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CURATIVE PROPERTIES OF BUTANONE EXTRACT OF *SOLENA AMPLEXICAULIS* FRUIT AGAINST ANTI-INFLAMMATORY DRUG INDUCED LIVER DISEASES

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Abstract:

*The Butanone extract of **Solena amplexicaulis** (Lam.) Gandhi (Cucurbitaceae) were evaluated by paracetamol 2000mg/kg intoxication in rats. The toxic group which received of paracetamol (PCT) by oral route and exhibited significant increase in serum alanine amino transferase (ALT), Aspartate amino transferase (AST), alkaline phosphatase (ALP) and total bilirubin (TB) levels. It also caused significant (P<0.001) decreased the protein levels. The groups received pre-treatment of SABE at a dose of 500 mg/kg b.w.p.o. had controlled the AST, ALT, ALP and total bilirubin levels and the effects were comparable with standard drug (Silymarin 100 mg/kg b.w.p.o). The total protein (TP) and albumin (ALB) levels were significantly increased in the animals received pre-treatment of the extract at the higher dose level. The animals received pre-treatment of the extract shown hepatocellular degeneration and decreased necrotic zones when compared to the liver exposed to higher dose Paracetamol intoxication alone. Thus the histopathological studies also supported the curative effect of the extract.*

Keywords: Liver toxicity, Paracetamol, Biochemical Parameters, Butanone fraction, Curative properties

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INTRODUCTION:

In India, Ayurveda, Siddha, Unani and Folk (tribal) medicines are the major system of indigenous medicines since ancient time. Among those Ayurveda describes 700 species, Unani 700 species, Siddha 600species, Amchi 600 species and Modern Medicine around 30 species. The *Rigveda* (5000 BC) has described 67 medicinal plants, *Yajurveda* 81species, *Atharvaveda* (4500-2500 BC) 290 species, *Charaka Samhita* (700 BC) and *Sushruta Samhita* (200 BC) had described 1100 and 1270species respectively, in compounding of drugs, these are still used in the classical formulations [1, 2, 3, 4]. The interest on synthetic chemical compounds is decreasing because of harmful side effects although, there are several medicinal plants mentioned in Ayurveda, Siddha, Unani, Homeopathy, Naturopathy and Folklore medicine are used as household remedies, other medicinal knowledge and practices all over the globe. The biochemical and pharmacological studies like hepatoprotective, antihepatotoxic and antioxidant activities were not fully established for those medicines. In spite of the significant popularity of these medicinal systems, they are still to be recognized is being universally acceptable treatment modalities for liver diseases. There are few effective plants that cure liver diseases, so considerable interests is developed for research of traditional plant remedies, which are useful for treatment. In recent years, investigations carried out to provide experimental evidence, which confirms hepatotoxic properties of plants [5, 6]. No effective measures are available for the treatment of liver diseases in modern medicine so far[7]. The drugs available in the modern system of medicine will give symptomatic relief. In allopathic medical practices, even in the absences of hepatoprotective drugs there are a number of herbal drugs and their formulations possess good therapeutic effects and important role in treatment of various liver diseases in ethanomedical practices and in the traditional system of medicine (Ayurveda) in India[8]. There is no satisfactory medication for serious liver diseases, so the research will continue for hepatoprotective drugs. According to this the present study was to evaluate the hepatoprotective and hepatotoxic effect of medicinal plants.

Liver functions and diseases: Liver is the largest glandular organ in the body contributing about1/50th of the total body weight, which regulates various important metabolic functions and performs many other functions. Liver is also known by a Greek word "*Hepato"*. It lies in the upper right quarter of the abdominal cavity. It is reddish-brown in colour and divided into four lobes of unequal sizes and shape[9]. The lobes of the liver are made-up of small lobules, just visible to the naked eye. The lobules are hexagonal from outside and are formed by cubical shaped cells which are Hepatocytes and are arranged in pairs of columns radiating from central vein. The sinusoids (blood vessels with an incomplete walls) containing a mixture of blood from the very small branches of the portal vein and hepatic artery. Their arrangement allows the arterial blood and venous blood (with a high concentration of nutrients) to mix and come into close contact with liver cells. The posterior surface of the liver is called portal hepatic, where various structures enter and leave the gland. The portal vein enters carrying blood from the stomach, spleen, pancreas and the small and large intestines. The hepatic artery enters, carrying arterial blood, is a branch from the celiac artery, which is a branch from the abdominal aorta. The right and left hepatic ducts leave, carrying bile form the liver to the gall bladder[10].

