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Research Article

HEPATOPROTECTIVE ACTIVITY OF LEAF OF METHANOL EXTRACT OF *LAURUS NOBILIS.L* AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Objective: The present study was conducted to evaluate the hepatoprotective activity of methanol extract of *Laurus nobilis* against paracetamol induced liver damage in rats.

Materials and Methods: The methanol extract of *Laurus nobilis* (200 mg/kg & 400 mg/kg) was administered orally to the animals with hepatotoxicity induced by paracetamol (400 mg/kg). Silymarin (25mg/kg) was given as reference standard. All the test drugs were administered orally by suspending in 0.5% Carboxy methyl cellulose solution.

Results: Paracetamol has enhanced the levels of ALT, AST, ALP and bilirubin, whereas the group receiving high dose (400 mg/kg body weight) methanol extract of *Laurus nobilis* was effective in protecting the liver against the injury induced by paracetamol in rats than that of low dose (200mg/kg body weight) treated group. This was evident from a significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin.

Conclusion: It was concluded from the result that the methanol extract of *Laurus nobilis* acts on the liver as a potent scavenger of free radicals to prevent the toxic effects of paracetamol induced hepatotoxicity in rats.

Keywords: Laurus nobilis, ALT, AST, ALP, hepatotoxicity.

INTRODUCTION

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects[1]. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders[2]. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow the systematic research methodology and to evaluate the scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.

Laurus nobilis.L (Bay leaf) belongs to the family Lauraceae, and is one of the most widely used culinary spices in all Western countries. The leaf of *Laurus nobilis* traditionally used as herbal medicine to treat rheumatism, earaches, indigestion, sprains, and to promote perspiration[3]. Recent research revealed that it can be used in treating diabetes and preventing migraine[4]. There is a lack of scientific reports on the hepatatoprotective role of methanol extract of leaves of *Laurus nobilis*. Hence, the objective of the present investigation was to evaluate the hepatoprotective activities of methanolic extract of *Laurus nobilis* in paracetamol induced rats.

MATERIALS AND METHODS

Plant materials

The leaf of *Laurus nobilis*, were collected from Bujang Valley, Archeological site, Merbok, Kedah, Malaysia. Taxonomic identification was made from USM, Malaysia (Specimen herbarium no: 11250). A voucher specimen is preserved in our laboratory for further reference at school of Pharmaceutical sciences, University Sains Malaysia.

Preparation of Extracts

The powdered plant materials were successively extracted with methanol by hot continuous percolation method in Soxhlet apparatus[5] for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Experimental Animals

Adult male Wistar rats weighing between 120-180gm were used for this purpose. The animals were housed in polypropylene cages and maintained at 24 ± 2°C under 12h light dark cycle and were fed *ad libitum* with standard pellet diet and had free access to water and use of animals as per the experiment was approved by the institutional Animal Ethics Committee.

Experimental design

The animals were divided into 5 groups of six rats each. The group I animals served as normal control and received distilled water for seven days. Group II animals orally received paracetamol (400 mg/kg body weight) for seven days. Group III & IV animals received 200 mg/kg & 400 mg/kg body weight of methanolic extract of *Laurus nobilis* along with paracetamol (400 mg/kg body weight). Group V animals received standard drug silymarin along with paracetamol (400 mg/kg body weight) for seven days.

Sample collection

At the end of the experiment the animals of all the groups were sacrificed by cervical dislocation method under mild anesthesia on the eighth day. Blood sample of each group was collected separately in sterilized dry centrifuge at 370 rpm, tubes and allowed to coagulate for 30 min.

Evaluation of effect on biochemical variables

The clear serum obtained after centrifugation was used for the estimation of serum alanine amino transferase[6], serum aspartate amino transferase[6], alkaline phosphatase[7], gamma-glutamyl transferase[8], Lactate dehydrogenase, serum protein, serum bilirubin (Malloy and Evelyn[9]) cholesterol and triglyceride.

Statistical Analysis

Results of biochemical estimations were reported as mean ± SD of six animals in each group. The data were subjected to one way Duncan ANOVA using the SPSS 14.5 version (SPSS, Cary, NC, USA) followed by Bonferroni's multiple comparison tests (BMCT). The P-Value was <0.01were considered statistically significant.

