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CHEMICAL AND PHARMACOLOGICAL EVALUATION OF AQUEOUS EXTRACT OF ROOT BARK OF *"OROXYLUM INDICUM"* VENT

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Abstract:

"Oroxylum indicum" Vent (Syonakh) belonging to the family Bignoniaceae, has been used in several traditional Ayurvedic and folk medicines. The decoction of this root bark used in jaundice treatment by tribal people. As a result of these findings the present study was undertaken to evaluate the hepatoprotective activity of crude aqueous extract of root bark of *"Oroxylum indicum"* Vent against liver damage in experimental animals. One new flavanoid compound was isolated from this extract which was elucidated by using modern spectral and chemical techniques and was named as Oroxylin B and this extract showed a significant hepatoprotective activity.

Key words: Paracetamol, *Oroxylum indicum*, Hepatoprotective, Silymarin.

Introduction:

Oroxylum indicum Vent (Syonakh) belonging to the Bignoniaceae family. It is an indigenous plant found throughout India in deciduous forests[1]. It is used in traditional Ayurvedic medicine such as in rheumatism, dysentery, anorexia, bronchitis, eruptive fevers, astringent, carminative, diuretic, antipyretic, and respiratory disorders[2]. Roots of *Oroxylum indicum* is one of the ingredients, of traditional Ayurvedic formulation viz Dasamula, Chyavanprasa, Brahma Rasayana, Dhanawatara, Awalwha and Narayana Taila[3].

The plant is reported to possess anti-inflammatory, diuretic, antiarthritic, antifungal and antibacterial activity[4]. The stem bark and leaves of this plant are reported to contain flavonoids namely baicalein, chrysin, Oroxylin A. and

Scutellarin[5,6]. Seeds of this plant are reported to contain ellagic acid[7]. Flavonoids such as baicalein is reported to possess an anti-inflammatory[8], anti ulcer[9], antioxidant[10], hepatoprotective[11], and immuno modulatory activity[12], whole chrysin and baicalein is reported to have antibacterial, antifungal and antiviral activity[13,14]. Ellagic acid is an important polyphenolic compound[15].

The present study was undertaken to evaluate hepatoprotective activity (400, 200 & 100mg/kg b.w) of the aqueous extract of root bark of *Oroxylum indicum*, in experimental rat model of liver injury induced by paracetamol and to chemically examine the above mentioned extract.

Materials and methods:

Plant extract:- The finely powdered (25g) aqueous extract of the plant produced from Laila Impex, Vijayawada, Andhra Pradesh.

Animals: Albino rats (wistar strain) and albino mice (Swiss strain) used in the present studies were produced from the animal house of Gosh enterprises, Kolkata. All animals were kept under standard laboratory conditions and fed on a standard diet supplied by Rayans Biotechnologies Pvt. Ltd, Hyderabad. The study was approved by Institutional animal ethical committee.(516/01/A/CPCSEA).

Experimental:

Extraction and isolation:- The finely powdered (20g) aqueous extract of the root bark was extracted with methanol by using Soxhlet apparatus. After evaporation of the solvent under reduced pressure 6g of crude extract was obtained. The resulting residue was dissolved in methanol and adsorbed on the 15g of silica gel (100-200 mesh). It was subjected to gradient column chromatography by hexane, ethyl acetate, methanol in increasing polarity. The fraction were collected and monitored on TLC. Upon purification of the collected fraction a new flavonoid, Oroxylin B was obtained along with sugar mixture and Ursolic acid. The structure was established by the interpretation of the spectral data (^{1}H NMR, ^{13}C NMR, IR and Mass).

Acute toxicity studies:

Albino mice (Swiss strain) were divided into five groups, six animals in each group. One group was set as control group and remaining four groups received (800, 1000 2000 and 3000 mg/kg) of the extract orally. The mice were observed continuously for 1hr for any gross behavioural changes and death, if any intermittently for

the next 6 hrs and then again at 24 hrs after dosing. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity and mortality were observed.

Paracetamol induced hepatotoxicity: The rats were divided into six groups I-VI, each group consisting of six rats. The rats in group-I served as vehicle control. Liver toxicity was induced in rats by administrating paracetamol orally in a 1% CMC at a dose of 2g/kg body weight for three days in all the groups except control (group-I).

