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Evaluation of Hepatoprotective Activity of Crocus

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ABSTRACT

The ethanolic extract of crocus was screened for anti-inflammatory in inflammatory animals induced via carbon tetrachloride. The degree of protection was measured by estimating biochemical parameters such as serum glutamate Oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), total protein (TP), total albumin (TA), alkaline phosphatase (ALKP) and the level of total serum bilirubin. The extract in addition reduced CCl_4 induced lipid peroxidation i*n-vivo* and *in-vitro*. The ethanolic extract (50 mg/kg, 100mg/kg,200mg/kg)exhibited significant inflammatory in carbon tetra chloride intoxicated rats in a dose-dependent manner. The anti-inflammatory effects of the extract were comparable with the standard drug silymarin.

Key Words: crocus, anti-inflammatory, Carbon tetrachloride, Silymarin.

INTRODUCTION

Early in the twenty century herbal medicine was a prime healthcare system as antibiotics or analgesics were not available. With the development of allopathic systems of medicine, herbal medicine gradually lost its popularity among people and it was based on the fast therapeutic actions of synthetic drugs. Almost a century has passed and we have witnessed limitations of allopathic systems of medicine. Lately herbal medicine has gained momentum and it is evident from the fact that certain herbal remedies peaked at par with synthetic drugs. It can be concluded that knowledge of Alternative and Complementary Systems of Medicines like Ayurveda, botany, pharmacognosy and phytochemistry, biochemistry, ethno pharmacology and toxicology is integral part of herbal medicine.

Recently we have witnessed explosive growth of herbal drug industry. Data and metaanalysis have shown that more and more people are consulting herbal practitioners. It"s cheering that the World Health Organization has also identified importance of herbal medicine. According to a study from U.S., 60-70% patients living in rural areas are dependent on herbal medicine for their day to day diseases [**1**] .Several authors have reported favorable results with herbal drugs (mostly in form of extracts) either in animal or in human studies. Ginkgo biloba L., Echinacea purpurea L., Hypericum perforatum L. and Cimcifuga racemosa (L.) Nutt, were subjected to clinical trials. Silybum marianum L., the reputed hepatoprotective, has remained a golden standard in the treated of liver ailments. Several years have passed but status of this herbal drug remains unquestioned. In India, a study reported that Picrorrhiza kurroa Royle. Is more potent than Silybum marianum as hepatoprotective agent (however, this study is not complete in all aspects) [**2**].Herbal drugs are significant source of hepatoprotective drugs. Mono and poly-herbal preparations have been used in various liver disorders. According to one estimate, more than 700 mono and poly-herbal preparations in the form of decoction, tincture, tablets and capsules from more than 100 plants are in clinical use. Surprisingly, several studies have appeared in journals addressing hepatotoxic potential of herbal drugs. These studies suggest that the drugs that were claimed to be hepatoprotective are actually hepatotoxic.In India, several steps have been taken to improve quality of Ayurvedic medicines. Good manufacturing practice (GMP) guidelines have been introduced so as to ensure quality control. Medicinal plant boards have been constituted at state and center level to inspire people, particularly the farmers for adopting cultivation of medicinal plants. Herbal gardens have been developed to make the common man conversant with the rich heritage of Indian system of medicine. Various institutes like NIPER, NBRI, CIMAP and CDRI are playing pivotal role in laying down standards for Ayurvedic system of medicine.To conclude it may be said that herbal drugs have provided us with potent weapons like atropine, codeine, taxol, vincristine and vinblastine. In the modern scenario, diseases are becoming drug-resistant and scientists are studying possible roles of plant based drugs for screening life saving drugs. The herbal system of medicine is a fully fledged system of medicine and it cannot be ruled out as quackery. Backing up this system is the fact that ancient findings and documentation have through the centuries provided us with leads on the development of lifesaving drugs.

