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NIRMAL K. NEOLIYA
YOGENDRA N. SHUKLA[#]
MAMTA MISHRA[#]

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DWIJENDRA SINGH*
SUCHETA S. MEHTA

Entomology Division,
[#]Phytochemistry Division,
Central Institute of Medicinal and
Aromatic Plants,
Lucknow 226 015, India
*For correspondence.
e-mail: dsinghko@sify.com

Hepatoprotective activity of *Sarcostemma brevistigma* against carbon tetrachloride-induced hepatic damage in rats

Liver diseases are a serious health problem. In the absence of reliable liver-protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethno-medical practices and in traditional system of medicine in India. However, we do not have satisfactory remedy for serious liver disease; most of the herbal drugs speed up the natural healing process of liver. So the search for effective hepatoprotective drug continues.

Sarcostemma brevistigma Wight (family Asclepiadaceae) grows throughout India and other tropical regions of the world. It is found to be active as anti-rheumatic, anti-allergy, anti-emetic and bronchodilator¹. Phytochemical studies reveal the presence of bergenin, brevine, brevinine, sarcogenin, sarcobiose and flavonoids^{2–4}. The present study was made to evaluate the effect of ethyl acetate extract of stem of *S. brevistigma* against CCl₄-induced hepatic damage in rats.

The stem of *S. brevistigma* was collected in June from the Kolli hills. The plant

was authenticated by the Botany Department, Tamil Nadu Agricultural University, Coimbatore. A voucher specimen has been preserved in our laboratory. The plant was dried and powdered. The dried and powdered plant was extracted with ethyl acetate using soxhlet apparatus and concentrated *in-vacuo*. Approximately, 0.50 g of dried ethyl acetate extract was obtained from 10 g of dried stem material. The extract was suspended in 5% gum acacia and used for studying hepatoprotective activity.

Male albino rats weighing between 150 and 175 g were used as animal models. The rats were divided into four groups, each group consisting of six animals. Hepatoprotective activity of *S. brevistigma* was evaluated using CCl₄-induced model⁵. Group one was kept on normal diet and served as control, the second group received CCl₄ (1.25 ml/kg) by oral route, the third and fourth group received silymarin (100 mg/kg; po) and extract of *S. brevistigma* (650 mg/kg; po) respectively once daily, for seven days. On the seventh day, CCl₄ was given by oral route 30 min after the administration of

silymarin and test drug. After 36 h of CCl₄ administration, blood was collected and serum separated was analysed for various biochemical parameters.

Biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT)⁶, serum glutamic pyruvate transaminase (SGPT)⁶, alkaline phosphatase⁷, total bilirubin⁸ and gamma glutamate transpeptidase (GGTP)⁹ were analysed.

The liver was examined grossly, weighed and stored in formalin 10% and were processed for paraffin embedding using the standard microtechnique¹⁰. A section of the liver (5 µm) stained with alummehematoxylin and eosin was observed microscopically for histopathological studies.

The results of biochemical parameters revealed the elevation of enzyme level in CCl₄-treated group, indicating that CCl₄ induces damage to the liver (Table 1). Liver tissue rich in both transaminase increased in patients with acute hepatic diseases, SGPT which is slightly elevated by cardiac necrosis is a more specific indicator of liver disease¹¹. A significant reduction ($P < 0.001$) was observed in SGPT, SGOT, ALP, total bilirubin and

Table 1. Effect of ethyl acetate extract of *S. brevistigma* on CCl₄-treated rats

Design of treatment	Liver (wt/100 g body wt)	Dose (mg/kg)	SGPT U/L	SGOT U/L	ALP U/L	Total Bil (mg%)	GGTP U/L
Control	3.4 ± 0.10	–	131.5 ± 1.98	45.3 ± 0.80	160.6 ± 3.79	0.70 ± 0.03	123.0 ± 4.10
CCl ₄	6.5 ± 0.28	1.25 ml/kg	217.3 ± 4.5	341.5 ± 3.8	388.6 ± 18.25	2.12 ± 0.01	251.0 ± 5.31
Silymarin + CCl ₄	3.8 ± 0.26*	100	138.0 ± 2.17**	81.3 ± 9.10*	218.6 ± 5.47**	0.8 ± 0.07*	109.6 ± 5.20**
Ethyl acetate extract + CCl ₄	4.1 ± 0.05*	650	115.3 ± 1.16*	67.0 ± 5.79*	292.6 ± 5.32*	0.83 ± 0.01*	133.3 ± 3.41**

N = 6 animals in each group.