Group-II served as paracetamol control.

Group –III received silymarin (25mg/kg, b.w) for seven days.

Group-IV-VI received aqueous extract (100, 200 and 400mg/kg, b.w) for seven days. All samples were administered orally.

Biochemical and histopathological parameters:

Blood samples of the rats were withdrawn on 1^{st}, 4^{th} and 10^{th} day from retro-orbital plexus. The blood and serum were separated by centrifugation and used for estimation of biochemical parameters that is SGOT, SGPT, ALP and bilirubin.

Statistical analysis: All the results were expressed as mean ± SEM. The siginificance of difference between mean values for the various groups was tested by using one way analysis of varience (ANOVA). The level of significance was $P < 0.05$.

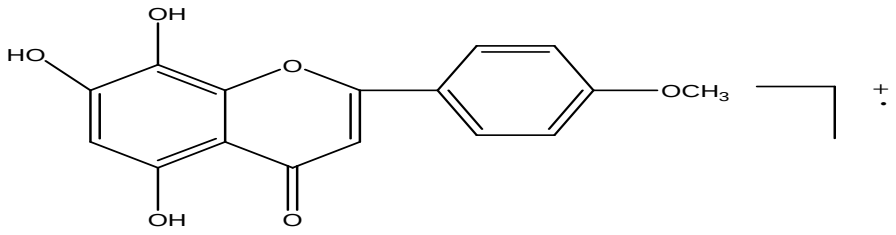
Histology: The liver tissue was excised from the animals, washed with normal saline to remove blood, fixed in 10% buffered neutral formalin for 12hours and processed for paraffin embedding. Section of 5µm thickness was cut using rotary microtome. The sections were processed and passed through graded alcohol series, stained with haematoxylin and eosin[8], cleaned in xylene and cover slipped in DPX. Histological examination was done under 10 X magnification using Trinocular Reseach zeiss Microscope (Gottingen, Germany).

Results:

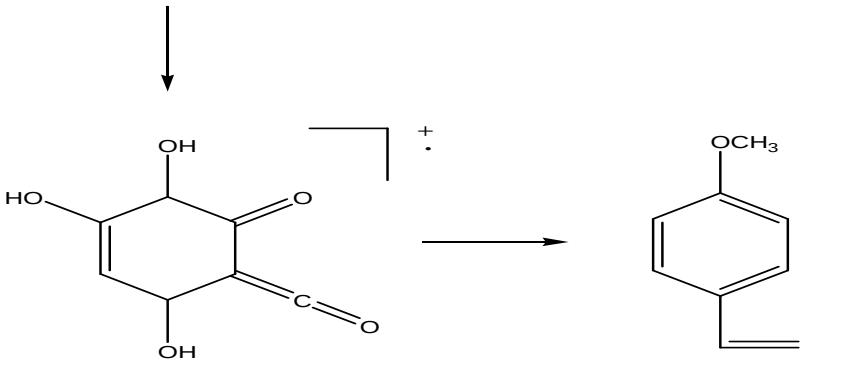
The aqueous extract of the root bark of *Oroxylum indicum* was re-extracted into methanol and the methanolic extract on concentration and column chromatography gave a new flavonoid closely related to *Oroxylin* A and was thus named as *Oroxylin* B. *Oroxylin* B was obtained as yellow needles with melting point 204-207 ^{o}C. It is analysed for $C_6H_{12}O_6$ supported by molecular ion *m/z* 300. It gave positive test for $FeCl_3$, Lead acetate,

Shinoda's test for flavonoids. Its IR spectrum exhibited bands at 3429 cm^{-1} (OH) and a band at 1656 (conjugated carbonyl) typical of a flavone skeleton. The UV spectrum exhibited absorption maximum α_{max} 217 nm (band II) and 320 nm (band I) characteristic of flavonoid skeleton, a bathochromic shift was noticed on band I on addition of aluminium chloride indicating to be a 3 or 5 hydroxy flavone. The presence of 7 hydroxy group were shown by bathochromic shift on band II to 278 nm.