Treatment options for common liver diseases such as cirrhosis, fatty liver, and chronic hepatitis are problematic. The effectiveness of treatments such as interferon, colchicine, penicillamine, and corticosteroids are inconsistent at best and the incidence of side-effects profound. All too often the treatment is worse than the disease. Conservative physicians often counsel watchful waiting for many of their patients, waiting in fact for the time when the disease has progressed to the point that warrants the use of heroic measures. Physicians and patients are in need of effective therapeutic agents with a low incidence of side-effects. Plants potentially constitute such a group.

Several hundred plants have been examined for use in a wide variety of liver disorders. Just a handful has been fairly well researched. The latter category of plants include: Silybum marianum (milk thistle), Picrorhiza kurroa (kutkin), Curcuma longa (turmeric), Camellia sinensis (green tea), Chelidonium majus (greater celandine), Glycyrrhiza glabra (licorice), and Allium sativa (garlic). This review will be divided into two parts. Silybum marianum and Picrorhiza kurroa will be reviewed in Part One. Curcuma longa, Camellia sinensis, Chelidonium majus, Glycyrrhiza glabra, and Allium sativa There are number of phytoconstituents from plants which have exhibited antihepatotoxic activity.

A number of recent reviews have focused on the adverse effects of herbal products. In the current review, we will highlight on herbs known to be hepatoprotective, mechanisms of hepatoprotectivity, and clinical documentation. In fact some herbal products claiming to be Hepatoprotective may actually be having some components with hepatotoxic potential. Silybum marianum, Picrrorhiza kurroa, Andrographis paniculata, Phyllanthus niruri, and Eclipta Alba are proven Hepatoprotective medicinal herbs, which have shown genuine utility in liver disorders. These plants are used widely in Hepatotprotective preparations and extensive studies have been done on them. Their discussion is beyond the scope of the article. India is known as a botanical garden in world and the largest producer of herbal medicines. India recognizes more than 3000 plant as medicinal use. It is estimated that more than 6000 plants in India are in use in traditional and herbal system of medicines. Herbal medicines are used in various forms in indigenous system such as Unani, Ayurveda, and Siddha.[2]

Around 25,000 effective herbal formulations are used in traditional and folk medicine in India. The demand for plant products is increased throughout the world and the pharmaceutical companies are currently carrying out research on plant material for the potential medicinal components. Even though they are not able to prove the therapeutic effects of many plants, research continues to screen the active ingredients which form the basis of drugs to fight disease like psychological disorder, neuro-developmental disorder, diabetes, cancer, AIDS and various more chronic disease **[3]**.

Herbal drug is the oldest form of health care known to mankind. Herbs had been used by all the cultures throughout the history. In modern civilization herbal drug is an integral part of the development. Primitive man observed and appreciated the great diversity of plants available to him. The most use of medicinal plant has been developed through observation of wild animal by trials and errors. As time moved on, each tribe added the medicinal power of herbs in their area based on their knowledge. They collected the information on herbs based on the method and well-defined it in herbal pharmacopoeia. Indeed, well into the 20th century most of the pharmacopoeia of scientific medicine was derived from the herbal lore of native place. Much of the drug commonly use now a day is of herbal origin. Most civilized country USA dispensed about 25% of prescription which contains at least one active ingredient derived from plant materials. Some are made from plant extract others are synthesized to mimic the natural plant compounds.

From last five thousand years human being has relied on natural product as the primary source of medicines. However, the last two centuries have brought an explosion to understand how the natural products are produced and how they react with other organisms. The World Health Organization (WHO) estimates that 80% of the world health populations presently use herbal medicines for some aspect of primary health care [4-6].

In recent years synthetic drugs are showing more adverse affect, to overcome this problem researchers are trying to avoid this risk of those drugs. Whenever a drug is prescribed to a patient they are facing risk of side effect, so long term use of these drugs patient should be careful. But in herbal medicine the toxic effects are negligible, so the uses of herbal industry are growing up. Indian, Chinese are using plant as medicine, as whole plant or its extract. Toxicity of herbal drugs is less when compared with the synthetic medicines **[7-10].**

MATERIALS AND METHODS

Plant Material collection and authentication

The leaves of *Crocus sativus*were collected at in the month of July, 2022. The specimens were submitted and identified as leaves of *Crocus sativus* family of Iridaceae, and authenticated by Dr. Madhav Chetty of the Department of Botany, Sri Venkateswar University, Tirupathi. The appession no. for the specimen is 490/BS/CS/16 has been preserved for future identification. The samples were shade dried so as to protect its chemical constituents not to get degrade at high temp.

