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DEVELOPMENT AND EVALUATION OF POLYHERBAL NUTRACEUTICAL TEA COMPRISING *SILYBUM MARIANUM* AND *ZINGIBER OFFICINALE*

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ABSTRACT

Paracetamol (acetaminophen) overdose remains one of the most common cause of drug induced hepatotoxicity worldwide due to formation of toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI) which is conjugated with glutathione. Due to depleted glutathione levels and Paracetamol overdose leads to NAPQI accumulation in hepatocytes leading to necrosis. The present study aims to develop and evaluate herbal neutraceutical tea comprising *Silybum marianum* and *Zingiber officinale* as hepatoprotective and anti inflammatory intervention against hepatotoxicity. The phytochemical silymarin isolated from *silybum marianum* exhibits potent hepatoprotective action while gingerol isolated from *Zingiber officinale* exibits anti-inflammatory as well as anti oxidant activity. Combination of these phytochemicals may exert synergistic hepatoprotective and anti-inflammatory action simultaneously targeting oxidative stress and hepatocellular

membrane integrity. The crude drug underwent authentication followed by preformulation studies. Post formulation validation conducted through infrared (IR) spectroscopy and *in vitro* hepatoprotective assay concludes that this polyherbal nutraceutical approach may offer a complementary strategy for overcoming drug induced hepatotoxicity and improve patient compliance. This neutraceutical offers a natural methodology for management of drug induced hepatotoxicity with potential benefits for liver protection and recovery.

INDEXTERMS- Acetaminophen, N-acetyl-p-benzoquinone imine (NAPQI), IR Spectroscopy, hepatoprotective, authentication, phytochemical, silymarin.

INTRODUCTION

The liver is an important organ that has a key role in the maintenance of homeostasis. It is responsible for multiple metabolic functions and physiological processes such as bile production, energy generation, vitamin storage, metabolism of carbohydrates, proteins, and lipids. After intestinal absorption is complete the blood is rich in nutrients and xenobiotics. The blood is then transported to the liver via portal vein, which carries multiple toxic substances including drugs and toxins to the liver. As a result, the liver is susceptible to toxicity and damage. Many people have been afflicted with some type of liver lesion. Examples of liver lesions include fatty liver, non-alcoholic steatosis, hepatitis A, B or C, cirrhosis, and hepatocellular carcinoma (the third leading cause of cancer-related mortality worldwide). Apart from this liver is responsible for metabolism of drugs. A number of drugs are metabolized by liver during phase 1 and 2 reactions which include complex processes involving cytochrome P450 enzymes. Additionally, drugs can also modify how the liver functions and cause dysfunction or even failure of the organ both by a direct effect on the liver or by alteration in liver blood flow. Paracetamol overdose can have severe and life threatening consequences for patients due to its effect on liver function. Paracetamol (acetaminophen) overdose remains one of the leading causes of drug-induced hepatotoxicity worldwide and represents a significant public health concern. Although paracetamol is considered safe and effective at therapeutic doses, excessive intake overwhelms normal metabolic pathways and results in accumulation of a highly reactive and toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI). Paracetamol is metabolised via one of three pathways: glucuronidation, sulfation or the hepatic cytochrome P450 enzyme system.

Conjugation events such as glucuronidation and sulphonation, these conjugates convert into nontoxic compounds which are excreted in urine. A small amount of Paracetamol is

metabolised by cytochrome P450 enzyme, mainly CYP 2E1 resulting into highly reactive and toxic metabolite NAPQI. At toxic dosages the cytochrome P450 system and glutathione become saturated resulting in NAPQI accumulation leading to oxidative stress, mitochondrial dysfunction and ultimately hepatic necrosis.

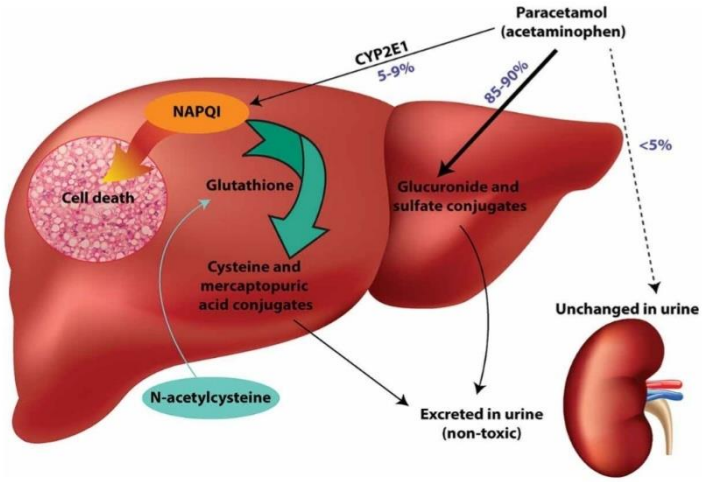

Fig. 1: Biotransformation of Paracetamol.

