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DETERMINATION OF HEPATOPROTECTIVE ACTIVITY OF CHRYSANTHEMUM BALSMITA AGAINST PARACETAMOL INDUCED HEPATO TOXICITY IN ALBINO WITSAR RATS

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Abstract:
The study was performed to determine hepatoprotective activity of Chrysanthemum Balsamita herb against paracetamol induced hepatotoxicity. It was found that that the extracts of test compound posses a significant hepatoprotective activity which is evident by the prevention in the elevation of serum bio chemical parameters like SGPT, SGOT, ALP and BIT. It can also be determined by the presence of steroidal compounds, flavanoids, tannins, sugar as in certain therapies they are being used for the treatment of liver diseases by regeneration of damaged liver cells.

Abbreviations: *IU/L international unit per year, OECD- organization for economic cooperation and development, PCM- paracetamol, SGOT- serum glutamate oxaloacetate transaminase, SGPT- serum Glutamate Pyruvate Transaminase, P.O-orally, LD50- lethal dose for 50, SIL-Silymarin, BIT- Total bilurubin, ALP- alkaline phosphatase, extract- chrysanthemum balsamita Ethanolic extracts.*

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INTRODUCTION:

The liver performs the normal metabolic homeostasis of the body as well as biotransformation, detoxification and excretion of many endogenous and exogenous compounds, including pharmaceutical and environmental chemicals. Drug-induced hepatotoxicity is a major cause of iatrogenic diseases. [1]

It is continuously and variedly exposed to xenobiotics, environmental pollutants, and chemotherapeutic agents because of its strategic placement in the body. If the natural protective mechanisms of the liver are overpowered during all such exposures, this will lead to hepatic injury. Liver diseases are worldwide problems, and conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. [2]

Despite of the great scientific achievement in the field of hepatology, management of liver diseases is still a challenge to modern medicine. Natural products are known to be used as alternative remedies in the treatment of liver toxicity. Silymarin (Si) is an herbal drug used almost in liver protection all over the world. It exerts its effect through antioxidation, anti-lipid peroxidation, antifibrotic, anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating mechanisms. Several studies have publicized that hepatoprotective effects are related to phytoextracts rich in natural triterpenoidal saponins such as Soyasaponins; that possesses a protective effect on liver injury.[3]

Plants and natural products have been used traditionally worldwide for the prevention and treatment of liver disease. Scientific research has supported the claims of the medicinal efficacy of several of these herbal compounds, as evidenced from the voluminous work on their hepatoprotective potentials. More than 700 mono- and polyherbal formulations from over a hundred different plants are available for use. [4]

Paracetamol is metabolically activated by cytochrome P450 to a reactive metabolite that covalently binds to protein. The reactive metabolite responsible for hepatotoxicity is N-acetyl-p-benzoquinone-imine which reacts with N-acetyl cysteine.

Although considered safe at therapeutic doses, in overdose, it produces a centrilobular hepatic necrosis that can be fatal. Various mechanisms leading to paracetamol toxicity includes

1. Increased formation of superoxide anions which cause lipid peroxidation (oxidative stress) via hydrogen peroxide formation
2. Decreased glutathione concentrations in centrilobular cells.
3. **D-galactosamine:** Galactosamine administration induces an inflammatory response in liver that biochemically and histologically resembles viral hepatitis A single administration causes hepatocellular necrosis and fatty liver causes appearance of specific lesions in liver cells, characterized by inhibition of nuclear RNA and protein synthesis
4. **Thioacetamide:** Thioacetamide, originally used as a fungicide is a potent hepatotoxic and is bioactivated by CYP450 and/or flavin-containing monooxygenase (FMO) systems to sulfine (sulfoxide) and sulfene (sulfone) metabolites, which causes centrilobular necrosis .This metabolite causes liver fibrosis. Thioacetamide interferes with the movement of RNA from the nucleus to cytoplasm which may cause membrane injury [5]

MATERIALS AND METHODS:

1 Collection of plant material: The dried herbs of the plant ChrysanthemumBalsamita of family Asteraceae was collected from botanical garden of Sri venkateshwar Reddy University, Tiupathi. The plant leaves were authenticated at botany college of srivenkateshwar Reddy University, Tirupathi by Dr K Mandhava Chetty professor of botany dept.