Extraction of Plant Materials

The air dried powdered material (100 g) was taken in 1000 ml soxhletapparatus and extracted with petroleum ether for 7 days to remove fatty material. At the end of 7th day the marc was taken out and it was dried and again subjected to extraction with absolute ethanol until the colour disappeared. Then the extract was concentrated by distillation. The final solution was evaporated to remove excess of remaining ethanol. Finally the colour consistency of ethanolic extract was noted [**11**].

ANIMALS

A total of 30 Albino Mice with an approximate age of 60 days were purchased from M/s. SreeVenkateshwaraEnterprises Pvt. Ltd, Bangalore. On their arrival a sample of animals was chosen at random and weighed to ensure compliance with the age requested. The mean weights of mice were25 - 30 g respectively. The animals were housed in metabolic cages (55 x 32.7 x 19 cm), with sawdust litter, in such a way that each cage contained a maximum of 5 animals of the same sex.All animals underwent a period of 7 days of observation and acclimatization between the date of arrival and the start of treatment. During the course of this period, the animals were inspected by a veterinary surgeon to ensure that they fulfilled the health requirements necessary for initiation of the Study.They were distributed among the experimental groups using a random distribution method. This procedure allows approximate equalization of initial bodyweights whilst allowing random allocation to experimental groups.

ACUTE ORAL TOXICITY STUDY [12]

Healthy Wistar rats (180-220 g) of both sex were used in acute toxicity studies as per OECD guidelines-425. The animals were fasted overnight and divided into 3 groups with 5 rat in each group. Extracts were administered at dose of 100, 500 and 2000 mg/kg, p.o. body weight. The mice were observed continuously for behavioural and autonomic profiles for 2 hrs and for any signs of toxicity or mortality up to 48 hrs (OECD-425, 2001).

ADMINISTRATION ROUTE AND PROCEDURE

The test substance was administered orally. The mice belonging to the control group were treated with the vehicle (distilled water) at the same administration volume as the rest of the treatment groups. The administration volume for oral administration was 10 ml/kg. The quantity of test substance administered to each animal was calculated from its body weight on the day of the treatment.

INDUCTION OF HEPATOTOXICITY

Pretreatment Group

All pretreated groups of albino mice except control groups receives a daily dose of Paracetamol (3g/ Kg of body weight) , Silymarin (100mg/kg) and Ethanolic Extract of *Crocus sativus* leaves (EECS) (250 and 500mg/kg) for 14 day.

Experimental Design

Group 1: Receives (Distilled water) as control for 14 days.

Group 2: Receives a daily dose of Paracetamol (3g/ Kg of body weight, p.o) for 14 days (p.o)

Group 3: Receives a daily dose of Paracetamol (3g/ Kg of body weight) and after one hour a daily dosage of Standard Silymarin (100mg/kg) of body wieght for 14 days (p.o)

Group4: Receives a daily dose of Paracetamol (3g/ Kg of body weight) and one hour a daily dosage of EECS 250mg / Kg of body weight for 14 days (p.o)

Group 5: Receives a daily dose of Paracetamol (3g/ Kg of body weight) and one hour a daily dosage of EECS 500mg / Kg of body weight for 14 days (p.o)

Sample collection

At the end of the 14th day treatment, samples of blood were withdrawn from the orbital sinus of mice from each group, under light ether anesthesia after fasting for 16 hours. The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP and BILIRUBIN. After separation of serum for biochemical estimation, the mice were sacrificed, liver of mice were isolated and washed with normal saline and stored for 12 h for in vivo antioxidant studies [13].

