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HEPATOPROTECTIVE ACTIVITY OF LEAVES OF *SYZYGIUM ALTERNIFOLIUM* AGAINST PARACETAMOL INDUCED TOXICITY

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ABSTRACT:

The liver, a vital organ responsible for numerous metabolic processes, is susceptible to damage from various toxins, drugs, and diseases. Hepatoprotective agents derived from plants have garnered significant attention due to their potential to mitigate liver damage and promote liver health. *Syzygium alternifolium* a medicinal plant with hepatoprotective potential used against paracetamol induced hepatotoxicity. Only in the last few decades, a reappearance of interest in plants as sources of medicines and of novel molecules for use in the illumination of physiological/ biological phenomena seen. There are number of reasons for this. First, there is a genuine expectation in developing countries that their health care problems can be solved through a sensible scientific exploitation of medicinal plants; some of which have been used for generations by local populations.

KEYWORDS:

Hepatoprotective, Syzygium alternifolium, Medicinal plant, Paracetamol

INTRODUCTION:

According to world health organization herbal medicine is still the mainstay of 75-80% of the world population, mainly in the developing countries, for primary health care needs (Kamboj, 2000). Since the medicinal plants are the backbone of traditional medicine, this mean that, 3300 million people in the under developed countries apply medicinal plants on a regular basis. This belief does not involve the developed countries where there has been a great interesting for the herbal medicines and dietary food supplements in the last decade (Dobriyal and Narayana, 1998).

Besides our rich heritage of health care systems, it was only for last 100-150 years that these systems slowly but steadily have been taken up at the industrial level. Prior to this, these systems were confined to be practiced by individuals like Vaidyas, Hakeems, Traditional Dais, Bone Setters, and Massagers etc. During initial decades of present century, various small and big manufacturing houses of traditional formulations have come up in this country (Narayana, 1998).

Some scientists thus expect that the plant kingdom holds the key to the understanding of complex human biochemistry/pathology and the cure of man’s perplexing diseases. The initial optimism, engendered by the idea that a

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sophisticated understanding of receptor systems and of the biochemistry of disease would pave the way to predictable drug development, has not been released., therefore, laboratories around the world are engaged in the screening of plants for biological activity with therapeutic potential.

Liver disease is still a worldwide health problem. Conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects (M Guntupali, 2006) in spite of tremendous advances made in discovery of new compounds. A few of these diseases can be mentioned like, hepatic disorders, viral infections, AIDS, rheumatic diseases etc., (Mohammed Ali, 1994). In the absence of a reliable liver protective drug in modern medicine there are number of medicinal preparations in ayurveda recommended for the treatment of liver disorders (TK Chatterjee, 2000). The available therapeutic agents only bring about symptomatic relief without any impact on the healthful process, thus, causing the risks of worsening and danger of untoward effects.

Many populations sicken, due to various reasons, from hepatic diseases and inflammatory conditions of known and unknown agent. The development of hepatoprotective drugs being a major effect area has drawn attention of majority of workers in the field of natural product research.

PLANT PROFILE:

Syzygium alternifolium is one of the dominant species endemic to Seshachalam hill ranges in Chittoor district, Andhra Pradesh. The Plant is also common on the hills of open dry deciduous forests of Kurnool, Kadapa district, Andhra Pradesh, Chengalpattu and North Arcot districts of Tamil Nadu and Bangalore District in Karnataka in India (Gamble 1957; Chitra 1983; Saldanha 1996; Reddy et al. 2006) up to an altitude of 930 M. The drug consists of dried leaves and bark of the plant.

The plant profile of the Syzygium alternifolium is as follows

Family: - Myrtacea

Kingdom: - Plantae

Order: - Myrtales

Genus: - Syzygium

Species: - Alternifolium

Synonym: - Eugenia alternifolium wight

The plant is commonly known as Mogi, Konda neredu, Adavi neredu.

