

## Hepatoprotective effect of coumestans isolated from the leaves of *Wedelia calendulacea* Less. in paracetamol induced liver damage

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Effect of coumestans isolated from the leaves of *W. calendulacea* was evaluated in paracetamol induced liver damage. The increased serum enzyme levels (lactate dehydrogenase, alanine and aspartate transaminase and alkaline phosphatase) by paracetamol induction were significantly lowered due to coumestans treatment. Results of this study revealed that coumestans of *W. calendulacea* afforded a significant protective action in the alleviation of paracetamol induced hepatocellular injury.

*Wedelia calendulacea* Less. (Compositae) known as (*Manjal karisalankanni*) (Tamil) and *Pila bhangra* (Hindi), is a perennial herb, with light camphor like odour and yellow head inflorescences (Fig.1). It grows in wet places in Uttar Pradesh, Assam, Arunachal pradesh and Tamil Nadu. This plant finds its place in indigenous Ayurvedic Medicinal System for its hepatoprotective efficiency<sup>1</sup> and the herbal extract was effective in curing induced liver injury *in vivo*<sup>2</sup>. The leaves are regarded as tonic and alternative, useful in cough, cephalgia and diseases of the skin. A decoction of the herb is used in uterine haemorrhage and menorrhagia. An ethanolic extract of the plant inhibits the growth of Ehrlich's ascites carcinoma. It is also found to affect the central nervous system<sup>3</sup>. In East and South Asia *W. calendulacea* is used to treat hepatitis, swelling, distended stomach, cough, headaches, skin diseases and baldness and in China it is used to treat diphtheria, pertussis and diarrhea<sup>4</sup>. This plant has also been used for its immuno-stimulatory activity<sup>5</sup>. In the present communication, we have further assessed the hepatoprotective activity of coumestans isolated from the leaves of *W. calendulacea* in particular *in vivo* by monitoring the changes in several liver enzyme markers in serum using paracetamol induced liver damage on Wistar rats as the experimental model.

*Collection of plant material*—The aerial part of *Wedelia calendulacea* was collected in November, the starting of winter season from the paddy fields near

Chennai. The taxonomic identification was done and the voucher specimen ERI. No. 1190 was deposited in the Herbarium of Entomology Research Institute, Loyola College, Chennai.

*Preparation of plant extract*—The leaves were collected from the field, dried at room temperature, ground and subjected to soxhlet extraction with methanol. The solvents was removed by rotary evaporator and the residue was suspended in water and heated on a steam bath (80°C) for 30 mins. Then the aqueous phase was partitioned with ethyl acetate. The organic phase was filtered and the solvent was evaporated to yield a light yellow powder and it was eluted with chloroform and methanol (70:30) in silica gel CC. This fraction was found to contain coumestans (Wedelolactone and Dimethyl wedelolactone). The presence of coumestans was confirmed using thin layer chromatography. The coumestans were quantitatively estimated by U.V. spectrophotometric method at 351 nm<sup>6</sup>. Coumestans of *W. calendulaceae* was dissolved in 1 % Carboxymethyl cellulose (CMC) (vehicle) and used for all the experiments. The drug was administered through a gastric tube. Control animals received an equal volume of the vehicle.

*Animal models*—Albino rats of both sexes (Wistar strain) weighing 100 to 200 gm were obtained from the Tamil Nadu, Veterinary Animal Maintenance Centre. They were fed with standard rat diet purchased from Lipton Ltd, Bangalore and water *ad libitum*. The rats were acclimatized to our laboratory conditions for a week before the experiments.

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Table 1—Effect of coumestans from the leaf extract of *W. calendulacea* in paracetamol induced liver damage.  
[Values expressed as mean  $\pm$  SE n=6]