Its ^1H NMR spectrum (400 MHz $CDCl_3$) showed the presence of one aromatic methoxy group at δ 3.83 (3H s), three phenolic hydroxyl groups at δ 12.99 (1H s) and δ 12.7 (2H, s). The NMR spectrum showed the characteristic 3H protons of a flavone at δ 6.71 in the aromatic region two protons each at δ 7.88 (2H, d 8Hz) and δ 7.52 (2H, d J 8Hz) accounting for AB quartet of a 4-substituted phenyl ring C-2. It further exhibited one aromatic proton as a singlet at δ 6.66 accounting for C-6 (H) between two hydroxyl at C-5 and C-7. From the above ^1H NMR spectral data the structure of *Oroxylin* B might be 5,7,8 trihydroxy 2-4H chromen-4-one. This structure still tentative and needs exact location of the methoxyl group at C-4', C-5', C-7 or C-8. Position C-5 can be ruled out in view of the presence of phenolic hydroxyl and the consequent shifts in UV maxima. The location of the methoxyl could be established to be at C-4' by observing the mass fragmentation at *m/z* 168 representing ring A. Thus the structure of *Oroxylin* B was firmly established as 5,7,8 trihydroxy-2-(4-methoxy phenyl)-4 H- chromen-4-one.



5,7,8-trihydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one



$C_7H_5O_4$ *m/z* 168 C_9OH_{10} *m/z* 134

Acute toxicity studies: The extract was found to be safe for further biological studies as no lethal effect was observed even at 3000 mg/kg, bw.

Serum enzymes:- In the present study, the aqueous extract of root bark of *Oroxylum indicum* has been found to reduce SGOT, SGPT, ALP and total Bilirubin in the treated groups is compared with the untreated groups (Table-1).

Histological examination: The hepatoprotective effect of the test drug was further confirmed by histopathological examinations of the liver sections of the control untreated group (group-I), paracetamol treated group-II and different doses of *Oroxylum indicum* extract + paracetamol treated groups IV-VI, and group-III treated with paracetamol + silymarin. The liver sections of paracetamol treated rats showed (Figure-II) centriolobular necrosis. Treatment with standard silymarin (Figure-III) and different groups of *Oroxylum indicum* (Figure IV-VI), there was less centriolobular changes and hepatocytes showing regeneration activity.

Discussion:

Paracetamol is one of the most commonly used hepatotoxin. The covalent binding of N-acetyl, P-benzo quinoneimine (oxidative product of paracetamol) to sulphydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity induced by paracetamol have been reported.

Although serum enzyme levels are not a direct measure of a hepatic injury they show the status of liver. The elevated levels of serum enzymes are indicative of cellular leakage and loss of function, loss of integrity of cell membrane of the liver.

Thus lower of enzyme contents in serum is a definite indication of hepatoprotective action of drug. High levels of SGOT indicate the liver damage such as due to viral hepatitis. SGPT catalyses the conversion aniline to pyruvate and glutamate, therefore SGPT is more specific to the liver and better parameter for detecting liver damage. Serum ALP and Bilirubin levels are also related to the status and function of hepatic cells. Increase in serum ALP is due to increased synthesis in presence of increased bilary pressure.

It is well documented that the hepato cellular enzymes serve as biomarkers of hepato cellular injury due to alcohol and drug toxicity. Administration of *Oroxylum indicum* root extract significantly enhanced the hepatic levels of glutathione dependent enzymes.

Conclusion:

The result of present study demonstrate that the aqueous root extract *Oroxylum indicum* has potent hepatoprotective activity against paracetamol induced liver damage in rats, it also showed that, it has great influence on liver blood parameters (SGOT, SGPT, ALP and Total Bilirubin). Thus it is proved that *Oroxylum indicum* aqueous extract has potent hepatoprotective activity and was found to be dose dependant. Further this could be associated with the new flavonoid Oroxylin-B which was obtained from 1% ethyl acetate-hexane fraction of this plant.