Measurement of Body weight

The body weight of the animals was monitored daily by weighing on an electrical balance with accuracy to ± 0.1 g. All measurements were made every day between 8.30 and 9.15 h, immediately before administration of the distilled water in the case of control and the drugs paracetamol.

STATISTICAL ANALYSIS

Statistical analysis was performed using GraphPad Prism version 7 for Windows (GraphPad Software, San Diego, CA, USA) and Microsoft Excel 2013. Raw data obtained from different wound models are expressed as mean ± SEM. Values less than 0.03 were considered to be statistically significant. The data were analyzed using GraphPad Prism version 7 for Windows and differences among groups were compared by one-way ANOVA followed by Dunnett's test.

RESULTS AND DISCUSSION

Physicochemical properties

Extractive Values and percentage yield of Crude Drugs

Solvent Extractive Values of Crude Drugs

Sl. No.	Name of the drug	Water soluble extractive value (% W/W)		Alcohol soluble extractive value (% W/W)	
		Theoretical	Obtained	Theoretical	Obtained
1	Crocus sativus	>11	22.9±0.53	>03	14.86±0.12

The water soluble extractive value indicated the presence of sugar, acids and inorganic compounds; the water soluble extractive value found to be 22.90±0.53 (Table 5.1). The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids. The alcohol soluble extractive value was found to be 14.86±0.12 which signify the nature of the phyto-constituents present in plant.

Percentage yield of Crude Drugs

Extract	**Nature**	**Percentage of yield**
EESC	Dark green	8.34

Preliminary phytochemical screening

Preliminary Phytochemical screening was performed for extracts of Crocus sativus leaves. It was noted that extracts of Crocus sativus leaves contains xanthones, flavonoids, coumarins, hydroxycinnamic acids, tannins, saponins, amino acids, proteins, triterpenoids and polysaccharides. (Table 5.3).

Qualitative analysis of ethanolic extract of Crocus sativus leaves

Sl. No.	**Groups of bioactive compounds**	**Result**
1	Alkaloids	-ve
2	Xanthones	+ve
3	Flavonoids	+ve
4	Coumarins	-ve
5	Hydroxycinnamic acids	+ve
6	Tannins	+ve
7	Saponins	+ve
8	Amino acids	+ve
9	Proteins	+ve
10	Triterpenoids	+ve
11	Polysaccharides	+ve

+ve: present; -ve: absent

Pharmacological assessment

Acute toxicity test

Administration of 2000 mg/kg, p.o. of the extracts did not produce any behavioral abnormalities and mortality. So the dose selected for further study was 100, 200 and 400 mg/kg, p.o. of the extracts.

Hepatoprotective activity

Hepatotoxicity is a common side effect of various drugs and xenobiotics. Paracetamol is a NSAID which is harmless in normal therapeutic doses and causes liver toxicity in high doses in humans.