Functions of the liver [11, 12]

Liver cells called as Hepatocytes are responsible for many functions that are pivotal to normal functioning of human body and they can be basically divided into 3 categories .a). Regulation, synthesis and secretions. b). Storage. c). Purification, transformation and clearance.

a. Regulation, Synthesis and Secretion: Hepatocytes are helpful for the regulation, synthesis and secretion of many substances important in maintaining the body's normal state.· Hepatocytes are metabolically active cells those uptake glucose, minerals and vitamins from portal and systemic blood and store them. Hepatocytes can produce blood clotting factors, I, II, V, VII, IX, XI, antithrombin, transporter proteins, cholesterol and bile components. Regulating blood levels of cholesterol and glucose. In the first trimester, foetus in pregnancy, the liver is the main site for RBC production.· Liver is responsible for immunological effects involving the reticuloendothelial system.

Paracetamol Induced Liver Injury: Paracetamol is caused due to its unstable toxic metabolites N-acetyl-p-benzoquinone imines (NAPQ-1), this metastable compound degenerated by cytochrome-p450-IIE1. This toxic metabolite is in activated y GSH, while GSH is depleted nearly 70% during the inactivation of the metabolite and allow there quinine to interact with nucleophilic functional groups primarily SH groups on hepatic proteins resulting in the formation of covalent adducts which reduces necrosis. The mechanism involve in the formation of the toxic metabolite from paracetamol is showing. the paracetamol induced hepatotoxicity is well indicated by unusually high serum transminase level histopathology indicates sign so acute hepatic necrosis especially zone-3-

necrosis. The hepatotoxicity of the paracetamol was found to be intensified. The hepatotoxicity of the paracetamol was found to be intensified by other agents like alcohol, drugs like isoniazide or malnutrition. Clinically treatment with N-acetyl glycine replenishes the depleted GSH levels to a considerable extent[13].

MATERIALS AND METHODS:

Collection of Plant Material: Fresh fruits of the S. amplexicaulis were collected from the forest area of Narsapoor (B) Utnoor mandal of Adilabad District, Telangana (India). The plant was authenticated by Prof. Raju S. Vastavaya Department of Botany, Kakatiya University, Warangal, Andhra Pradesh (India) and a specimen voucher (C.No.1028/Param and V.S. Raju) was deposited for future reference.

Preparation of plant extract: The fruits of the ***Solena amplexicaulis*** were made into a coarse powder and extracted with Butanone by successive solvent fractions. The obtained fraction was evaporated by using Rotavapour (BUCHI, Germany) under reduced pressure.

Phytochemical Screening: The Butanone extract was subjected to preliminary phytochemical investigation to identify the presence of various phytoconstituents i.e. Alkaloids, Glycosides and Steroids present in the fraction.

Acute toxicity study: Acute toxicity studies were carried out using acute toxic class method as per OECD guideline 425.

Wistar albino mice of 25 to 30 g were divided into ten groups of six animals each. Acute toxicity study was carried out according to the method described. The Butanone fraction of fruits of S. amplexicualis were suspended in 5% gum acacia in doses of 100, 200, 400, 600, 800, 1000, 1200, 1400, 1800 and 2000 mg/kg and were given orally to albino mice. The animals were observed continuously for any change in autonomic or behavioral responses for first few hours and later at 24 h intervals for a period of 48 h. At the end of this period, the mortality rates in all groups were noted[14].