RESULTS AND DISCUSSION

Hepatic cells participate in a variety of metabolic activities and contain a host of enzymes. AST and ALT are reliable markers of liver function which are found in higher concentrations in the cytoplasm and an altered form of AST also exists in the hepatocyte mitochondria. Although both transaminase enzymes are widely distributed in other tissues of the body, the activities of ALT outside the liver are low and therefore this enzyme is considered more specific for hepatocellular damage. During liver injury, transport function of the hepatocytes is disturbed which leads to leakage of plasma membrane, thereby causing an increased enzyme level in serum and soluble enzymes like AST will also be similarly released. Estimation of these enzymes in serum is a useful quantitative marker for the extent and different types of hepatocellular damage[10]. The present study elevated activities of AST and ALT in serum were observed in paracetamol administered rats which indicates increased permeability, damage and/or necrosis of hepatocytes[11].

ALP and GGT are membrane bound enzymes which are released unequally depending on the pathological phenomenon. Generally, ALP is excreted by the liver via bile and hence when the hepatic cells get damaged, this enzyme is not excreted through the bile therefore ALP is released into the blood stream. Thus, serum ALP is a measure of the integrity of the Hepatobiliary system and the flow of bile into the small intestine. GGT a key enzyme in the metabolism of GSH has been reported to be high in alcoholic liver disease and its measurement has been claimed to be an extremely sensitive test and marker of ethanol induced hepatic damage[12].

Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. It provides the useful information on how well the liver is functioning[13]. Bilirubin is toxic and needs conjugation before excretion. Under normal conditions, circulatory bilirubin is bound to albumin, which protects cells against its potential toxicity[14]. Liver conjugates bilirubin with glucuronic acid, which is subsequently excreted via the bile. Several human diseases are reported to be caused by abnormally increased levels of bilirubin. In the present observation, paracetamol intoxicated rats showed a significant increase in the levels of serum bilirubin as compared to all other groups. Elevated levels of bilirubin are an indication of biliary obstruction and haemolysis, this may be a sequel to reduced blood supply to hepatocytes.

Treatment with methanolic extract of *Laururs nobilis* (200 mg/kg & 400 mg/kg) significantly alleviates the increased activities of serum enzymes and the bilirubin levels (Table 1) to near normal, which may be a consequence of the stabilization of plasma membrane and maintaining the functional status of the liver from paracetamol toxicity. This effect is in agreement with the commonly accepted view that serum levels of tranaminases would return to normal after the healing of hepatic parenchyma and regeneration of hepatocytes. Thus, from the above findings it is evident that the methanolic extract of *Laururs nobilis* (200 mg/kg & 400 mg/kg) has a remarkable hepatoprotective effect against liver damage.

Table 1: Effect of methanol extract of *Laurus nobilis* on hepatic marker enzymes and bilirubin in serum of control and experimental animals

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	Bilirubin (mg/dl)
Control	76.17 ± 7.16	28.14 ± 1.59	89.29 ± 8.81	2.47± 0.26	0.99 ± 0.08
Paracetamol (400 mg/kg b.wt)	145.18± 13.27	71.45 ± 7.61	177.41±16.23	7.91 ± 0.78	1.89 ± 0.18
Paracetamol+ MELN (200mg/kg b.wt)	140.77± 11.46	63.47 ± 5.17	159.73±15.94	6.14 ± 0.41	1.63 ± 0.11
Paracetamol+ MELN (400mg/kg b.wt)	128.43± 12.13	35.12 ± 3.24	131.42±12.34	3.89 ± 0.32	1.33 ± 0.03
Paracetamol+Silymarin (25mg/kg b.wt)	92.16 ± 9.83	32.06 ± 3.81	124.48±11.93	2.99 ± 0.27	0.98 ± 0.09

Values are expressed as means ± S.D. For six rats in each group; MELN: methanolic extract of *Laurus nobilis;*

The kidney is an important organ actively involved in maintaining homeostasis of the body by reabsorbing important material and excreting waste products. It has been reported that habitual consumption of large amount of alcohol was associated with an increased risk of kidney failure in the general populations[15]. Kidney functional markers such as urea, uric acid and creatinine are the main indicators of renal dysfunction. Urea is the main end product of protein catabolism, uric acid the major product of purine nucleotides. Creatinine is endogenously produced and released into the body fluids where its clearance is measured as the indicator of glomerular filtration[16,17]. In our result paracetamol administrated rats showed a significant increase in the levels of urea, uric acid and creatinine which are considered as a significant marker of renal dysfunction. This increase is mainly caused by increased production of ROS and acetaldehyde which are the mediators of tissue damage and finally leads to altered kidney function and renal failure[18][. In contrast to the increase in paracetamol fed rats, we found that renal markers were brought back to normal on treatment with a methanolic extract of *Laururs nobilis* at the dose of 200 mg/kg & 400 mg/kg (Table 2). Thus it is inferred that the methanolic extract of *Laururs nobilis* (200 mg/kg & 400 mg/kg) preserves the functional capacity of the kidney against paracetamol toxicity.