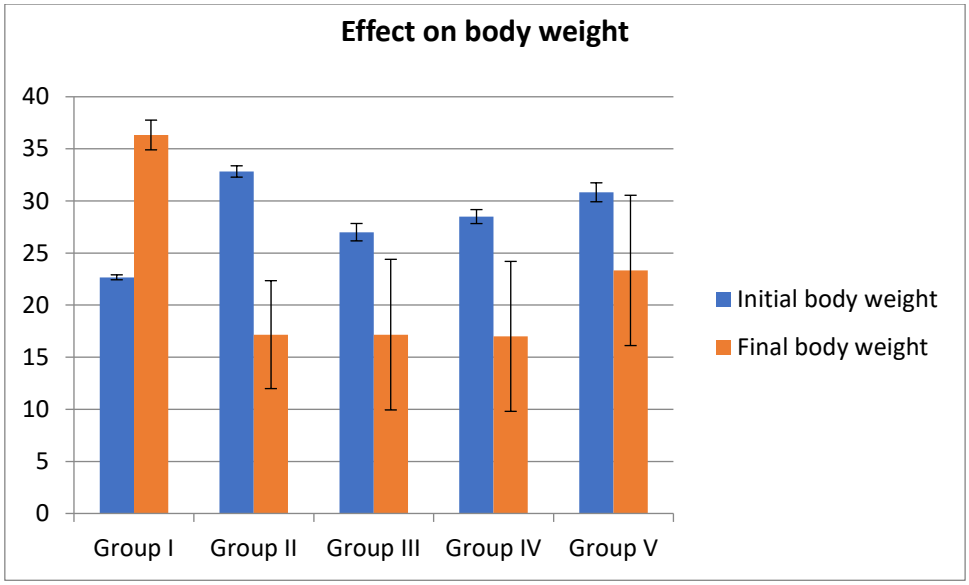
Effect on body weight

Effect of ethanolic extract of Crocus sativuson Body Weight

Group	**Initial body weight**	**Final body weight**
Group I	22.67±0.236	36.333±1.429
Group II	32.83±0.543	17.166±5.179*
Group III	27.00±0.837	17.166±7.231
Group IV	28.50±0.671	17.000±7.197
Group V	30.83±0.910	23.333±7.214

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way

ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01,***P < 0.05 calculate by comparing treated group with control group.



Liver damage is caused when paracetamol is administered to albino mice. This is manifested in a lower body weight. The body weight of normal mice showed significantly increased in mice following paracetamol treatment (17.166±5.179). In EECS treated mice at the doses of 250 and 500mg/kg treated mice, the final body weights became (17.000±7.197) and (23.333±7.214) respectively. However, administration of paracetamol with standard silymarin and the low dose and high dose EESC significantly reduced the relative body weight.

Effect on liver weight

Effect of ethanolic extract of Crocus sativus on Liver weight in paracetamol induced hepatotoxicity

GROUP	LIVER WEIGHT
Group I	0.720±0.236
Group II	1.520±0.524
Group III	1.032±0.463
Group IV	0.856±0.385**
Group V	1.187±0.398*

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnet's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with CONTROL group

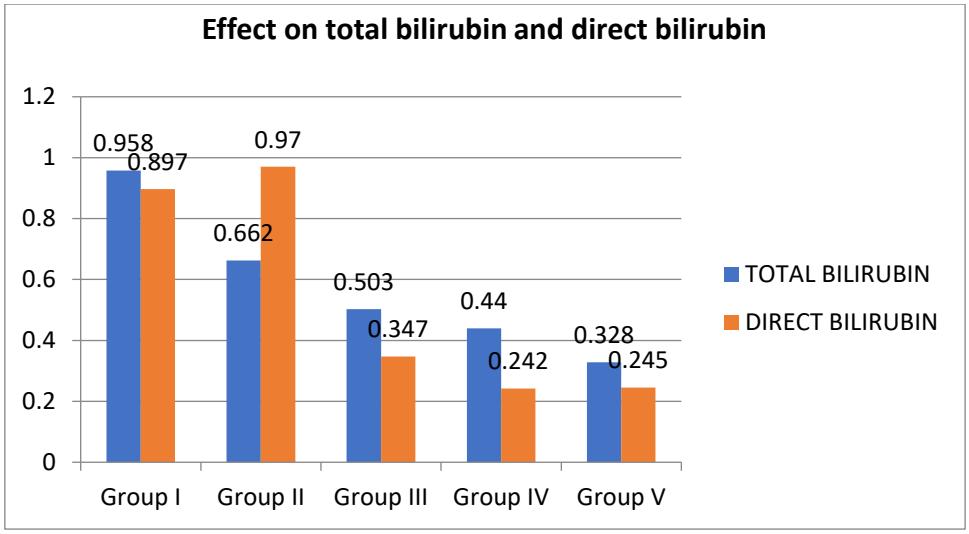
The relative liver weight of the Acetaminophen treated groups shows an increase in relative liver weight when compared to the control group. A further significant increase in relative liver weight is shown in the Silymarin + Acetaminophen treated group (1.032±0.463) when compared to the control group. The EECS treated groups clearly shows a decrease in weight for low dose 250mg/kg (0.856±0.385) and a medium increase in relative liver weight at 500mg/kg (1.187±0.398) which is the higher dose (HD) administered.