2 Successive solvent extraction: The herb was dried in shade, powdered and stored in air tight containers for the study.

The powdered material was subjected to maceration. The solvents used were ethanol and distilled water. The powdered material of ChrysanthemumBalsamita herb was kept in contact with the solvents in cool vessel for 7 days by occasional stirring. The liquid was strained and was clarified by filtration. Then the excess liquid is evaporated and concentrated. The concentrated extracts were air dried at room temperature, weighed and percentage yield was obtained. The color and the consistency of extracts were also noted.

3 Experimental design: Albino Wistar rats of either sex weighing between 250-300gm were procured from animal house of Shadan Medical college, Peerancheru. After procuring, the animals were acclimatized for seven days under standard husbandry conditions with 12 hour light/dark cycle. The animals were fed with standard diet and free

access to water was provided under strict hygiene conditions. After obtaining permission from Animal ethical committee (IAEC), studies were performed in accordance to guidelines of CPCSEA 425and proposal reference number was CEAD/SES/2016/63.

4 Study design: The rats were selected randomly and were divided into 5 groups each containing 6 animals.
Group -1normal control
Group 2- toxicant- Paracetamol (2.5g/kg) orally
Group 3-Silymarin 125mg/kg
Group 4-Chrysanthemum Balsamita **250mg/kg** orally
Group 5- Chrysanthemum Balsamita**500mg**/kg orally

5 Chemicals: Aqueous extracts of Chrysanthemum Balsamita were used. Silymarin 125mg/kgwas used as standard drug. Paracetamol 2.5g/kg was used to induce hepatotoxicity.

6 Paracetamol induced hepatotoxicity: The dose of paracetamol to induce hepatic damage was selected as 2.5g/kg body weight for four days with three days interval

RESULTS:

LD50 studies were conducted in albino Wistar rats by OECD guidelines NO 425 for Chrysanthemum balsamita extracts. it was found that the extracts even at 2000mg/kg dose had not shown any mortality confirming it is practically non-toxic in nature.

Hepatoprotective study: Elevated levels of serum biochemical parameters like SGPT, SGOT, ALP, and BIT were observed in paracetamol induced hepato toxicity and were analyzed. Silymarin a standard drug significantly produces decrease in the above parameters
.
The Ethanolic extracts showed significant hepato protective activity at dose of 500mg/kg when compared to 250mg/kg against paracetamol induced hepato toxicity at dose 2.5/kg.

Solvent extraction observation:

Table 1: Percentage yield, consistency and solubility of ChrysanthemumBalsamita herb extracts; the results are expressed as mean±SEM... The difference means was

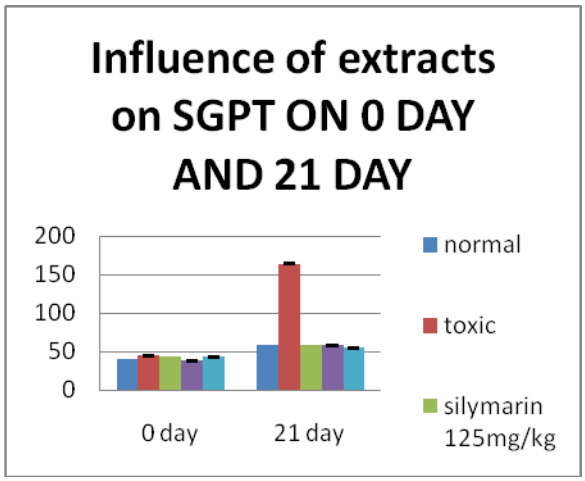
Plant	Extracts	% yield	Color	Consistency	Solubility in water
ChrysanthemumBalsamita herb	Alcohol	8.3%	Dark green	Sticky	Soluble
	water	8.1%	brown	Dry powder	Highly soluble

Table 2: Phytochemical screening

SNO	Screening for	Result in aqueous extracts
1	sterols	Positive
2	Alkaloids	Negative
3	Saponins	Negative
4	Glycosides	positive
5	Tannins	positive
6	Flavanoids	Positive
7	sugars	positive

Table 3: Influence of ChrysanthemumBalsamita Ethanolic extracts on serum biochemical parameters inparacetamol induced hepatotoxicity: (i) SGPT (ii)SGOT