Fig-1: *Syzygium alternifolium*

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Chemical constituents:

Syzygium alternifolium has a diverse chemical composition, particularly in its essential oils. Here are some key components:

Monoterpene Hydrocarbons:

- β-mercene (24.04%)
- β-pinene (9.23%)
- β-trans-ocimene (9.2%)
- Cyclofenchene (7.21%)
- β-cis-ocimene (2.1%)
- Sesquiterpene Components:
- Viridiflorol (15.05%)
- α-cubebene (7.71%)
- β-caryophyllene

These compounds contribute to the plant's antimicrobial and antioxidant properties

MATERIALS AND METHODS:

Plant material collection:

Pharmacognostic evaluation is a primary step to certify the identity of the crude drug and to assess the quality and purity of it, so that the chosen plant drugs were therefore first subjects to Pharmacognostic evaluation.

The fresh entire plant and leaves of *Syzygium alternifolium* was procured from the boot hills of the Tirumala hills, Andhra Pradesh, India, and the identity was confirmed by Dr. Madhava setty, Botany department, SV University, Tirupathi. The voucher specimen (COG/TML/04/SVSK/2013) has been deposited in the herbarium of the institute.

Study design:

The grouping of animals and treatment protocol is similar for all groups which contain 5 wistar rats of either gender:

The protocol for Paracetamol- induced hepatotoxicity

Group	Day 1	Day 2	Day 3	Day 4	Day 5
Control	Vehicle	Vehicle	Vehicle	Vehicle	Withdrawal of blood
PCML	Vehicle	Vehicle	Vehicle +PCML	Vehicle	
Standard	Silymarin	Silymarin	Silymarin +PCML	Vehicle	
Test	Extract	Extract	Extract +PCML	Vehicle	

Histopathological Examination:

The animals have been sacrificed by anesthesia and livers were excised and placed in 10% formalin solution and given for histopathological examination.

Statistical Analysis:

Overall values have been mentioned as mean ± S.E.M and were applied utilizing Graph pad prism 5.03 for windows. One way ANOVA followed by the Tukey posttest was utilized to estimate statistical significance between various groups. Difference was taken statistically significant when $p<0.05$.

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RESULTS:

Proximate analysis

Proximate analysis helps to set up certain standards for the crude drugs to avoid batch variations and to judge their quality and purity.

The results of phenolic and flavonoid contents of leaves of *Syzygium alternifolium* was recorded in below

Phenolic content of leaves of *Syzygium alternifolium.*

Total phenolic content	% W/W
Ethanol extract	0.589
Ethanol fraction ethanol extract	1.219

The phenolic content of ethanol extract of leaves of *Syzygium alternifolium* was found to be 0.58 % w/w and EFEE extract of leaves of *Syzygium alternifolium* was found to be 1.21 % w/w, representing the presence of various phenolic compounds like poly phenols, flavonoids, phenolic acids etc.

The results of flavonoid content of leaves of *Syzygium alternifolim* was recorded in below

Flavonoid content of leaves of Syzygium alternifolium

Extracts	AlCl3 method	2,5- DNPH method	Total flavonoids % W/W
Ethanol extract	0.671	0.582	1.253
Ethanol fraction ethanol extract	0.928	0.838	1.766

The flavonoid content of EE & EEFE of leaves of *Syzygium alternifolium* was found to be 1.253 % w/w, & 1.766 % w/w respectively.

Qualitative evaluation of extract and fractions

The results obtained from the qualitative evaluation of ethanolic extract and its fractions of *Syzygium alternifolium* are recorded in below. The results denoted the presence of steroids, flavonoids and alkaloids in leaves of *S.alternifolium.*

Qualitative evaluation of ethanolic extract

Constituents	*S.alternifolium*		
	EE	CFEE	EFEE
Alkaloids	+	-	+
Phenolics and flavonoids	+	-	+
Terpenoids and steriods	+	+	-
Anthracene glycosides	-	-	-
Coumarins	-	-	-
Cardenolides	-	-	-

Acute toxicity studies

The chloroform fraction of ethanol extract (CFEE) and ethanol fraction of ethanol extract (EFEE) of *S.alternifolium* were subjected to acute toxicity determination as per OECD guidelines. None of this showed mortality even at the dose level of 2000 mg/kg and therefore considered safe.