Animals Adult Swiss albino mice (both sex) weighing 25-30 g are used in these studies. The animals were maintained at 28 ± 2°C at a 12 h light and dark cycle. Animals were housed in groups of four per cage and had free access to food and water. All the animals were acclimatized to laboratory conditions prior to experimentation. All protocols for animal experiment have been approved by institutional animal ethical committee.

Protective Effect of Selective Bioactive Butanone Fraction SABE (*Solena amplexicaulis* Butanolic Extract) against Paracetamol Induced Hepatic Damage[15]

The protective effect of selected fractions against paracetamol induced liver damage was carried out in healthy albino wistar rats. The animals maintained under standard conditions and were divided in to eight groups. The animals in various groups except those in toxic group were first treated with vehicle/Silymarin/test SABE fraction orally for 7 days and on the 8th day, an acute oral dose of paracetamol (2g/kg; b.wt.) in1% w/v gum acacia was given for inducing liver damage. Silymarin and SABE were dispersed in 2% w/v of gum acacia in water, whereas suspension of paracetamol was prepared in 1% w/v of the same suspending agent. **Control group** received the vehicle alone (2% w/v gum acacia 1 ml/kg. b.wt. *p.o*) for 8 days. **Toxic group** received the vehicle for 7 days followed by paracetamol (2.0 g/kg; b.wt.) in 1% gum acacia on the 8th day alone. **Standard group** received with 50 mg/kg of Silymarin *p.o*. for 7 days followed by paracetamol (2.0 g/kg; b.wt.) in 1% gum acacia on the 8th day. **Test groups** received 100 mg/kg; b.wt. *p.o*. of the selected by oral route for 7 days, followed by paracetamol (2.0 g/kg; b.wt.) in 1 % w/v gum acacia on the 8th day. The total duration of the study was 8 days and the administration of fractions, standard, vehicle or

Paracetamol was only once on the days specified. The blood was withdrawn 24 hrs after the administration of paracetamol. The withdrawal of blood, separation of serum, dissection of liver and calculation of percentage protection of the biochemical parameters were carried out as mentioned under the hepatoprotective activity.

Biochemical Parameters: Hepatic damage produced by paracetamol was evident in to toxic groups showing a significant increasing in serum levels of enzymes i.e. SGPT, SGOT, ALP, TB and TP, ALB, The test groups SABE-500mg/kg dose showed significantly reversed the serum levels of biochemical parameters, the degree of protection was maximum with SABE similar to that of the standard-Silymarin.

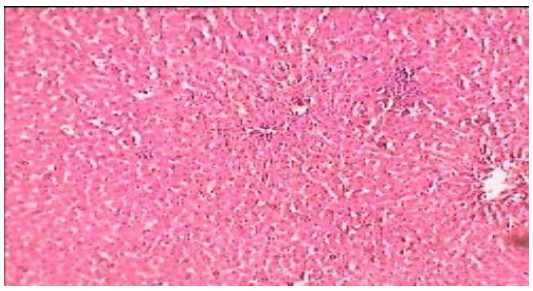
Histopathological studies: The Histopathological studies of the paracetamol treated rats (toxic group) revealed the damage of Centrilobular vein with focal necrosis and ballooning degeneration of hepatic parenchyma and the standard group Silymarin and Test group SABE showed a remarkable protection against paracetamol induced liver toxicity.

Results and Discussions:

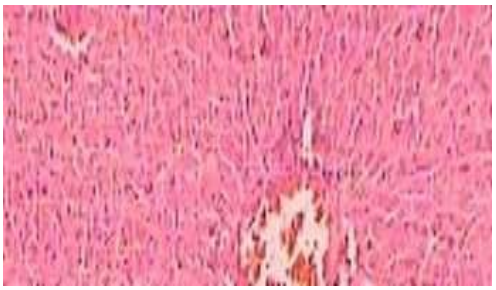
GROUPS	SGOT (IU/L)	SGPT (IU/L)	ALP (KA/dL)	TB (mg/dL)	ALB (gm%)	TP (gm%)
NORMAL	30.10±2.60	27.01±3.22	30.91±1.40	0.42±0.07	3.91±0.14	6.24±0.21
TOXIC(PCT)	129.8±9.88	117.7±5.49	112.1±9.11	2.21±0.16	1.05±0.11	3.03±0.04
SILYMARIN 50mg/kg	64.21±2.51***	57.87±3.35***	46.03±7.07**	0.87±0.17**	3.38±0.22**	5.44±0.28***
SABE 100mg/kg	77.23±6.20***	64.72±9.36***	65.89±7.53**	1.30±0.08**	3.01±0.24**	4.81±0.24***