Group	Treatment	Serum			
		Lactate dehydrogenase $\mu\text{mole} \times 10^{-1}$ of pyruvate liberated/min/ mg protein	Alanine transaminase $\mu\text{mole} \times 10^{-2}$ of pyruvate liberated /min /mg protein	Aspartate transaminase $\mu\text{mole} \times 10^{-2}$ of pyruvate liberated/ min /mg protein	Acid phosphatase $\mu\text{mole} \times 10^{-2}$ of phenol liberated / min / mg protein
1.	Normal control	6.55 $\pm$ 0.53	1.35 $\pm$ 0.10	0.73 $\pm$ 0.06	3.55 $\pm$ 0.28
2.	Paracetamol control	11.62 $\pm$ 0.89*	2.94 $\pm$ 0.25*	5.54 $\pm$ 0.43*	6.15 $\pm$ 0.46*
3.	Paracetamol + 125 mg	8.75 $\pm$ 0.69 <sup>z</sup>	1.95 $\pm$ 0.16 <sup>z</sup>	2.75 $\pm$ 0.22 <sup>z</sup>	5.01 $\pm$ 0.43 <sup>z</sup>
4.	Paracetamol + 250 mg	7.14 $\pm$ 0.56 <sup>+</sup>	1.49 $\pm$ 0.18 <sup>+</sup>	1.05 $\pm$ 0.09 <sup>+</sup>	3.85 $\pm$ 0.28 <sup>+</sup>

Group 1 and group 2; group 2 and group 3 and 4 and group 3 and 4 were statistically compared .  
Data followed by different symbols are statistically significant at  $P < 0.05$ .



Figs 1.2—(1) A twig of *Wedelia calendulacea* Less; and (2) TLC plate showing the presence of coumestans (Upper layer- wedelolactone and lower layer- dimethyl wedalolactone).

**Experimental schedule**—Rats were divided into four groups of each consisting of 6 animals. Group 1 was normal control and group 2 was treated as a vehicle control. Group 3 and group 4 received the coumestans i.e. 125 and 250 mg/kg respectively. The animals were treated for 6 days and on the 7<sup>th</sup> day, group 2, 3 and group 4 were starved for 12 hours and oral intubation of paracetamol was given to induce live injury. Twenty-four hours after paracetamol

administration, the rats were anesthetized and blood was withdrawn from the vena cava and centrifuged at 3000 rpm for 5 min to separate the serum. Lactate dehydrogenase<sup>7</sup> (LDH), alanine and aspartate transaminase<sup>8</sup> (ALT and AST) and acid phosphatase<sup>9</sup> (ALP) were colorimetrically estimated.

TLC studies showed the presence of two compounds i.e. wedelolactone and dimethyl wedelolactone in upper and lower layers respectively,

collectively known as coumestans (Fig. 2). Table 1 clearly shows the levels of serum enzyme in the paracetamol control rats and paracetamol + coumestan pre-treated rats. Rats treated with a single dose of paracetamol developed significant hepatic damage as observed from the elevated serum levels of hepatospecific enzymes, which serve as the enzyme markers for liver damage. Paracetamol (acetaminophen), a widely used antipyretic-analgesic drug produces acute liver damage if accidental overdoses are consumed. The covalent binding of N-acetyl-*p*-benzoquinoneimine, an oxidation product of paracetamol, to sulphhydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity have been reported earlier<sup>10,11</sup>.

Liver cells participate in a variety of metabolic activities and therefore contain a host of enzymes. Paracetamol damage to liver raises the serum levels of lactate dehydrogenase, alanine transaminase, aspartate transaminase and acid phosphatase by releasing them into the blood stream. Coumestans-treatment seems to preserve the integrity of liver membrane in paracetamol induced rise of LDH, ALT, AST and acid phosphatase. Pre-treatment with coumestans attenuated the increased enzyme activities produced by paracetamol and the subsequent recovery towards normalization of these enzymes strongly suggest the possibility of coumestans being capable of conditioning the hepatocytes so as to cause accelerated regeneration of parenchyma cells, thus protecting against membrane fragility decreasing the leakage of marker enzymes into the circulation.

Acid phosphatase is frequently employed as a marker enzyme to assess the lysosomal changes *in vivo* because it is localized almost exclusively in the particles and its release parallels that of lysosomal hydrolases<sup>12</sup>. A significant reduction in the acid phosphatase as a result of coumestans pre-treatment against paracetamol induced liver damage indicates protection against the rupture of lysosomes and the leakage of this enzyme. These altered biochemical profiles due to paracetamol treatment were significantly reversed towards normalization by the pre-treatment with coumestans of *W. calendulacea*

leaves. The maximum protection against paracetamol induced hepatic injury was afforded by the 250 mg/kg dose which reversed the elevated values of liver enzyme markers to near normal values. It can be concluded that the coumestans of leaves of *W. calendulacea* exhibit significant hepatoprotective property as it reduces cell membrane disturbances induced *in vivo* by paracetamol. Therefore the hepatoprotective effect of *W. calendulacea* as claimed by the traditional systems has a sound scientific basis.

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