Table 2: Effect of methanolic extract of *Laurus nobilis* on renal function markers in the serum of control and paracetamol treated rats

Groups	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Control	28.78 ± 1.59	1.39 ± 0.06	0.89 ± 0.06
Paracetamol (400 mg/kg b.wt)	47.66 ± 2.86	2.93 ± 0.35	1.98 ± 0.07
Paracetamol+MELN (200mg/kg b.wt)	41.25 ± 2.98	2.85 ± 0.70	1.36 ± 0.04
Paracetamol+MELN (400mg/kg b.wt)	33.81 ± 2.33	2.67 ± 0.16	0.89 ± 0.08
Paracetamol+Silymarin (25mg/kg b.wt)	28.76 ± 2.71	1.83 ± 0.13	0.79 ± 0.06

Values are expressed as means ± S.D. for six rats in each group; MELN: methanolic extract of *Laurus nobilis;*

Oxidative stress due to the formation of free radicals was incriminated as one of the mechanisms underlying ethanol induced toxicity. Chronic alcohol intake generates excess production of free radicals where the antioxidant defenses are impaired which resulted in sequential degradation of cell membranes by a process known as lipid peroxidation. This process may destroy the integrity of the membranes both within and surrounding the cell, seriously compromising cell function[19]. Researchers have demonstrated that chronic alcohol consumption induces lipid peroxidation in rats and that the degree of lipid peroxidation is related to the extent of liver injury[20]. In agreement with these findings, our results showed increased levels of lipid peroxidative markers such as TBARS and lipid hydroperoxides in circulation and tissues of alcoholics when compared to control. On the other hand, treatment with SNFEt and silymarin significantly declined the levels of lipid peroxidation products to near normal. This protective effect is probably based on the antioxidant activity of the extract which reduces the oxidative damage by blocking the production of free radicals and inhibits lipid peroxidation

It is well known that a natural antioxidant systems are inactivated by lipid peroxidation and ROS[21]. Generally, antioxidants are the cell's defense against free radicals. Enzymatic antioxidants such as SOD, CAT and GPx are the first line of defense against oxidative injury. SOD is the first antioxidant enzyme to deal with oxyradicals with accelerating the dismutation of superoxide radicals (O_2^-) to hydrogen peroxide (H_2O_2). In the present study, we observed a significant decrease in SOD activity in the erythrocytes and tissues of paracetamol treated rats. This decrease could be due to inefficient scavenging of ROS which might be implicated to oxidative inactivation of enzymes[22]. CAT acts as a preventive antioxidant and plays an important role in protecting against the deleterious effects of lipid peroxidation. Reports have shown that a significant decrease in the activity of catalase during paracetamol ingestion indicates inefficient scavenging of H_2O_2 [23, 24]. Our results were also in correlation with the above observations in ethanol fed groups. GPx has a well established role in protecting cells against oxidative stress and this in turn requires glutathione as a cofactor. GPx catalyses the oxidation of GSH to GSSG at the expense of H_2O_2[25]. Decreased GPx activity was also observed in paracetamol treatment. Our findings agree with the above observations. Thus paracetamol consumption diminishes the activity of these enzymatic antioxidants and renders the cells more susceptible to free radical induced injury.