n=6,Values expressed as Mean±SEM Significant*(P<0.05),**(P<0.01),***(P<0.001) compared with standard and toxic group using one-way ANOVA (Dunnett'stestmethod)

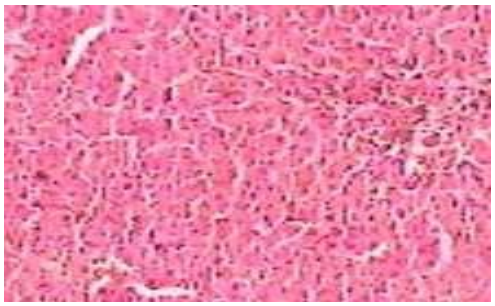
Effect of post-treatment of bioactive fractions of SABE on different biochemical parameters in Paracetamol induced liver damage in rats (values expressed as Mean±SEM)



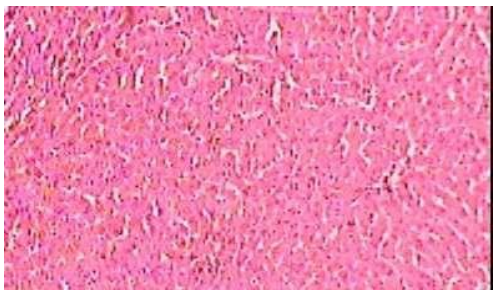
NORMAL-CONTROL



TOXIC (PCT 2000mg/kg.b.w.p.o)



STANDARD (Silymarin-100 mg/kg.b.w.p.o)



SABE 500mg/Kg.b.w.p.o

Effect of selected bioactive fraction of SABE on histopathological changes in paracetamol induced liver toxicity

Discussion: The recovery effect of SABE-500 was comparable to Silymarin which showed almost normalization.

Biochemical Parameters: Hepatotoxicity produced by paracetamol was evident in toxic group showing a significant increase in serum levels of enzymes (SGOT, SGPT and ALP), TB, lipids (CHOL) as well as decrease in serum proteins (TP, ALB). The test group BNF-SABE significantly reversed the serum levels of biochemical parameters. The degree of protection was maximal with BNF-SABE similar to that of the Silymarin.

However, the hepatoprotective effect of other fractions was comparable to that of Silymarin.

Histopathological Studies: The histopathological studies of the paracetamol treated rats (toxic group) revealed the damage of Centrilobular vein with focal necrosis and ballooning, degeneration of hepatic parenchyma. The test BNF-SABE and standard Silymarin group showed a remarkable protection against paracetamol induced hepatotoxicity.

CONCLUSION:

Fruit of *Solena amplexicualis* Butanone extract/ fraction (SABE) were found to be safe and no toxicity was exhibited in mice up to 2 g/kg b.w.p.o exhibited significant hepatoprotective effects against paracetamol induced toxicity in rats.

The fraction BNF-SABE also exhibited a significant protective effect against paracetamol induced hepatotoxicity. The hepatoprotective effect of extracts/some fractions was well comparable to that of a known phytogenic hepatoprotective drug, Silymarin (100 mg/kg).

The fraction is found to possess hepatoprotective effect. The activity of the fractions was attributed to the different classes of compounds present in them such as steroids/triterpenoids, flavonoids and phenolic compounds confirmed in phytochemical screening. The studies substantiate the use of *Solena amplexicualis* butanone extract in traditional medicine for the treatment of liver disorders.

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