* $P < 0.001$; ** $P < 0.01$ when compared with control.

Values are expressed as mean ± SE.

GGTP levels in the groups treated with silymarin and ethyl acetate extract of *S. brevistigma*. The enzyme levels were almost restored to the normal.

It was observed that the size of the liver was enlarged in CCl₄-intoxicated rats but it was normal in drug-treated groups. A significant reduction ($P < 0.001$) in liver weight supports this finding.

It was found that the extract decreased the CCl₄-induced elevated levels of the enzymes in group third and fourth, indicating the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extract.

Histopathological examination of the liver section of the rats treated with toxicant showed intense centrilobular necrosis and vacuolization. The rats treated with silymarin and extracts along with toxicant showed sign of protection against these toxicants to considerable extent as evident from formation of normal hepatic cords and absence of necrosis and vacuoles.

Decrease in serum bilirubin after treatment with the extract in liver damage indicated the effectiveness of the extract

in normal functional status of the liver. The preliminary phytochemical studies revealed the presence of flavonoids in ethyl acetate extract of *S. brevistigma*; various flavonoids have been reported for their hepatoprotective activity¹². So the hepatoprotective effect of *S. brevistigma* may be due to its flavonoid content.

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M. G. SETHURAMAN*
K. G. LALITHA†
B. RAJ KAPOOR‡

*Department of Chemistry,
Gandhigram Rural Institute,
Gandhigram 624 302, India

†Annai J.K.K. Sampoorani Ammal
College of Pharmacy,
B. Komarapalayam 638 183, India

‡Department of Pharmacology,
Vinayaka Mission's College of Pharmacy,
Yercaud Main Road,
Salam 636 008, India

*For correspondence. (e-mail:)

***Plasmodium ovale*: First case report from Assam, India**

The most prevalent species of human malaria parasite reported in India is *Plasmodium vivax* accounting for nearly 65% cases in the country followed by *Plasmodium falciparum* contributing about 35% malaria case load and *Plasmodium malariae* with only a few thousands cases recorded from few foothill areas in Orissa state¹. Occurrence of *Plasmodium ovale*, the fourth malaria parasite species, has not been very common in India and till date only three reports of *P. ovale* are available from Kolkata², Orissa³ and more recently from Delhi⁴. Here we report the finding of a case of *P. ovale* from Jorhat district of Assam, which is the first from the northeastern region of India.

During our longitudinal malaria epidemiologic investigation (April 2001–October 2002) in a village under Titabor Primary Health Centre of Jorhat district, the blood smear from 'GT', a 28-year-old male, was collected on 17 February 2002 by the surveillance worker during routine active case detection visit in that

village. At the time of blood smear collection the patient gave the history of intermittent high fever for the past 4–5 days accompanied by chill and rigor, bodyache and vomiting. He was administered presumptive treatment of 600 mg chloroquine after collecting the blood smear. The blood smear was stained with JSB (Jaswant Singh & Bhattacharya) stain and examined on 22 February 2002 in the field laboratory. The smear was positive for malaria parasite which looked like *P. vivax* in thick smear at first glance. However, careful examination of thin smear revealed it as *P. ovale* on the basis of specific morphological characteristics⁵. Many infected red blood corpuscles were oval in shape, some were fimbriated on one or both ends with heavy coarse Schuffner's stippling even in early trophozoite stage. The cytoplasm of the growing parasite was thick, compact and usually not amoeboid (Figure 1) and schizonts had 7–8 merozoites. All these features of the parasite and infected

RBCs were confirmatory for *P. ovale*. Subsequently, the identification of *P. ovale* was confirmed at Faculty of Tropical Medicine, Mahidol University, Bangkok and at Wellcome-Mahidol University-Oxford Tropical Medicine Research Unit, Bangkok. On 23 February 2002, the patient was clinically examined, a follow-up slide was taken and treated radically with 1200 mg chloroquine and 75 mg primaquine (15 mg × 5 days). The moderately anaemic patient during clinical examination was found with resolved symptoms. His liver was unpalpable whereas the spleen was soft, tender and one finger enlarged. No malaria parasite was seen in the follow-up slide, indicating that the patient responded positively to the 600 mg presumptive treatment of chloroquine. The patient was followed up at fortnightly intervals until October 2002 during which he was neither found to suffer from any febrile episode of fever nor was presence of malaria parasite detected in blood smears.