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Hepatoprotective and Inhibition of Oxidative Stress in Liver of *Prostechea michuacana*

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Abstract: Methanol, hexane and chloroform extracts of *Prostechea michuacana* (EMM) were studied against carbon tetrachloride (CCl4) induced hepatic injury in albino rats. Pre-treatment with methanolic extract reduced biochemical markers of hepatic injury like serum Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels demonstrated dose dependant reduction in the in vivo peroxidation induced by CCl4. Likewise, pretreatment with extracts from EMM on paracetamol induced hepatotoxicity and the possible mechanism involved in this protection were also investigated in rats. The extracts of EMM at 200, 400 and 600 mg/kg were administered. The degree of protection was measured by using biochemical parameters such as serum transaminase (GOT and GPT), alkaline phosphatase (ALKP) and bilirubin. The methanol extract of orchid produced significant hepatoprotective effect by decreasing the activity of serum enzymes, and bilirubin. These results suggest that EMM could protect from paracetamol-induced lipid peroxidation eliminating the deleterious effects of toxic metabolites from paracetamol. Hexane and chloroform extracts did not show any effect. This hepatoprotective activity was comparable with sylmarin. The results obtained in the present study indicate that the MEMC can be a potential source of natural hepatoprotective agent.

Keywords: *Prostechea michuacana;* orchid;hepatoprotective activity; biochemical parameters.

1. Introduction

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences [1]. Liver diseases are mainly caused by toxic chemicals, excessive consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid

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peroxidation and other oxidative damages [2]. In spite of tremendous advances in modem medicine, there are no effective drugs available that stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells [3]. In absence of reliable liver-protective drugs in modem medicine, there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver ailments [4].

The medicinal plants that have been used since Colony and they appears nowadays in the traditional markets of Oaxaca, Mexico, where Zapotec people live, could be an adequate option. Local botanical knowledge of medicinal plants is interrelated with unknown chemical substances useful for human curable uses. These plants may have some pharmacological substance that had probed their effectiveness for a lot of years by the local people.

Prostechea michuacana (Lex.) W. E Higgins belonging to the family Orchidaceae. is an orchid species that has been common in traditional market of Oaxaca since Colony years. It is used as an anti-inflammatory, diuretic, antidiabetic, and also against liver disorders. The folk botanical common uses are for the nativity decoration in December for to quench one's thirst on the field and Burns. [5].The two last uses mentioned are uncommon in orchid family, and some unknown chemical substances of pharmacological use could be in this species. Previous studies reported that *P. michuacana* contains 8-C-(6-deoxy-β-D-glucopyranosy) apigenina, l-(3'-hydroxy-5'-methoxyphenyl)-2-(4"-hydroxy-5"-methoxy phenyl) ethane and 2-(4-hydroxybenzyl) malic acid [6].The current investigation is an attempt to evaluated the hepatoprotective effect of the hexane, chloroform and methanol extracts of seudobulbs of *Prostechea michuacana* in carbon tetrachloride and paracetamol induced hepatic injury model in rats.

2. Materials and Methods

2.1 Plant material

P. michuacana were collected from Oaxaca State, Mexico in December 2007. A voucher specimen (No.6478) was deposited in the Herbarium of the CIDIR-Oaxaca for further reference.

2.2 Animals

The study was conducted in male Wistar strain albino rats, weighing about 180- 225 g. Before and during the experiment, animals were fed with normal laboratory diet and water *ad libitium*. The animals were acclimatized for a period of 3 days in the new environment before initiation of experiment. The study was approved by the Institutional Animal Ethics Committee.

2.3 Preparation of plant extracts

Aerial parts and seudobulbs (100 g) were dried and powdered in a mechanical grinder. The powdered material was extracted by 500 ml of hexane, chloroform, methanol and water consecutively using soxhlet apparatus. These extracts was filtered and concentrated by rotary vacuum evaporator and kept in a vacuum dessicator for complete removal of solvent. Aqueous suspension of EMM was prepared using 2% (v/v) Tween-80 and used for oral administration.

2.4 Hepatoprotective activity
Induction of in vivo carbon tetra chloride hepatotoxicity

The animals were divided into control, carbontetrachloride (CCl_4) and test groups (CCl_4 + extracts or silymarin each containing 6 animals in all the sets of experiments. CCl_4 solution (50% v/v) in olive oil was used for administration.

Animals from the control group received single daily dose of aqueous tween-80 (4% w/v) solution (1ml/kg s.c.) for four days and olive oil (1 ml/kg s.c) day 2 and 3.

