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Hepatoprotective and Safety Evaluation Studies on Sarsaparilla

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ABSTRACT

The effect of the ethanol extract of sarsaparilla *(Smilax regelii)* has been studied on carbon tetrachloride (CCi_4)-induced hepatocellular damage in rats. Pretreatment with an ethanol extract of sarsaparilla significantly inhibited CCl_4-induced biochemical changes. Acute and chronic toxicity studies were also undertaken to determine the safety of prolonged use of sarsaparilla. Acute administration of sarsaparilla extract in the dose range of 0.5 to 3.0 g/kg did not produce any adverse effects or mortality in mice over a period of 24 hours. Animals treated with sarsaparilla extract (100 mg/kg/day) for a period of 90 days in drinking water showed no symptoms of toxicity. There was no significant change in body weight and hematological parameters in the chronically treated animals as compared to the control group. These findings suggest that sarsaparilla, besides having hepatoprotective potential, has no untoward effects in rodents.

INTRODUCTION

The uses of sarsaparilla *(Smilax regelii* Killip & Morton, Fam. Liliaceae) as tonic, antirheumatic, diuretic, and for the treatment of skin and liver diseases have long been known (Al-Baitar 1871; Ghani 1921; Kritikar and Basu 1918; Nasir 1882). This plant was introduced in Europe by Spaniards in the early sixteenth century (Trease and Evans, 1972). Sarsaparilla was included in the British Pharmacopoeia in 1864. In 1942, the drug was added to the United States Pharmacopoeia (Wallis, 1955). In Greco-Arab and Indian systems of medicine, the drug is still used for the treatment of liver diseases, inflammation and renal deficiency (Ghani, 1921; Raza, 1933; Albert, 1952). Besides its medicinal use, sarsaparilla is often used as a flavouring agent in non-alcoholic drinks (Stuart, 1979). A decoction made from the roots is used as a vehicle in the preparation of syrups which have been reported to have cooling properties (Clause, 1971). Recently, Ageel *et al.* (1989) have observed significant anti-inflammatory and anti-rheumatic activity of an ethanol extract of sarsaparilla in rats. However, the much claimed activity against liver disorders has not been scientifically documented. The present investigation has been undertaken to study the hepatoprotective and toxic potential of an ethanol extract of sarsaparilla in rodents.

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MATERIALS AND METHODS

The roots of sarsaparilla were procured from a local market and identified at the Medicinal, Aromatic and Poisonous Plant Research Centre of King Saud University.

The powdered drug (500 g) was extracted using percolation with 96% ethanol. The solvent was removed at low temperature under reduced pressure and the extract stored in a refrigerator prior to pharmacological and toxicity studies. The extract yield was 11.6% (w/w) in terms of starting material. All doses are expressed in terms of the extract.

Animals and exposure conditions

Male Wistar albino rats, weighing approximately 185-200 g and Swiss albino mice, weighing 25-30 g (home bred), were fed Purina diet and tap water *ad libitum.* The animals were housed at a temperature of 25 "C + l'C, with a 12-h light/dark cycle (light from 0600 to 1800 h).

Carbon tetrachloride (CCl_4)-induced hepatocellular damage

The rats were divided into three groups of six rats each. The first group served as control; the second and third groups were injected intraperitoneally with $CC1_4$ in a dose of 3 ml/kg body weight (10% solution of $CC1_4$ in light liquid paraffin). The third group was pretreated orally for 3 consecutive days with 500 mg/kg of the ethanol extract of sarsaparilla (5 ml/kg) before the injection of $CC1_4$ on the fourth day. The animals were anaesthetized with ether 18 h after the $CC1_4$ injection, and blood was collected by heart puncture for biochemical evaluations. Livers were excised and fixed in buffered formalin for histopathological assessment.

The biochemical parameters included liver glycogen (Montgomery, 1957); serum transaminases (Rietman and Frankel, 1957), serum bilirubin (King, 1951), serum cholesterol (Zlatkis *et al.* 1953) and blood urea (King, 1951).

Safety evaluation studies

Acute toxicity

Acute toxicity testing was performed on 3 groups of mice consisting of 6 animals per group. The ethanol extract was administered orally in doses of 500 mg, 1 g and 3 g/kg body weight. The behavioural changes, symptoms of toxicity and mortality were observed for 24 h (Shah *et al.,* 1988).

Chronic toxicity

A total of 40 mice (20 male and 20 female) were randomly allotted to different treated and control groups. The extract in each case was administered in drinking water. The dose selected was 100 mg/kg body weight per day, which is 1/5 of the pharmacologically active dose (Parmar *et al.,* 1987), for a period of 3 months (WHO, 1967). The animals were observed for symptoms of toxicity and mortality. The average pre- and post-treatment body weights of the animals were recorded and compared with the control group. At the end of the treatment, 5 animals from each group were sacrificed 24 h after the last exposure. The blood was analysed for RBC and WBC counts, and haemoglobin level, with a Contraves degicell 3100h.

Phytochemical screening

The preliminary phytochemical screening of the roots of sarsaparilla was conducted to determine the presence or absence of alkaloids, cardiac glycosides, flavonoids, tannins, coumarins, anthraquinones, saponins, volatile oil, volatile bases, cyanogenic glycosides, glucosinolates, sterols and/or triterpenes according to the methods described by Famsworth (1966).

RESULTS AND DISCUSSION

The treatment of animals with $CC1_4$ produced a significant increase in serum transaminases (Table 1). The hepatocellular enzymes, especially GPT, are tightly bound to particular organelles, and an hepatotoxin like $CC1_4$ is known to disturb

Table 1. Effect of pretreatment with ethanol extract of sarsaparilla (500 mg/kg) on CCl_4-induced hepatocellular damage in rats.

