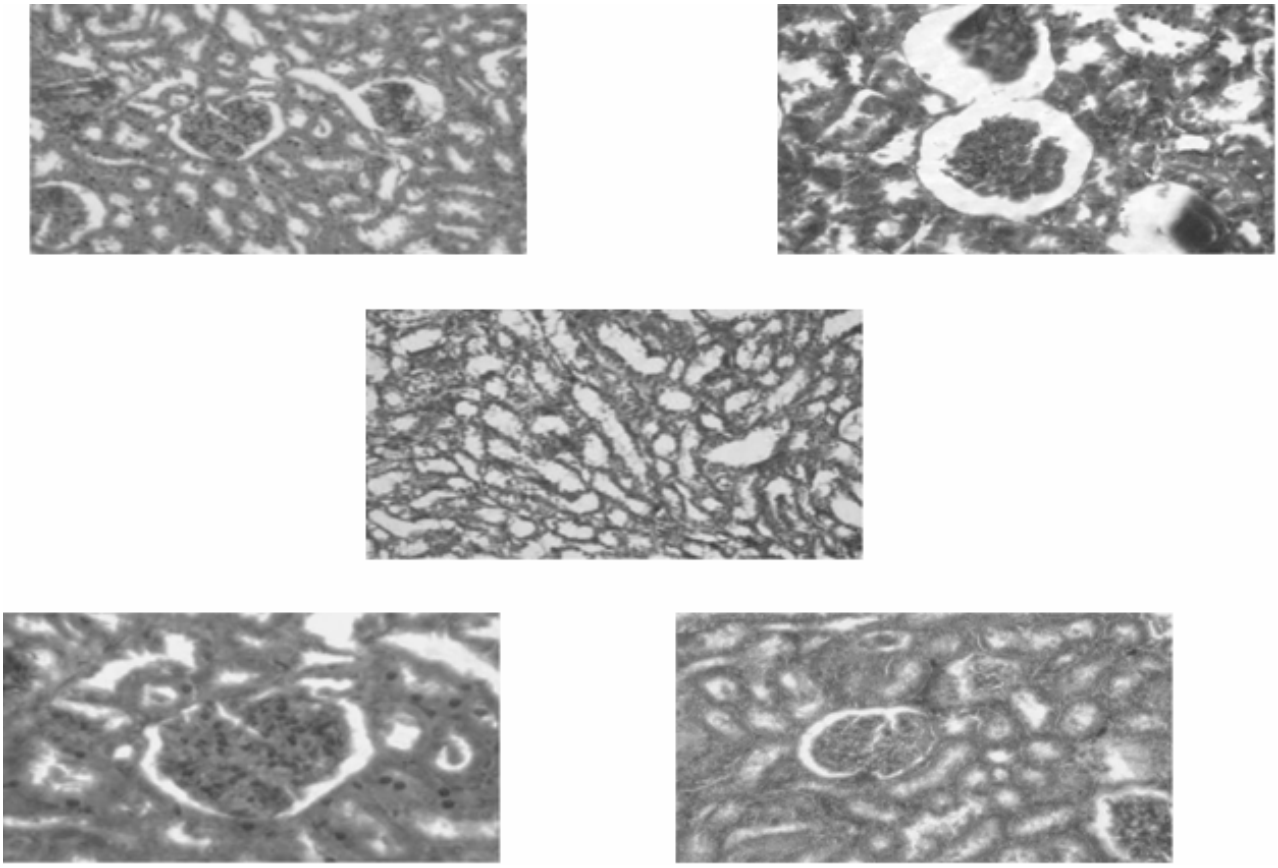

Fig 2. Histopathology of kidney (a) Normal rats showing normal glomeruli and normal tubules (20X) (b) Normal rats + vanillic acid showing normal glomeruli and tubules (20X) (c) APAP toxic rats showing cloudy swelling of tubules and glomerulosclerosis (20X) (d) APAP rats treated with vanillic acid showing mild inflammation of parenchymal cells and normal glomeruli and tubules (20X), (e) APAP rats + silymarin treatment showing normal glomeruli and normal tubules.