Table-1: Effect of crude aqueous extract of root bark of *"Oroxylum indicum"* on serum parameters in paracetamol induced (2gms/kg) hepatic damage in rats.

parameters	Control Group-I (1ml/kg)	Paracetamol Group-II (2gms/kg)	Silymarin Group-III (25mg/kg)	Extract Group-IV (100mg/kg)	Extract Group-V (200mg/kg)	Extract Group-VI (400mg/kg)
T.Bilirubin (mg/dl)	O.61±1.08*	2.39±1.16*	0.94±1.21*	1.7±O.17*	1.2±0.115*	1.1±0.145*
SGOT/AST (IU/L)	130±6.54*	222±11.12***	144±17.34***	210±9.49***	182±7.05***	168.6±4.63***
SGPT/ALT (IU/L)	31±5.29***	55.75±7.85***	36.75±5.34**	57.3±9.49***	48.6±7.05***	43.3±4.63***
ALP (IU/L)	82±7.82***	133±12.83***	106.5±10.43**	169±9.86***	139.6±7.44***	124.6±12.13***

The data were represented as mean± S.E.M of four animals in each group. student's *t* – test is used for the statistical analysis of blood serum parameters. The symbol * represented the value were $P < 0.05$ consider to be significant. The symbol ** represented the value were $P < 0.01$ consider to be significant. The symbol *** represented the value were $P < 0.001$ consider to be significant.

Normal control

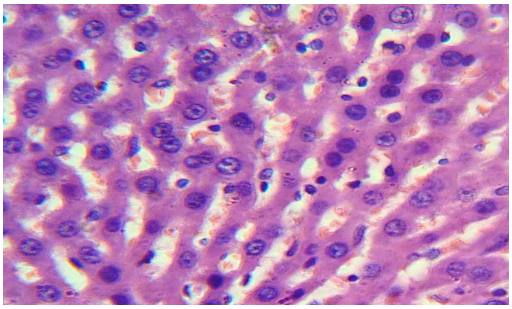


Fig 1: The parenchymatous tissue showing the regenerative changes by emptying of cytoplasm of Hepatocytes and nucleus was centrally located.

Paracetamol treated.

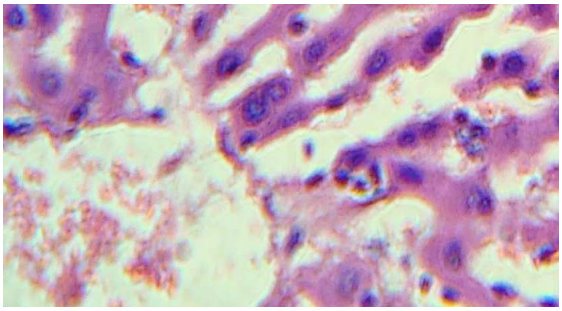


Fig 2: Liver showing mild congestion, increased space of canaliculi moderate vacuolation and foci of necrosis.

Paracetamol+ Silymarin treated

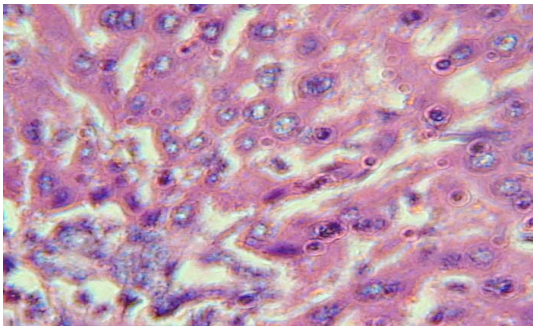


Fig 3: The sheets of hepatocytes were positioned in typical radiation pattern. Hepatocytes were individually demarked with canalicular space.

Paracetamol + aqueous extract (100mg/kg)

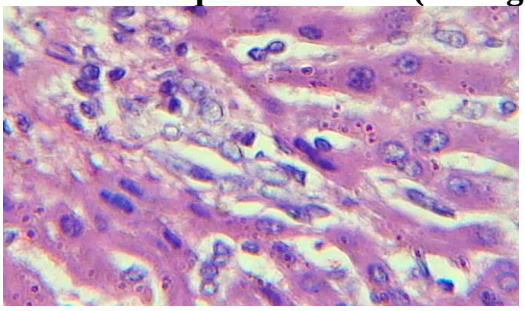


Fig 4: Hepatic parenchyma revealing a large area of necrosis and serve degenerative changes. Normal space of canaliculi was observed.