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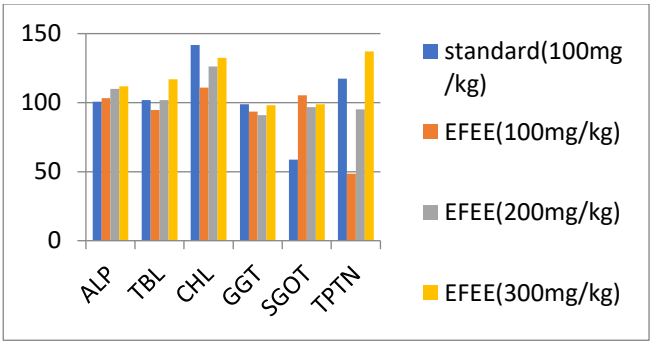
Both, CFEE and EFEE were investigated for hepatoprotective activity in rats using Paracetamol model.

Paracetamol induced hepatotoxicity

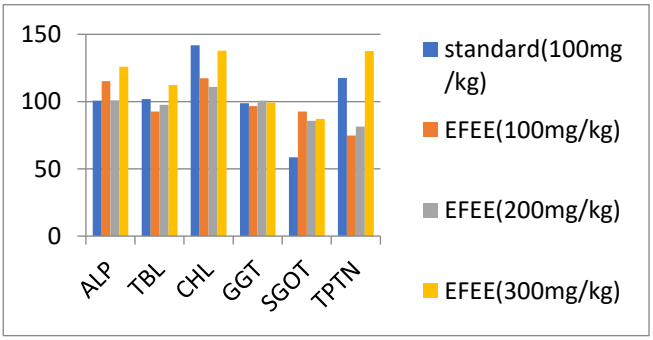
Paracetamol intoxication (3 g/kg p.o.) induced a marked increase in the serum levels of GOT (107.46 ±32.69 to 558.28 ±118.57), ALKP (349.67 ±41.79 to 1371.09± 134.88), TBL (7.91± 1.01 to 18.22 ±4.74), GGT (5.56 ±1.36 to 37.93 ±16.37) and CHL (57.25 ±8.12 to 96.68 v12.48) and decrease in the levels of TPTN (21.32 ±2.60 to 10.62 ±0.89) when compared to normal rate indicating acute centrilobular necrosis. The groups of rats which received CFEE at dose levels of 100, 200 and 300 mg/kg p.o. and EFEE at dose levels of 100, 200 and 300 mg/kg p.o. showed a significant decrease ($p<0.05$) in all the elevated levels of biochemical parameters and significant ($p<0.05$) increase in depleted TPTN levels similar to that observed in the case of rats of Silymarin treated group. The activity exhibited by CFEE was statically similar the activity exhibited by EFEE. The results obtained are shown in below.

Histological examination of liver sections of rats of control group, revealed normal cellular architecture while those intoxicated with Paracetamol (3 g/kg p.o.) showed disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis and bridged necrosis, characterized by bands of necrosis linking one central vein to another, sinusoidal hemorrhages, and dilatation. Treatment with CFEE (100, 200 and 300 mg/kg) and EFEE (100, 200 and 300 mg/kg) followed by Paracetamol intoxication resulted the absence of necrosis, sinusoidal dilation and lesser degree of disarrangement and degeneration of hepatocytes indicating marked protective activity similar to that observed in Silymarin treated rat liver sections.

Percentage restoration of various parameters by CFEE against Paracetamol-induced hepatotoxicity



Percentage restoration of various parameters by EFEE against paracetamol-induced hepatotoxicity



The percentage restoration of various biochemical parameters showed by CFEE and EFEE at various dose levels against Paracetamol-

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induced hepatotoxicity are represented in above graphs. The maximum percentage restoration, in the levels of ALKP, GOT, TBL and TPTN was observed with CFEE at dose level of 300 mg/kg, while EFEE at a dose level of 300 mg/kg afforded highest percentage restoration in the levels of GGT, and CHL levels.