Silymarin is an active phytoconstituent obtained from plant *Silybum marianum* is one of the oldest plants of ancient times used for the treatment of liver toxicity. It possess diverse pharmacological activities including hepatoprotective and antioxidant action. Silymarin has membrane stabilizing property which promotes hepatocyte regeneration and inhibits fibrogenesis. Gingerol obtained from *Zingiber officinale* is a phytoconstituent responsible for exhibiting anti-inflammatory and antioxidant activity. Combination of these phytochemicals exert synergistic action and may help to maintain hepatocellular integrity by reducing oxidative stress.

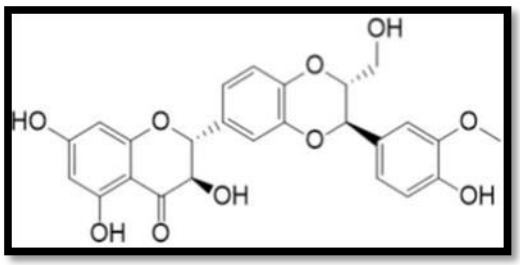

Fig. 2: Chemical structure of Silymarin.

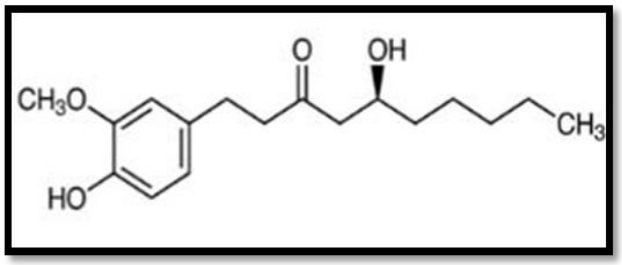

Fig. 3: Chemical structure of Gingerol.

MATERIALS AND METHODOLOGY

1. Authentication and Preformulation

The crude drugs were authenticated and Preformulation studies were performed.

2. Monograph analysis

The crude drugs were analyzed in accordance with the monograph specified in the Indian Pharmacopoeia.

3. Formulation

The crude drugs were subjected to comminution. Then combined in a specific ratio and packaged into tea bags.

4. Evaluation

a) *in vitro* hepatoprotective assay

i. HepG2 Cells were incubated at a concentration of 1×10^4 cells/ml in culture medium for 24 hrs at 37°C and 5% CO_2. 1% Paracetamol was used to induce a cellular stress in a cell lines.

ii. Cells were seeded at a concentration (100µl) 10^4 cells/well) in 100µl culture medium and 20, 40, 60, 80, 100 µg/ml of samples into micro plates respectively (tissue culture grade, and 96 wells).

iii. Control wells were incubated with Dimethyl sulfoxide (0.2% in phosphate buffer saline) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture.

iv. Cell cultures were incubated for 24 hrs at 37°C and 5% CO_2 in CO_2 incubator.

v. After incubation, the medium was completely removed and added 20µl of MTT reagent(5mg/ml PBS).

vi. After addition of MTT, cells incubated for 4 hours at 37°C in CO_2 incubator.

vii. Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark colored formazan by viable cells only.

viii. After removing the medium completely. Added 200µlof DMSO (kept for 10 min) and incubate at 37°C (wrapped with aluminum foil).

ix. Triplicate samples were analyzed by measuring the absorbance of each sample by a micro plate reader at a wavelength of 550 nm.

b) Infrared spectroscopy

Infrared spectroscopy was used for confirmation of formulation stability.

RESULTS

- **Authentication**

The submitted crude drug (p 26021239) was identified as the achene of Silybum marianum (L.) Gaertn of family Asteraceae, commonly known as milk thistle.

- **Preformulation parameters**

Bulk density	0.48 kg/m³
Tapped density	0.60 kg/m³
Angle of repose	38.23°
Carr's index	20 %
Hausner's ratio	1.25

- **Monograph analysis**

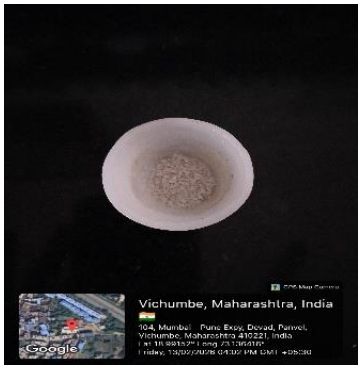

Fig. 4: LOD (loss on drying). Fig. 5: Total ash. Fig. 6: Alcohol soluble extractive.