Effect on total bilirubin and direct bilirubin

Effect of ethanolic extract of *Crocus sativus* on total bilirubin, direct bilirubin

GROUP	TOTAL BILIRUBIN	DIRECT BILIRUBIN
Group I	0.958±0.456	0.897±0.237
Group II	0.662±0.299	0.970±0.012^{ns}
Group III	0.503±0.232	0.347±0.064^{ns}
Group IV	0.440±0.207	0.242±0.021*
Group V	0.328±0.206	0.245±0.086*

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with control group.



Bilirubin is an orange-yellow pigment, a waste product primarily produced by the normal breakdown of heme. Heme is a component of hemoglobin, which is found in red blood cells (RBCs). Bilirubin is ultimately processed by the liver to allow its elimination from the body. Any condition that accelerates the breakdown of RBCs or affects the processing and elimination of bilirubin may cause an elevated blood level.

The levels of bilirubin is decreased in the group 2 (only paracetamol) (0.662±0.299) and shows gradual decrease on treatment with standard drug silimarin, low dose EECS and high dose EECS. This test shows that there is reduced breakdown of total bilirubin pointing to hepato protective activity.

Bilirubin that is bound to a certain protein is called unconjugated, or indirect, bilirubin. Conjugated, or direct, bilirubin travels freely through the bloodstream to the liver. Most of this bilirubin passes into the small intestine. The levels of direct bilirubin show significant reduction on treatment with standard drug silymarin and low dose EECS (0.242±0.021) and high dose EECS (0.245±0.086).

Effect on serum protein

Effect of ethanolic extract of *Crocus sativus*. On serum protein in paracetamol induced hepatotoxicity

GROUP	**TOTAL PROTEIN**
Group I	8.90±0.153
Group II	8.60±0.404
Group III	8.22±1.02
Group IV	9.89±1.81
Group V	9.03±0.463

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with CONTROL group.

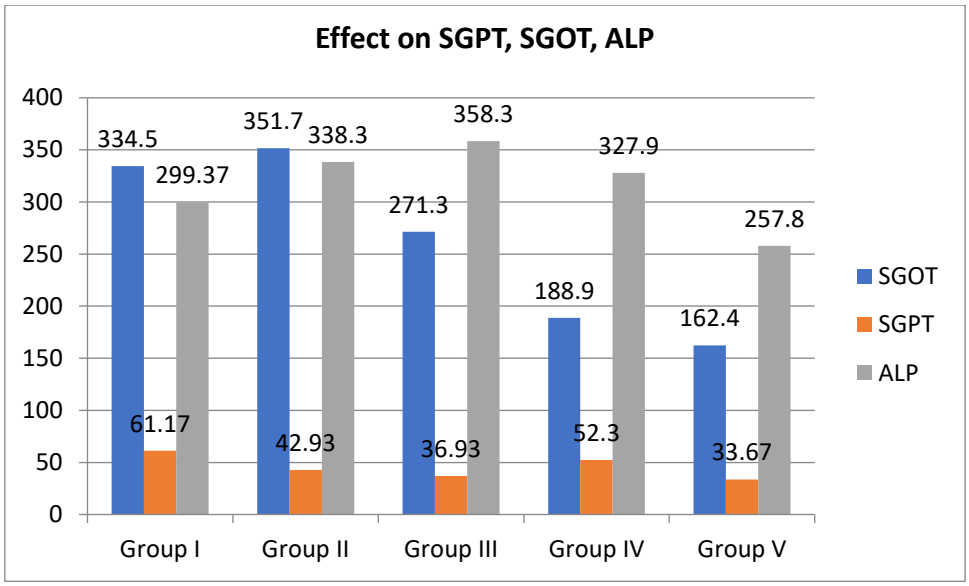
The level of serum protein which includes albumin and globulin are largely unchanged after treatment with paracetamol. The group 3 which received paracetamol and standard drug silymarin showed a slight decrease (8.22±1.02**)** and the group 4 (paracetamol + low dose) shows a slight elevation in the level of total proteins (9.89±1.81).

