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# Valproic acid prevents the deregulation of lipid metabolism and renal renin–angiotensin system in L-NAME induced nitric oxide deficient hypertensive rats

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ABSTRACT

The present study was aimed to investigate the antihyperlipidemic and renoprotective potential of valproic acid against N$^{\omega}$-nitro-L arginine methyl ester hydrochloride (L-NAME) induced hypertension in male albino Wistar rats. In hypertensive rats, mean arterial pressure (MAP), kidney weight, levels of oxidative stress markers in tissues were increased. Dyslipidemia was also observed in hypertensive rats. Moreover, enzymatic and nonenzymatic antioxidant network also deregulated in tissues. Valproic acid (VPA) supplementation daily for four weeks brought back all the above parameters to near normal level and showed no toxicity which was established using serum hepatic marker enzyme activities and renal function markers. Moreover the up regulated expression of renin–angiotensin system (RAS) components were also attenuated by VPA treatment. All the above outcomes were confirmed by the histopathological examination. These results suggest that VPA has enough potential to attenuate hypertension, dyslipidemia and renal damage in nitric oxide deficiency induced hypertension.



## 1. Introduction

Hypertension is an over whelming global challenge, which ranks third as a means of reduction in disability-adjusted life-years. Hypertension and its related conditions such as coronary artery disease, stroke, heart failure, and chronic kidney disease is a growing public health issue for which successful treatment often remains inadequate (Mehmet et al., 2012). It affects more than 600 million people and results in 13% of total deaths globally, and it is estimated that there will be 29% of the world's adult with hypertension by 2025 (Mittal and Singh, 2010).

Hypertension, a prevalent risk factor for CVD, frequently occurs in conjunction with metabolic disturbances and in particular with dyslipidemia (Chapman and Sposito, 2008). Dyslipidemia is a broad term which implies imbalance between elevated circulating levels of cholesterol and it occurs concomitantly in over one-third of patients with hypertension (Wong et al., 2006). In humans, hypertension and

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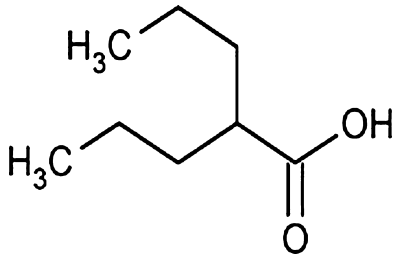


**Fig. 1 – Structure of valproic acid (2-propylpentanoic acid).**

hyperlipidemia are frequent causes of CVD and major risk factors for atherosclerosis; the presence of both conditions accelerates atherosclerosis (Kwon et al., 1998). Epidemiological studies provide a large body of evidence for the independent relationship between lipid profile and cardiovascular risk (Seventh Report of the Joint National Committee, 2003). Moreover, lipoproteins can stimulate the release and/or expression of endothelial mediators which may, in turn, contribute to the development of endothelial dysfunction and hypertension (Jagla and Schrezenmeir, 2001). Recent evidence indicates that oxidative stress as the main mechanism is responsible for cardiovascular complications such as alteration in lipid metabolism (Palmieri et al., 2006).

Valproic acid (VPA) (Fig. 1), a short branched chain fatty acid, was originally synthesized as an analog of valeric acid extracted from *Valeriana officinalis* and it has been used worldwide for decades, in the form of sodium valproate, as an antiepileptic drug with therapeutic values. Suberoylanilide hydroxamic acid (SAHA) and valproic acid were shown to lower mean arterial pressure in deoxycorticosterone acetate (DOCA)-treated and spontaneously hypertensive rats, respectively (Cardinale et al., 2010; Iyer et al., 2010). Recent study indicates that, VPA-mediated neuroprotection against *I/R* injury in the retina may involve cytoprotective Hsp70 induction via transcriptional activation and inhibition of the mitochondria-mediated apoptosis pathway (Zhang et al., 2012).

In our previous study, we established the effects of valproic acid on systolic and diastolic blood pressure, heart rate, water intake, heart weight, body weight, nitric oxide metabolites, ET-1 mRNA expression in aorta, lipid peroxidation products (heart and aorta), and antioxidant status (heart and aorta) in L-NAME induced hypertensive rats (Rajeshwari et al., 2013). In continuation of our previous work, in this study we have concentrated on the effects of valproic acid on mean arterial pressure (MAP), lipids, lipoproteins, hepatic and renal function markers, lipid peroxidation products (liver and kidney), and antioxidant status (liver and kidney), and histopathology of liver, kidney against L-NAME induced hypertensive rats. Thus, this is a novel study with different set of animals and entirely different from the previous study.

## 2. Materials and methods

### 2.1. Source of chemicals and animals

L-NAME and valproic acid were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All other chemicals used in this study were of analytical grade obtained from Merck and Himedia, India. Male albino (9 week-old) rats of Wistar strain with a body weight ranging from 180 to 220 g, were procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and were maintained in an airconditioned room (25 ± 1 °C) with a 12 h light/12 h dark cycle. Feed and water were provided ad libitum to all the animals. The study was approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No.160/1999/CPCSEA, Proposal number: 900), Annamalai University, Annamalainagar.

### 2.2. Induction of hypertension and administration of VPA

Animals were given L-NAME in drinking water at a concentration of 0.4 mg/ml to account for a daily intake of 40 mg/kg throughout the experimental period (30 days) (Rosanky, 1990; Saravana kumar et al., 2010). Valproic acid (100 mg/kg) was dissolved in 0.9% saline and administered to rats orally using an intragastric tube daily for a period of four consecutive weeks (Rajeshwari et al., 2013).

### 2.3. Measurement of blood pressure by non-invasive method

MAP was recorded every week during the entire period of the study by tail-cuff method (IITC, model 31, Woodland Hills, CA, USA).

### 2.4. Experimental time line

After the successful induction of experimental hypertension, the rats were divided into four groups of six animals each as given below.

Group I—control rats (animals that received only normal diet and water);

Group II—control + valproic acid (100 mg/kg/body weight);

Group III—L-NAME control rats;

Group IV—L-NAME + valproic acid (100 mg/kg/bodyweight).

At the end of the fourth week, all the rats were anesthetized with intraperitonial injection of urethane (1.2 g/kg) and sacrificed by cervical dislocation. Blood was collected in two different tubes, i.e., one with anticoagulant for the separation of plasma and another without anticoagulant for the serum. Plasma and serum were separated by centrifugation for the estimation of various biochemical parameters. Liver and kidney tissues (250 mg) were sliced into pieces and homogenized in appropriate buffer in cold condition (pH 7.0) to get 20% homogenate (w/v). The homogenate was centrifuged at 56 × *g* for 10 min at 0 °C in refrigerated centrifuge. The supernatant was separated and used for various biochemical estimations.

### 2.5. Biochemical estimations

Plasma and tissue lipids were extracted by the methods of Folch et al. (1957). Plasma and tissue total cholesterol, triglycerides, free fatty acids, and phospholipids were estimated by the methods of Allain et al. (1974), McGowan et al.