Animals from CCl_4 group received single daily dose of tween-80 (4% w/v) solution (1ml/kg s.c.) for four days and CCl_4 solution 2 ml/kg s.c day 2 and 3, 30 min after the administration of aqueous tween-80 solution.

Animals from the test groups received single daily dose of the extracts (200, 400 600 mg/kg s.c) and silymarin (50 mg/kg s.c) for four days. The animals were also administered toxicant CCl_4 (2 ml/kg s.c) 30 min after the administration of the test extracts.

2.5. Induction of in vivo paracetamol hepatotoxicity

The animals were divided into control, paracetamol (Pcl) and test groups (Pcl + extracts or silymarin) each containing 6 animals in all the sets of experiments. Pcl was suspended in 60% w/v aqueous sucrose solution.

Animals from the control group received single daily dose of 4% w/v aqueous acacia solution (1ml/kg i.p.) on all three days and single dose of 60% w/v sucrose solution (1 ml/kg p.o.) on day 3.

Animals from Pcl group received single daily dose of 4 % w/v aqueous acacia solution (1ml/kg i.p.) for three days and single dose of Pcl suspension 3 g/kg p.o. on day 3, 60 min later administration of aqueous tween-80 solution.

Animals from the test groups received single daily dose of the extracts (200, 400 y 600 mg/kg i.p.), silymarin (50 mg/kg i.p) for three days. The animals were also administered toxicant single dose of Pcl suspension (3 g/kg p.o.) on day three, 60 min after the administration of the test extracts [7].

2.6 Biochemical studies

At the end of the experimental period (CCl_4 hepatotoxicity), animals were sacrificed by cervical decapitation, blood collected and serum separated for biochemical analyses. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed by the colorimetric test of Rodier and Mallein [8] as toxicity marker enzymes. The hepatoprotective activity was calculated as:

$$[1- (ALTdrug - ALTcontrol/ALT\ CCl_4 - ALTcontrol)] \times 100$$

In the case of induction of *in vivo* paracetamol-induced the activity was assessed by estimating serum transaminases viz. glutamyl pyruvate transaminase (GPT) and glutamyl oxalacetate transaminases (GOT) using Reitman and Frankel [8]. Alkaline phosphatase (ALKP) [9] and total bilirubin (TB) [10] were also determined.

2.7 Statistical Analysis

The results of Biochemical analysis are presented as mean ± S.D. and percent reduction was calculated by considering the difference between the control and toxicant as 100 % reduction. The statistical significance of the difference was analysed through one way analysis of variance (ANOVA). The difference between the test group and control was determined by least significant difference method at $p<0.05$ confidence levels .

3. Results and Discussion

The present study evaluated the hepatoprotective activities of *P. michuacana* in CCl_4 and paracetamol induced liver toxicity.

As dipicted in Table 1, administration of CCl_4 induced a marked increase in the levels of ALT and AST significantly ($p<0.01$) as compared to the control group. However, pretreatment of rats with different doses (200,400 and 600 mg/kg) of methanol extract or sylimarin (100 mg/kg) caused a significant reduction of these elevated levels in a dose-related manner. Methanol extract at 600 mg/kg reduced the levels of ALT and AST by 25.7and 37.4 %, respectively. Hexane or chloroform extracts failed to show any effect on the enzyme levels.

Administration of paracetamol elevated the levels of GPT, GOT, AKKP and TB by three to four folds indicating development of hepatotoxicity. Administration of different doses of methanol extract caused a significant reduction in the biochemical parameters. However, hexane and chloroform extracts again failed to reduce the elevated biochemical parameters indicating no protection against paracetamol-induced hepatotoxicity. Pretreatment of animals with methanol extract at 600 mg/kg, significantly reduced the levels of GPT (43.17 %), GOT (38.13 %), ALKP (31.08 %) and TB (57.64%) as compared to paracetamol treated animals (Table 2).

It is established that hepatotoxicity induced by CCl_4 depends on the cleavage of the carbon-chlorine bond to generate tricloromethyl free radical ($.CCl_3$) that reacts rapidly with oxygen to form a trichloromethyl peroxy radical ($.CCl_3O_2$). This metabolite possibly attack membrane polyunsaturated fatty acids thereby causing lipid peroxidation leading to impairment of membrane function and liver injury [11].

CCl_4-induced hepatic injuries are commonly used animal models for the screening of hepatoprotective plant extracts and the magnitude of hepatic damage is assessed by measuring the level of released cytosolic transaminases including ALT and AST in circulation [12]. When administrated prophylacticaly, methanol extract exhibited protection against CCl_4 induced liver injuries as manifested by the reduction of toxin-mediated rise in serum enzymes in rats.