Paracetamol produces hepatic necrosis when ingested in large doses. It is metabolised in the liver primarily to glucuronide and sulphate conjugates. Paracetamol toxicity is due to formation of toxic metabolites when a part of it is metabolised by cytochrome P_{450}. Induction of cytochrome P_{450} or depletion of glutathione is a prerequisite for Paracetamol induced hepatotoxicity (Savides *et al*., 1983; Rao and Mishra, 1998).

Therefore, the hepatoprotective activity of the CFEE, and EFEE of aerial parts of *S. alternifolium* against Paracetamol induced hepatotoxicity may be due to inhibition of cytochrome P_{450}; stimulation of hepatic regeneration or activation of the functions of reticuloendothelial systems.

Thus, the hepatoprotective activity of these extracts may be due to their ability to affect the cytochrome P_{450} mediated functions or stabilisation of endoplasmic reticulum resulting in hepatic regeneration.

In accordance with these results, it may be hypothesized that flavonoids with their anti-oxidant properties and steroids which are present EFEE, and CFEE, of *S. alternifolium* are responsible for the hepatoprotective activity.

Effect of CFEE and EFEE of S.alternifolium on paracetamol- induced hepatotoxicity in rats

GROUP	ALP (IU/L)	GGT (IU/L)	CHL (mg/dl)	SGOT (IU/L)	TBL (mg/dl)	TPTN (g/dl)
Normal	349.67±41.79	5.56±1.36	57.25±8.12	107.46±32.69	7.91±1.01	21.32±2.60
Paracetamol(3g/kg)	1371.09±134.88	37.93±16.37	96.68±12.48	558.28±118.57	18.22±4.74	10.62±0.89
Silymarin(100mg/kg)	342.93±149.02 *	7.56±1.91*	40.81±5.35*	293.85±133.93*	7.72±1.08*	23.19±3.46**
CFEE 1	316.90±53.61 *#	7.65±1.36*#	52.92±7.24*#	83.11±21.96*#	8.45±1.19*#	15.82±3.34
CFEE 2	247.51±70.96 *#	8.52±1.33*#	46.91±9.73*#	122.29±27.26*#	7.72±2.20*#	20.79±1.42
CFEE 3	228.82±89.17*#	6.14±0.86*#	44.41±7.39*#	112.57±27.26*#	6.17±1.41*#	25.28±2.87**#
EFEE 1	193.71±72.10*#	6.63±1.01*#	50.39±8.72*#	140.80±44.06*#	8.68±2.18*#	18.63±1.15
EFEE 2	340.01±70.19*#	5.37±1.69*#	52.91±5.62*#	171.52±35.41*#	8.15±0.58*#	19.33±2.69
EFEE 3	270.74±84.81*#	5.77±1.51*#	42.35±6.94*#	165.49±43.58*#	6.65±1.20 *#	25.04±3.54**#

Data represents the mean ± SEM of six animals.

CFEE1, CFEE 2 and CFEE 3: Chloroform fraction of ethanol extract 100,200 and 300mg/kg.

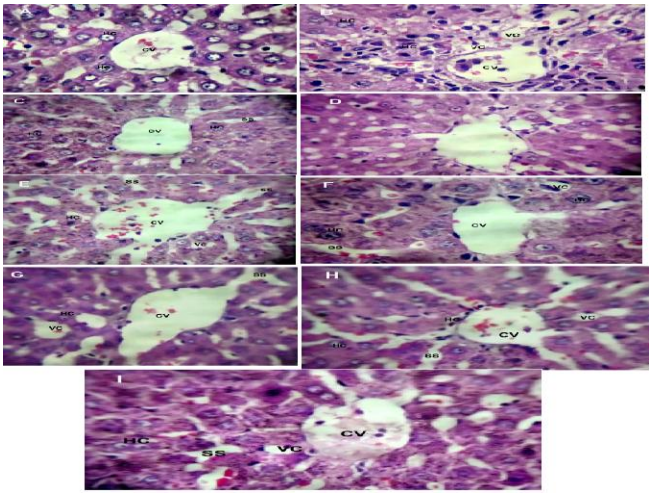
EFEE 1, EFEE 2 and EFEE 3: Ethanol fraction of ethanol extract 100, 200 and 300mg/kg.