DISCUSSION

Acetaminophen (APAP), a widely used antipyretic-analgesic drug produces acute hepatic damage on accidental over dosage. It is established that, a fraction of acetaminophen is converted via the cytochrome P_{450} pathway to a highly toxic metabolite; N–acetyl–*p*–benzoquinamine (NAPQI) which is normally conjugated with glutathione and excreted in urine. Overdose of acetaminophen depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction and the development of acute hepatic necrosis. Several P_{450} enzymes are known to play an important role in APAP bioactivation to NAPQI. P_{450}2E1 have been suggested to the primary enzymes for acetaminophen bioactivation in liver microsomes.[22] Studies demonstrated that APAP-induced hepatotoxicity and nephrotoxicity can be modulated by substances that influence P_{450} activity. Protection against APAP-induced toxicity has been used as a test for potential hepatoprotective activity by several investigations. [23]

Hepatotoxicity and nephrotoxicity are the potential complications of APAP, which is widely, used in general medicine, and an assessment of its relative toxicity is important. A number of drugs or chemicals such as melatonin, vitamin E and N-acetyl-cysteine have been used to prevent APAP-induced hepatic and renal injury

[24]. Our earlier investigations demonstrated the protective role of vanillic acid against APAP induced hepatotoxicity and oxidative stress (data not shown) in rats. In the present study, we assessed whether the nephrotoxic effects caused by acute administration of APAP could be prevented or ameliorated by treatment with vanillic acid. Our results showed that serum urea, uric acid and creatinine levels were significantly increased in groups treated with APAP alone, demonstrating the deterioration of the renal function, in comparison with those of the control and APAP + vanillic acid groups. These findings are consistent with the results of a previous study in which APAP was administered to rats[24]. Administration of vanillic acid modified the serum urea, uric acid and creatinine levels towards normal.

The liver is the major site for the synthesis and metabolism of cholesterol, bile acids and phospholipids. An elevation in the levels of total cholesterol in both serum and tissue of APAP intoxicated animals may be due cholesterolemia a condition often associated with liver disorder due to the impairment of hepatic cells to remove cholesterol from blood. Modest triglyceridemia occurs frequently in hepatocellular diseases as described in viral and drug induced toxic hepatitis. The major disorder encountered in APAP-induced hepatitis is fatty accumulation in the liver, which develops either due to excessive supply of lipids to the liver or interference with lipid deposition. The pathogenesis is multifactorial, reflecting complex biosynthetic, enzymatic and catabolic derangement in lipoprotein metabolism. In the present study, the levels of total cholesterol and LDL-cholesterol were significantly higher in APAP treated rats as compared to that of normal rats indicating the APAP-induced hypercholesterolemic condition. Also the level of HDL-cholesterol was slightly reduced in APAP treated rats. This is in line with an earlier study.[25] Increased cholesterol levels in the liver might be due to increased uptake of LDL from the blood by the tissues.[26] The abnormal cholesterol deposition is favored by the dangerous tendency of cholesterol to passive exchange between the plasma lipoproteins and the cell membranes.

The levels of triglycerides were significantly ($p<0.001$) higher in plasma and tissues of APAP rats as compared to normal animals. Increased lipolysis of depot triglycerides liberates free fatty acids from adipose tissue stores and the free fatty acids liberated by the adipose tissue are also epidemic condition [25, 26]. In the present study, a significant ($p<0.001$) reduction was noticed in the levels of triglycerides and free fatty acids in plasma and tissues of vanillic acid and silymarin administered rats as compared to that of APAP- induced hepatotoxic rats, indicating the anti-hyperlipidemic effect of vanillic acid.

Histopathological examination of APAP rats' kidney showed cloudy swelling of tubules and glomerulosclerosis but vanillic acid and silymarin treated group showing mild inflammation of parenchymal cells with normal glomeruli and tubules. This shows the protective effects of vanillic acid against APAP toxicity. Further the administration of vanillic acid did not show any significant effect on normal kidney. In conclusion, our study reveals that vanillic acid possess good nephroprotective activity and showed beneficial effects on lipid profile, in APAP-induced hepatotoxic rats and these properties are comparable with the standard drug silymarin.

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