Paracetamol + aq. Extract (200mg/kg)

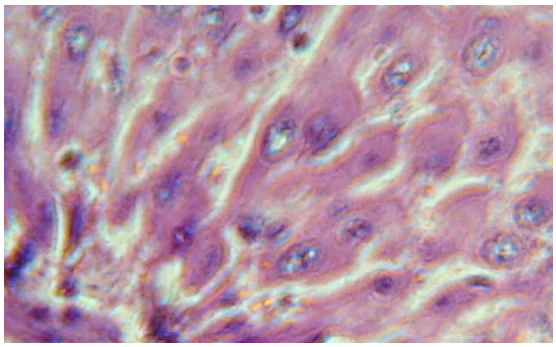


Fig 5: Hepatic parenchyma revealing a large area of necrosis and serve degenerative changes. Normal space of canaliculi was observed.

paracetamol +aq.extract (400mg/kg)

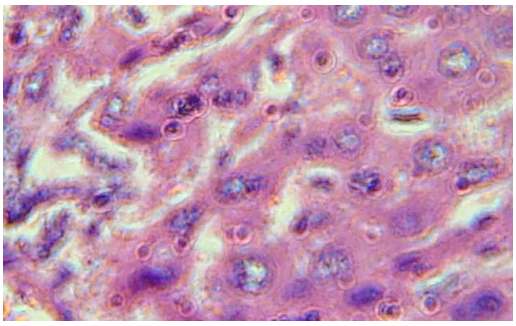
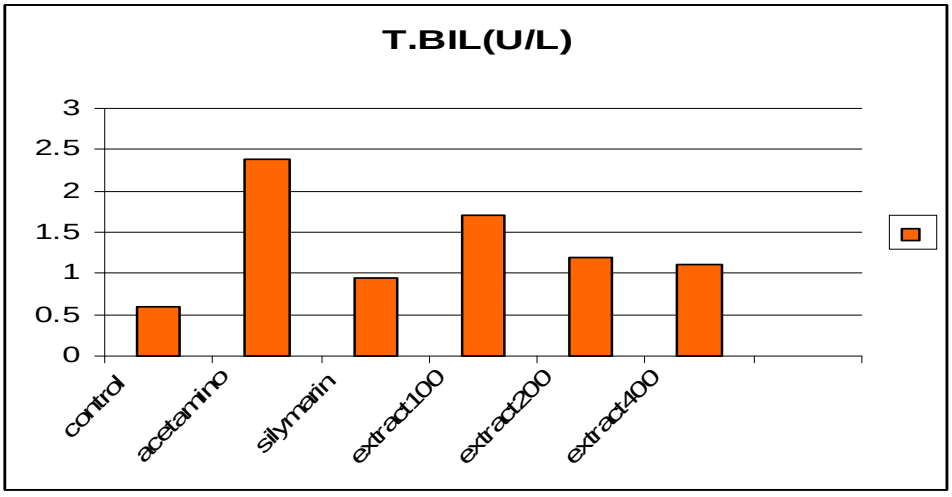
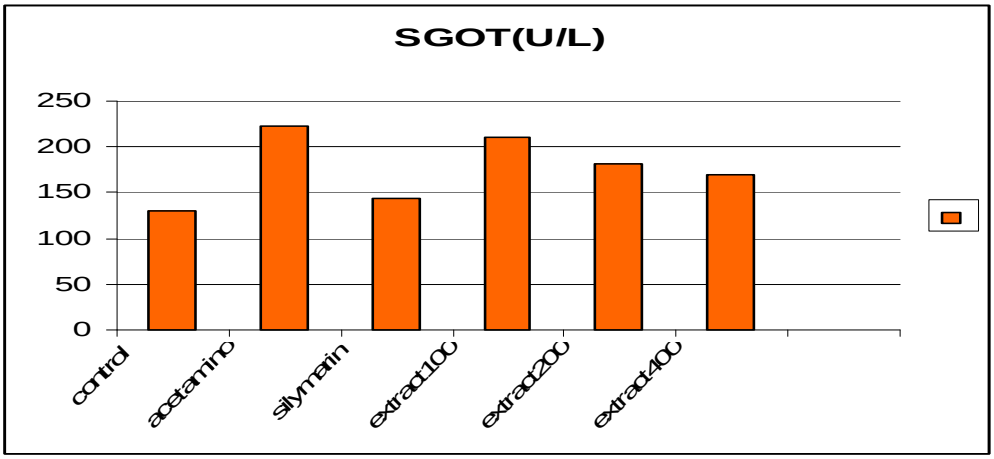