Paracetamol is a common antipyretic agent, which is safe at therapeutic doses whereas, at higher doses it may produce hepatic damage or necrosis in rodents and man [13]. Protection against paracetamol-induced toxicity has been used as a reliable test for screening hepatoprotective agents 14]. Apparently, the covalent binding of N-acetyl-*p*-benzoquinoneimine, (an oxidation product of paracetamol), to sulfhydryl groups of protein lead to cell necrosis and lipid peroxidation. This has been shown to be due to decline in the glutathione levels in the liver responsible for hepatotoxicity [15]. Indeed, glutathione is one of the most important natural antioxidants in hepatocytes and its unavailability renders the cell remarkably susceptible to oxidative stress.

In the assessment of liver damage by paracetamol, the determination of enzyme levels such as GOT and GPT is largely used. Necrosis or membrane damage releases the enzymes into circulation. High levels of GOT indicates liver damage, it may be also due to viral hepatitis, cardiac infarction or muscle injury. On the other hand, GPT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Thus, GPT is more specific to the liver, and is a better parameter for detecting liver injury [16]. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [17]. Serum ALP and bilirubin levels on the other hand are related to the functioning of hepatic cells [18].

Acute administration of paracetamol produced a marked elevation in the serum levels of GOT, GPT, ALKP, bilirubin, as compared to the control group. Treatment with methanol extract at 200, 400 and 600mg/kg significantly reduced the elevated levels of the enzymes towards the respective normal value indicating stabilization of plasma membrane as well as repair of hepatic tissue damage induced by paracetamol. These changes may be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells.

Effective control of alkaline phosphatase (ALKP) and bilirubin levels points towards an early improvement in the secretary mechanism of the hepatic elaborate.

Considering the overall results, this study indicates that methanol extract of orchid demonstrates strong hepatoprotective activity. These findings thus establish a potential medicinal value to the plant *P. michuacana* that is used in indigenous Mexican system of medicines. Further detailed investigations on this plant are needed in order to justify its use in polyherbal formulations prescribed in the treatment of liver disorders.

Table 1. Hepatic protection of different doses of methanol extract from seudobulbs of *P. michuacana* (EMM) on CCl_4-induced ALT and AST increase in rats

Group (mg/kg)	ALT (IU/L)	AST(IU/L)
Control	24.68 ± 3.23	60.17 ± 2.17
CCl_4	103.65 ± 2.84 **	142.38 ± 5.32**
EMM 200	91.54 ± 1.57* (11.7)	113.78 ± 4.19* (20.1)
EMM 400	81.73 ± 1.49* (21.14)	97.71 ± 3.52* (31.4)
EMM 600	77.03 ± 0.99* (25.7)	89.12 ± 2.51* (37.4)
Sylimarin 100	87.56 ± 2.31* (15.23)	101.43 ± 1.87* (28.8)

Each value represents the mean ± SEM, n = 5; *p< 0.05 significantly different values from CCl_4 group. **p<0.01 significantly different values from control group. Values given in the parenthesis are percent reduction.

Table 2. Hepatic protection of different doses of methanol extract from seudobulbs of *E. michuacana* (EMM) on biochemical parameters in rats intoxicated with paracetamol

		% Reduction		
Group (mg/kg)	GPT (U/L)	GOT (U/L)	ALKP (U/L)	TB (U/L)
Control	68.43 ± 2.38	109.76 ± 3.12	45.10 ± 4.64	0.23 ± 0.56
Paracetamol	127.12 ± 7.63**	240.15 ± 5.67**	173.32 ± 6.65**	1.70 ± 0.15**
EMM 200	115.56 ± 2.18* (9.09)	201.54 ± 3.89* (16.07)	155.71 ± 1.87* (10.16)	1.43 ± 0.034* (15.9)
EMM 400	86.71 ± 3.45* (31.8)	174.34 ± 4.01* (27.4)	134.79 ± 2.03* (22.23)	1.15 ± 0.091* (32.35)
EMM 600	72.23 ± 2.89* (43.17)	148.56 ± 2.87* (38.13)	119.45 ± 1.93* (31.08)	0.72 ± 0.87* (57.64)
Sylimarin 50	67.32 ± 2.09* (47.04)	140.32 ± 4.11* (41.56)	105.34 ± 2.55* (39.22)	0.60 ± 0.33* (64.7)

Each value represents the mean ± SEM, n = 5; *p< 0.05 significantly different values from paracetamol group. **p<0.01 significantly different values from control group. Values given in the parenthesis are percent reduction.

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