Groups	Liver Glycogen (ug)	GOT (I.U./Litre)	GPT (I.U./Litre)	Bilirubin (mg/100 ml)	Cholesterol (mg/100 ml)	Blood urea (mg/100 ml)
Control	18.20 ± 1.06	30.53 ± 3.23	26.50 ± 2.39	1.05 ± 0.18	264.0 ±16.31	59.0 ± 14.26
$CC1_4$	6.40 + 0.43<=	55.47 + 4.73[a]	58.21 ± 5.72[b]	2.75 ± 0.29[b]	334.0 ± 26.94[c]	160.0 ± 13.87[b]
$CC1_4$+ Sarsaparilla extract	17.60 ±3.21[b]	47.97 ±4.39	30.85 + 4.62°	1.20 ± 0.18[b]	267.2 ± 19.30°	131.0 ±24.20

a = P < 0.05; b = P < 0.01 and c = P < 0.001. Student's t-test.
Six animals were used in each group.

the membrane integrity and produce leakage of this enzyme, suggesting tissue destruction (Choudhury and Poddar, 1983; Bang, 1958). Treatment of animals with extract significantly inhibited the CCl_4-induced rise in GPT. This enzyme is one of the best indices of liver injury. Liver GPT activity represents 90% of the whole body content (Cornelius, 1963). Unlike GPT, the extract failed to inhibit the CCl_4-induced increase in GOT activity. However, this enzyme is not totally specific to the liver. Its activity may be increased in pathogenic conditions of skeletal muscle, diaphragm, and heart muscle, as well as liver injury. It is also increased in stress situations (Cornelius, *et al.* 1959).

There were also increases in serum bilirubin, cholesterol and urea levels. Elevation of these parameters following CCl_4-induced hepatotoxicity is well documented (Osar *et al,* 1965; Nisa, 1985). Pretreatment of the rats with sarsaparilla extract significantly protected the liver against CCl_4-induced heptocellular metabolic changes. These findings were further confirmed by our histopathological studies in which the animals treated with the extract before the $CC1_4$ challenge had well-defined cellular borders with minor mononuclear infiltration; only a few cells showed architectural abnormalities. In contrast, the liver of the rats treated with $CC1_4$ showed centrilobular necrosis with mononuclear infiltration in the portal area with fatty deposition, and a loss of cell boundaries.

The chemical constituents of Sarsaprilla responsible for hepatoprotection and their mechanism of action are not clear. Our phytochemical studies have revealed the presence of a high concentration of saponins along with flavonoids, tannins, sterols and triterpenes. Treaesche *et al.* (1969) have isolated saponins from radix sarsaparilla, a species closely related to *Smilax regelii.* Saponins have recently been shown to possess highly significant immunomodulating activity, promoting the ability of the animals to combat pathogens and xenobiotics (Bomford, 1988; Chavali and Campbell, 1987; Tanaka and Kasai, 1984; Price and Johnson; 1987).

The immunostimulating action of sarsaparilla was also suggested in an earlier report from our laboratory in which Ageel *et al.* (1989) showed a significant inhibition of carrageenan-induced edema and cotton pellet granuloma. On the other hand, some flavonoids have been reported to have anti-oxidant activity (Harborne, 1967; Das and Ratty, 1986). Kiso *et al.* (1983) attributed hepatoprotective activity of several plants to their antioxidant properties. However, further studies are required to determine the active constituents and their mechanism(s) of action.

Our results on acute toxicity of the ethanol extract of sarsaparilla, following a single oral dose (0.5, 1.0 or 3 g/kg) produced no significant adverse effects nor mortality in the animals during 24 hours. Sarsaparilla is widely used as a vehicle and large quantities are employed in the manufacture of nonalcoholic drinks (Trease and Evans, 1972), suggesting frequent exposure of people to the plant for a longer period. Chronic treatment of mice in the dose of 100 mg/kg/day for 90 days did not show any significant change in the behaviour and growth rate of the animals. The gain of the body weights of the mice in the treated and control

Table 2. Effect of chronic treatment (90 days) of an ethanol extract of sarsaparilla on the body weight of mice.

Treatment and dose (100 mg/kg/day)	Pretreatment body weight Mean ± *S.E.*		Post-treatment body weight Mean + *S£.*	
	Male	Female	Male	Female
Control	28.3 ± 1.0	27.8+ 1.2	31.1 ± 1.2	30.0 ±1.5
Sarsaparilla extract	32.7 ±0.7	21.1 ± 0.9	33.4 ± 1.6	27.1 ± 1.2*

* P< 0.01, Student's t-test.
Twenty mice were used in each group.

Table 3. Effect of chronic treatment (90 days) of an ethanol extract of sarsaparilla on haemoglobin level, RBC and WBC counts in mice.

Treatment	Oral dose mg/kg/day	Haemoglobin (mg/lOOml)	RBC ($X10^5$)	WBC ($X\ 10^6$)
Control		13.10 ±0.53	6.83 ± 0.30	4.28 ±0.60
Sarsaparilla extract	100	11.4 ± 0.23	6.92 ±0.33	4.92 ±0.60

Five animals were used in each group.

groups were similar. However, the gain in body weight of extract-treated female mice was higher as compared to control animals (Table 2).

Sarsaparilla has been used as a tonic and an appetite stimulant in Unani system of medicine (Raza, 1933). Our studies on hematology and survival following the chronic treatment of mice with sarsaparilla extract revealed no significant change in RBC or WBC counts and haemoglobin levels of the animals as compared to control group (Table 3). The rate of mortality in the test groups over a period of 90 days was same as in the control group (20%, each group). These findings suggest that the sarsaparilla, besides having hepatoprotective potential, does not cause any deliterious effect on animals.

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