Fig 6: Hepatocytes were regenerative and showed a milder degree of vacuolation but prominent nuclei, indicating returning to normalcy.

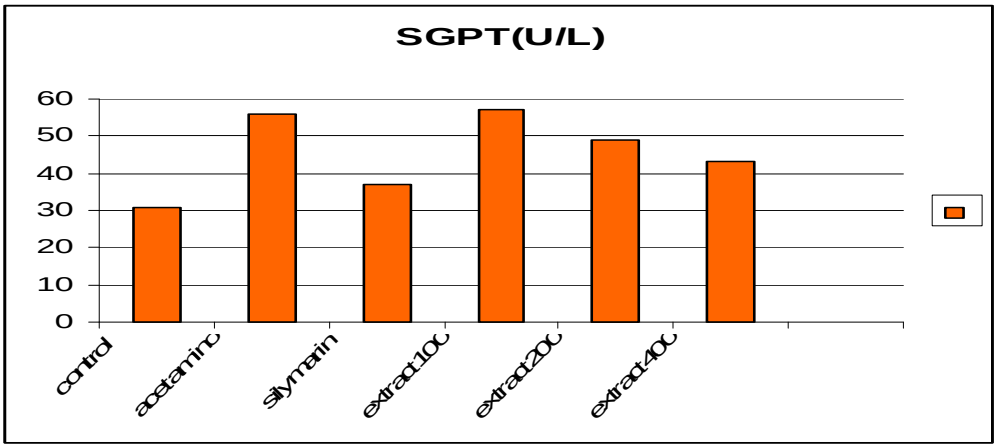
Graph-1: The effect of various groups on Bio-chemical parameter, such as T.Bilirubin in Rat serum.



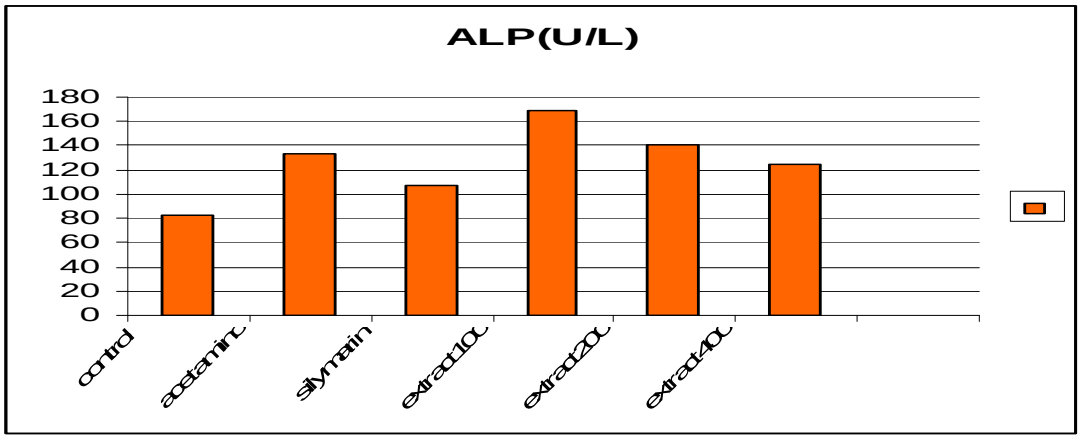
Graph-2: The effect of various groups on Bio-chemical parameter, such as SGOT in Rat serum.



Graph-3: The effect of various groups on Bio-chemical parameter, such as SGPT (U/L) in Rat serum.



Graph-4: The effect of various groups on Bio-chemical parameter, such as ALP (U/L) in Rat serum.



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