Apart from enzymatic antioxidants, non-enzymatic antioxidants play an excellent role in protecting the cells from oxidative damage. Non enzymatic antioxidant systems such as GSH, Vitamin C and E are considered as the second line of defense against free radicals. GSH a major non-protein thiol, presumed to be an important endogenous defense against peroxidative destruction of cellular membranes. Glutathione reacts directly with ROS and electrophilic metabolites, protects essential thiol groups from oxidation and serves as a substrate for several enzymes including GPx. In our study, the concentration of GSH was significantly reduced in paracetamol treated rats, which was in constituent with other reports[26, 27]. The reduced form of GSH therefore becomes readily oxidized to GSSG on interacting with free radicals[28]. Antioxidants other than GSH may also play a role in preventing lipid peroxidation under experimental conditions. Vitamin C and E are naturally occurring free radical scavengers[29]. Vitamin C (ascorbic acid) is an important H_2O soluble antioxidant in biological fluids and an essential micronutrient required for normal metabolic functioning of the body. It is shown to react directly with superoxide[30, 31], hydroxyl radicals[32] and singlet oxygen[33]. Vitamin C undergoes synergistic interactions with tocopheroxyl radical in the regeneration of α-tocopherol[34]. Decreased levels of vitamin E were observed in plasma and tissues of paracetamol treated rats which may be due to reduced concentration of Vitamin C and GSH levels which can result in reduced conversion of α-tocopheroxyl radical to α-tocopherol.

Co-administration of methanolic extract of *Laururs nobilis* (200 mg/kg & 400 mg/kg) with paracetamol significantly modulates the antioxidant in erythrocytes, plasma and tissues suggesting the enhancing effect of methanolic extract of *Laururs nobilis* (200mg/kg & 400 mg/kg) on cellular antioxidant defenses (Table 3). The protective activity of the extract was compared with that of silymarin. Elevation of antioxidant status by methanolic extract of *Laururs nobilis* (200 mg/kg & 400 mg/kg) presumably offers protection against lipid peroxidation by quenching and detoxifying the free radicals. The antioxidant property of the extract may therefore be due to the presence of high content of polyphenolic compounds such as flavonoids and steroids, vitamin C and β-carotene. From these findings it can be inferred that the methanolic extract of *Laururs nobilis* (200 mg/kg & 400 mg/kg) positively modulated the antioxidant status by quenching and detoxifying the free radicals and restoring it to near normal (Table 4).

Table 3: Effect of methanolic extract of *Laurus nobilis* on TBARS and lipid hydroperoxide in Plasma and tissues of control and paracetamol administered rats

Groups	Plasma (mmoles/dl)		Liver (mmoles/100g wet tissue)		Kidney (mmoles/100g wet tissue)	
	TBARS	Lipid hydroperoxides	TBARS	Lipid hydroperoxides	TBARS	Lipid hydroperoxides
Control	0.18 ± 0.05	14.21 ± 4.29	0.76 ± 0.06	97.52 ± 9.57	1.55 ± 0.16	83.95 ± 8.88
Paracetamol (400mg/kg b.wt)	0.49 ± 0.03	24.72 ± 2.80	2.88 ± 0.22	171.83 ±73.60	2.98 ± 0.28	167.93 ± 16.63
Paracetamol+MELN (200mg/kg b.wt)	0.43± 0.05	22.45 ± 2.71	2.62 ± 0.09	154.11 ± 54.07	2.80 ± 0.07	140.74 ± 11.72
Paracetamol+MELN (400mg/kg b.wt)	0.22 ± 0.02	19.31 ± 1.95	1.76 ± 0.08	117.31 ±11.31	2.15 ± 0.19	125.18 ± 12.36
Paracetamol+Silymarin (25mg/kg b.wt)	0.19 ± 0.02	18.51 ± 1.75	1.77 ± 0.07	111.02 ±10.17	1.91 ± 0.09	117.51 ± 11.80

Values are expressed as means ± S.D. for six rats in each group; MELN: methanolic extract of *Laurus nobilis;* Values not sharing a common superscript differ significantly at $p<0.05$ (DMRT)

Table 4: Effect of methanolic extract of *Laurus nobilis* on the activities of SOD, CAT and GPx in the erythrocyte of control and paracetamol administered rats

Groups	Erythrocyte		
	SOD (U*/mg Hb)	CAT (U#/mg Hb)	GPx (U$/mg Hb)
Control	6.07 ± 0.65	169.42 ± 16.94	13.98 ± 1.32
Paracetamol (400 mg/kg b.wt)	3.12 ± 0.03	98.53 ± 9.63	7.73 ± 0.70
Paracetamol+MELN (200mg/kg b.wt)	4.23 ± 0.22	145.31 ± 7.91	9.54 ± 0.49

Paracetamol+MELN (400mg/kg b.wt)	4.53 ± 0.42	155.81 ± 14.97	10.34 ± 0.09
Paracetamol+Silymarin (25mg/kg b.wt)	4.41 ± 0.47	151.14 ± 15.25	11.76 ± 1.17