TREATMENT	SGPT(IU/L)		SGOT(IU/L)	
	0 DAY	21 DAY	0 DAY	21 DAY
normal	40. 55±0.41	58.52±1.11	63.46±1.06	7.45±1.93
toxic	44.42±0.73	163.9±3.98	64.62±1.30	365.1±55.61
silymarin 125mg/kg	43.56±0.40	58.31±189	68.43±2.05	91.8±1.17
extracts chrysanthemum balsamita 250mg/kg	37.8±1.08	58.03±1.19	68.2±1.37	90.44±1.11
extracts chrysanthemumbalsamita 500mg/kg	42.63±0.93	54.71±1.45	66.34±2.08	69.64±3.22



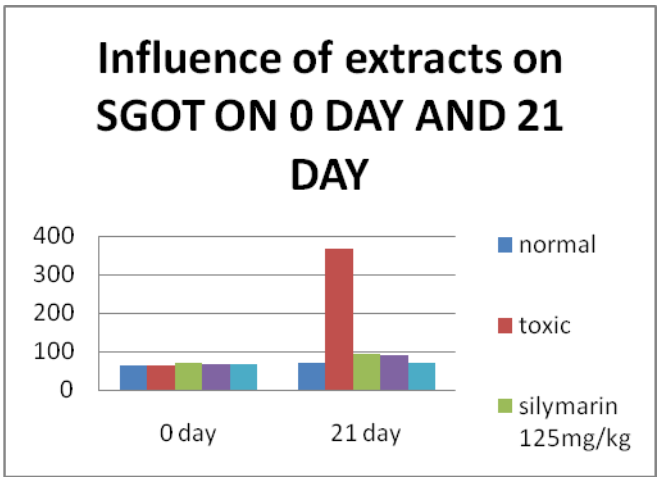
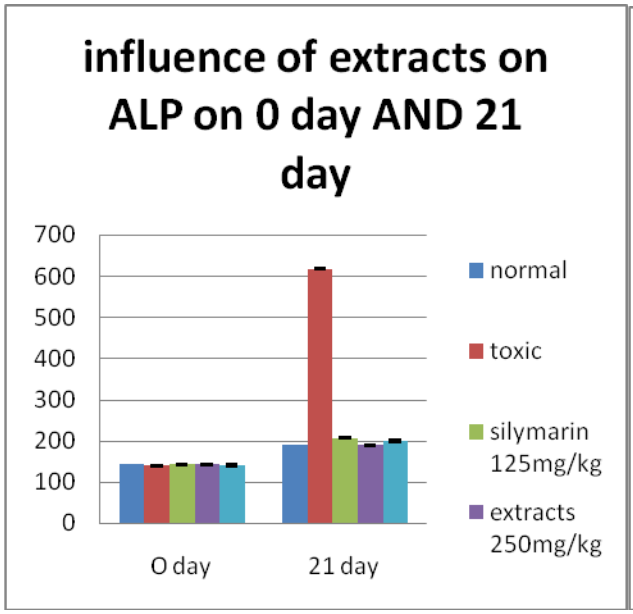


Table 4: Table 3: Influence of Chrysanthemum Balsamita Ethanolic extracts on serum biochemical parameters in paracetamol induced hepatotoxicity: (iii) ALP (iv)BIL

TREATMENT	ALP(IU/L)		BIT(IU/L)	
	0 DAY	21 DAY	0 DAY	21 DAY
normal	143.6±0.44	188.29±1.02	0.747±0.10	0.83±.0.09
toxic	138.76±1.18	617.55±9.98	0.65±0.09	3.19±0.22
silymarin 125mg/kg	142.8±1.97	206.7±5.28	0.69±0.17	0.99±0.17
extracts of chrysanthemum balsamita **250mg/kg**	141.79±1.4	189.4±3.61	0.64±0.15	0.84±0.09
extracts of chrysanthemum balsamita **500mg/kg**	140.64±3.18	200.09±3.24	0.44±0.12	0.96±0.15