* Significant reduction compared to paracetamol (P<0.05)

**Significant increase compared to paracetamol (P<0.05)

not significant compared to silymarin (p>0.05)

Photomicrographs representing effect of CFEE and EFEE against paracetamol- induced hepatotoxicity in rats



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A. Normal rat liver section; B. Liver section of the rat intoxicated with paracetamol; C. Liver section of rat treated with silymarin and intoxicated with paracetamol; D. Liver section of the art treated with CFEE 100 mg/kg and intoxicated with paracetamol; E. Liver section of the rat treated with CFEE 200 mg/kg and intoxicated with paracetamol; F. Liver section of the rat treated with CFEE 300 mg/kg and intoxicated with paacetamol. G. Liver section of the rat treated with EFEE 100 mg/kg and intoxicated with paracetamol; H. Liver section of the rat treated with EFEE 200 mg/kg and intoxicated with paracetamol; I. Liver section of the rat treated with EFEE 300 m g/kg and intoxicated with paracetamol

DISCUSSION:

Many of the essential drugs of modern medicine are obtained from plants as their constituents or those which are the modified form of the original constituents like digoxin, vincristine, quinine, taxol derivatives, artemisin and its derivatives etc. Investigations on plants for their biological utility therefore they become an important task throughout the world on a scientific basis. Generally, the lead for these investigations in order to select the plant species comes from among the folklore practitioners. This information provides a basis of screening these natural products or plants following a well-accepted protocol in order to eliminate useless ones from useful one. Modern system of medicine although extremely equipped for combating many disorders by providing effective medicaments, still certain disease like hepatic disorders, viral infections, rheumatic disorders etc. could only be treated symptomatically. The available agents in alternative systems therefore need to be tapped for obtaining effective medicaments.

A significant number of populations in the country suffer from hepatic disorders of known and unknown origin and the curative agents are in developing stage the discovery of new entity from both natural and synthetic sources to combat hepatic disorders has become identified area of thrust of many research groups.

In all the test models, conditions for liver damage are implemented and an attempt is made to counteract this toxicosis with the substance/preparation under test. The magnitude of the protective effect can be measured by estimating the enzyme activities and the rate of survival and can be verified histologically. The available methods are in vivo, ex vivo& in vitro methods. All these methods are used to study the protective or curative effects of any compound under test. In order to test for hepatoprotective activity the test substance and the hepatotoxin are administered simultaneously whereas in case of antihepatotoxic or curative activity the test substance is generally administered after induction of hepatotoxicity.

Chloroform fraction (100, 200 and 300mg/kg) and ethanol fraction (100, 200 and

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300mg/kg) of ethanol extract of *S.alternifolium* were prepared to determine the hepatoprotective activity against paracetamol induced toxicity, after subjecting them to acute toxicity determinations as per OECD guidelines. Silymarin was used as positive control. Chloroform and ethanolic fractions at all the dose levels tested can exhibit the hepatoprotective activity against paracetamol induced toxicity.

The assessment of hepatoprotective activity was carried out by estimation of various biochemical parameters i.e. Glutamic oxloacetic transaminase (GOT), alkaline phosphate (ALKP), total bilirubin (TBL), total cholesterol (CHL), total protein (TPT), and gamma glutamyl transaminase (GGT) in serum. The biochemical observations were supported by histological of liver sections of rats.

SUMMARY AND CONCLUSION:

In present investigations chloroform fraction and ethanol fractions of ethanol extract of *S.alte rnifolium* exhibited hepatoprotective activity in vivo against paracetamol induced toxicity. The activities were comparable to silymarin at doses tested. Hence it may hypothesize that flavonoids and steroids, are responsible for the exhibited hepatoprotective activity of the plant. Overall, these findings offer a scientific proof with regard to usage of the plant in treatment of liver disorders.

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