Values are expressed as means ± S.D. for six rats in each group; MELN: methanolic extract of *Laurus nobilis;* Values not sharing a common superscript differ significantly at *p*<0.05 (DMRT)

Lipids are a heterogenous group containing active metabolic substances which play an important role in the pathogenesis of alcoholic liver disease. The most common lipid abnormalities during chronic alcohol consumption are known to produce hypercholesterolaemia and hypertriglyceridaemia[35,36]. In our study, we observed increased levels of plasma and tissue lipids such as TC, TG, FFA where as the phospholipids were increased in plasma and decreased in the tissues of paracetamol treated rats.

Paracetamol intoxicated groups showed increased levels of cholesterol in plasma and tissues while LDL-cholesterol was remarkably increased in plasma and HDL-cholesterol were slightly reduced. The increased cholesterol during paracetamol ingestion is attributed to increased β–hydroxyl methyl glutaryl CoA [HMG CoA] reductase activity which is the rate limiting step in cholesterol biosynthesis[37].

With this above observation we found that administration of methanolic extract of *Laurus nobilis* restored the lipid levels to near normal indicating the efficacy of methanolic extract of *Laurus nobilis* showing antihyperlipidemic activity (Tables 5 & 6). The possible reason for lowering of cholesterol level is that the HMG CoA reductase activity might be lost through phospholigation by cAMP-dependent protein kinase (PKA) which was activated by methanolic extract of *Laurus nobilis* glycoprotein[38]. Therefore we speculate that the methanolic extract of *Laurus nobilis* can modulate lipid abnormalities by inhibiting the activity of hepatic HMG-CoA reductase and brings the lipid levels to normal.

Table 5: Effect of methanolic extract of *Laurus nobilis* on lipid profile in the liver of control and paracetamol administered rats

Groups	Liver (mg/g of tissue)			
	Total cholesterol	**Triglycerides**	**Phospholipids**	**Free Fatty acids**
Control	5.98 ± 0.30	3.61 ± 0.35	17.74 ± 1.66	6.78 ± 0.62
Paracetamol (400 mg/kg b.wt)	6.59 ± 0.58	7.34 ± 0.73	9.76 ± 0.32	11.45 ± 1.31
Paracetamol+MELN (200mg/kg b.wt)	6.14 ± 0.43	6.71 ± 0.36	8.74 ± 0.95	10.88 ± 1.08
Paracetamol+MELN (400mg/kg b.wt)	5.49 ± 0.38	4.44 ± 0.55	21.68 ± 1.89	9.82 ± 0.90
Paracetamol+Silymarin (25mg/kg b.wt)	5.19 ± 0.37	4.15 ± 0.40	20.39 ± 1.71	8.65 ± 0.53

Values are expressed as means ± S.D. for six rats in each group; MELN: methanolic extract of *Laurus nobilis;* Values not sharing a common superscript differ significantly at *p*<0.05 (DMRT)

Table 6: Effect of methanolic extract of *Laurus nobilis* on lipid profile in the kidney of control and paracetamol treated rats

Groups	kidney (mg/g of tissue)			
	Total cholesterol	**Triglycerides**	**Phospholipids**	**Free Fatty acids**
Control	4.79 ± 0.57	4.77 ± 0.65	16.73 ± 1.62	3.75 ± 0.77
Paracetamol (400 mg/kg b.wt)	6.63 ± 0.67	6.88 ± 0.59	7.55 ± 0.56	7.96 ± 0.32
Paracetamol+MELN (200mg/kg b.wt)	6.17 ± 0.57	6.26 ± 0.24	7.04 ± 0.09	7.11 ± 0.75
Paracetamol+MELN (400mg/kg b.wt)	5.45 ± 0.54	5.74 ± 0.74	20.27 ± 2.53	5.39 ± 0.92
Paracetamol+Silymarin (25mg/kg b.wt)	5.48 ± 0.62	5.16 ± 0.58	18.63 ± 1.89	4.76 ± 0.56

Values are expressed as means ± S.D. for six rats in each group; MELN: methanolic extract of *Laurus nobilis;* Values not sharing a common superscript differ significantly at *p*<0.05 (DMRT)

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that a methanolic extract of leaves of *Laurus nobilis*, exhibits significant hepatoprotective activities. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further investigations need to be carried out to isolate and identify the compounds present in the plant extract.

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