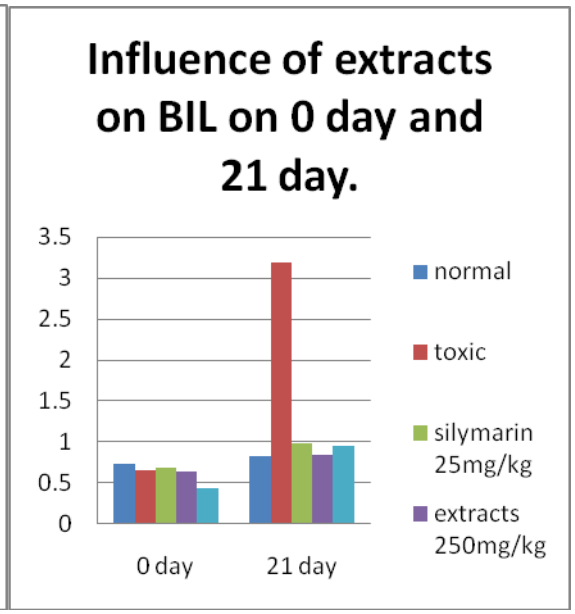
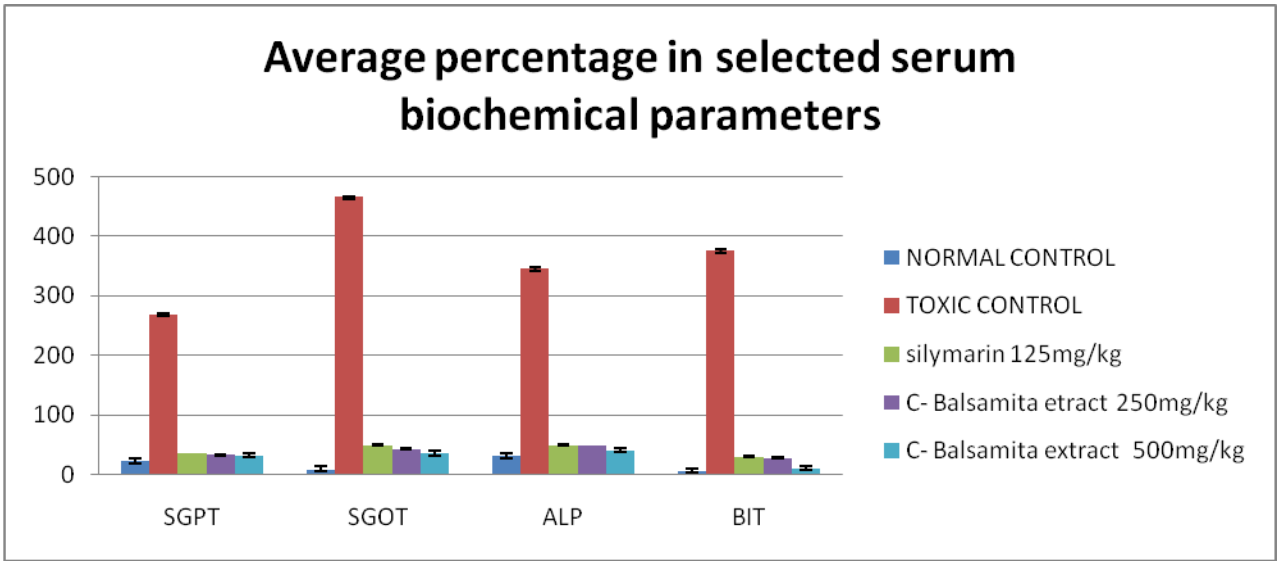


Table 5: Average change in the selected serum biochemical parameters in paracetamol induced hepatotoxicity in rats

GROUP	SGPT	SGOT	ALP	BIT
normal	22.83±2.57	9.31±1.51	31.78±0.86	6.67±15.70
toxic	268.6±12.91	464.76±11.33	345±4.482	374±32.13
silymarin 125mg/kg	35.82±44.2	49.28±3.68	49.77±4.07	30.66±4.85
extracts of chrysanthemum balsamita**250mg/kg**	33.35±5.3	42.91±3.68	48.71±3.60	29.78±30.6
extracts of chrysanthemum balsamita**500mg/kg**	32.91±3.71	35.52±4.94	41.01±4.02	11.62±14.98



SUMMARY:

Chrysanthemum balsamita belonging to family Asteraceae is a small deciduous plant. In our study, wedetermined the hepato protective activity of the herb.

Preliminary photochemical investigation and screening of chrysanthemum balsamita leaves state the presence of steroidal compounds, Flavanoids, tannins, sugars. The plant extract and some of its constituents can be used for the treatment of liver by regeneration of damaged liver cells.

The extracts posses statistically significant hepatoprotective activity as evident by prevention in the elevation of serum biochemical parameters like SGPT, SGOT, ALP AND BIL in paracetamol induced hepato toxicity rats which were compared with standard Silymarin drugs.

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