The sample complies with monograph of Indian Pharmacopoeia

LOD	**1%**
Total ash	**4%**
Alcohol soluble extractive	**16%**

- ***in vitro* hepatoprotective assay**

SR NO	SAMPLE CODE	Conc. (µg/ml)	OD			Mean	% Of Inhibition	% Of Viability
1	Control		1.534			-	-	-
2	Standard	20	1.566	1.566	1.567	1.566	NE	NE
	Silymarin	40	1.496	1.497	1.495	1.496	2.47%	97.53%
		60	1.448	1.445	1.446	1.446	5.73%	94.27%
		80	1.395	1.396	1.394	1.395	9.06%	90.94%
		100	1.340	1.343	1.338	1.340	12.64%	87.36%
3	A1	20	1.521	1.524	1.518	1.521	0.85%	99.15%
		40	1.489	1.485	1.483	1.485	3.19%	96.81%
		60	1.461	1.464	1.459	1.461	4.76%	95.24%
		80	1.437	1.433	1.440	1.436	6.39%	93.61%
		100	1.388	1.385	1.388	1.387	9.58%	90.42%

Fig. 7: Effects of compounds against HepG2 cell lines.

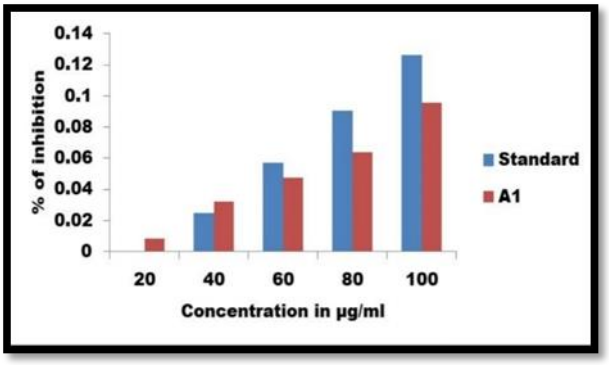

Fig. 8: Graphical data of % inhibition Vs concentration.

The MTT-based hepatoprotective assay performed on HepG2 cells shows a clear concentration- dependent increase in percentage inhibition for both the standard drug and the test compound A1 across the range of 20–100 µg/ml. At 20 µg/ml, minimal protective activity is observed for both treatments. At 40 µg/ml, A1 exhibits slightly higher activity compared to the standard. However, from 60 µg/ml onward, the standard demonstrates progressively greater hepatoprotective efficacy than A1. At 80 µg/ml, both treatments show marked improvement, and at 100 µg/ml, maximum protection is observed, with the standard achieving the highest percentage inhibition, followed by A1 with comparatively lower but significant activity. Overall, the data indicate that A1 possesses notable hepatoprotective

potential in HepG2 cells, although its efficacy is moderately lower than that of the standard drug, suggesting dose-dependent cytoprotective activity.

- **Infrared spectroscopy**

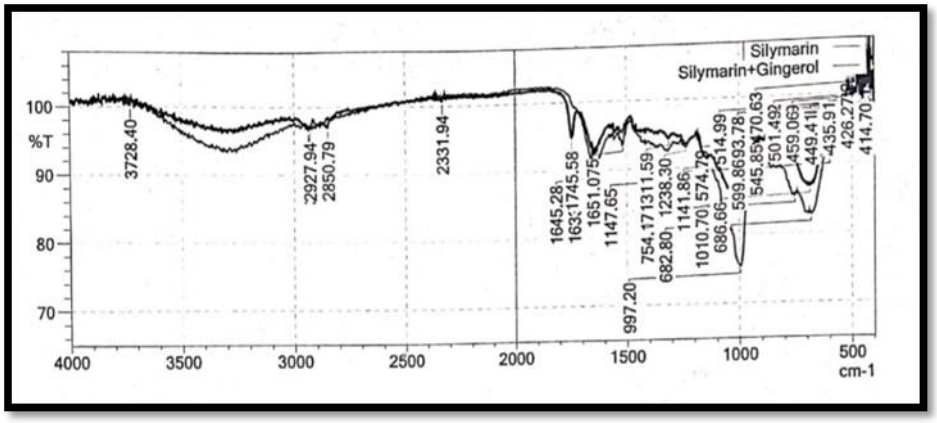

Fig. 9: Purity index for Silymarin Vs Silymarin and Gingerol.

Purity index = 0.9748

Confirming spectral compatibility and formulation stability

CONCLUSION

The developed polyherbal nutraceutical tea formulation containing *Silybum marianum* and *Zingiber officinale* demonstrated significant in-vitro hepatoprotective potential in HepG2 liver cells against Paracetamol. The formulation exhibited a clear dose-dependent protective effect. The IR purity index of 0.9748 indicates high compatibility and stability. This formulation exhibits hepatoprotective action and stability. This nutraceutical tea may serve as a promising natural therapy for drug induced hepatotoxicity and needs further mechanistic and clinical evaluation.

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