EFFECT ON SGOT, SGPT, ALP

Effect of ethanolic extract of *Crocus sativus*on SGOT, SGPT, ALP

GROUP	SGOT	SGPT	ALP
Group I	334.5±45.87	61.17±19.92	299.37±54.57
Group II	351.7±89.96	42.93±19.61	338.3±27.89
Group III	271.3±44.92*	36.93±4.313**	358.3±35.41
Group IV	188.9±60.03**	52.30±22.27	327.9±29.27*
Group V	162.4±3.467**	33.67±2.00**	257.8±79.96**

Values are expressed as the mean ± S.D; Statistical significance (p) calculatedby one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P <0.01, ***P < 0.05 calculate by comparing treated group with CONTROL group.



The level of AST in the paracetamol only group on AST/SGOT shows anincrease pointing to possible hepatotoxicity (351.7±89.96). The AST levels arelowered than the control in the groups 3(paracetamol with std) with an even moresignificant decrease in 250mg/kg EECS (188.9±60.03) and 500mg/kg EECSgroups (162.4±3.467). This graph shows significant hepatoprotective activity ofethanolic extract of *Crocus sativus*.

The level of serum ALT is lower than the control groups of mice in thestudy. Paracetamol with silymarinstandard (36.93±4.313) and Paracetamol withHigh dose EECS (33.67±2.00) show comparable levels of hepatoprotectivity.In severe tissue damage ALT activity is higher than AST and the ALT:AST ratio becomes ≥1(normally <1). Some increase in the activities of ALT and AST areseen in extrahepatic cholestasis. In both cirrhosis and carcinoma activity of AST isfound to be higher than the ALT. ALT is a more liver specific enzyme as increasedALT activity in serum is hardly seen in tissues other than liver cell damage.The mice that were administered only paracetamol showed a higher value of alkaline phosphatase than the control (338.3±27.89). The paracetamol and standard drug silymarin group showed an even higher increase in ALP (358.3±35.41)

This shows liver damage in these groups. Damaged liver cells release increased amounts of ALP into the blood. ALP is especially high in the edges of cells that join to form bile ducts. If one or more of them are obstructed, for example by a tumor, then blood levels of ALP will often be high. The paracetamol and Low Dose EECS (327.9±29.27) group showed a slightincrease in ALP compared to groups 2 and 3. However, paracetamol with High doseEECS showed significant protection from liver damage by registering a lower levelof ALP than the control groups itself (257.8±79.96). This is a significant find in the evaluation of hepatoprotective activity of *Crocus sativus*.

Effect on activity of LDH

Effect of ethanolic extract of *Crocus sativus*on LDH activity

GROUP	**LDH**
Group I	2426±325.6
Group II	1808±325.6
Group III	3966±682.9*
Group IV	1638±65.86**
Group V	4159±698.5*

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with CONTROL group.

LDH is an enzyme found in all living tissue. Because it is released during tissue damage, it is a marker of common injuries and disease.

In our study, we see group 2 mice having a low level of LDH showing the pharmacological effect of paracetamol. Group 3 shows that paracetamol and silymarin combination causes marked elevation of LDH. Paracetamol and HD of EECS (4159±698.5) show marked elevation as well. Paracetamol and low dose EECS lowers Lactate dehydrogenase levels showing significant hepatoprotective property. (1638±65.86)

Effect on Homogenised Liver Tissue

Effect of ethanolic extract of *Crocus sativus*On homogenised liver tissue in paracetamol induced hepatotoxicity

GROUP	TOTAL PROTEIN
Group I	0.368±0.164
Group II	0.504±0.225
Group III	0.248±0.111**
Group IV	0.306±0.136*
Group V	0.293±0.131*

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with CONTROL group.

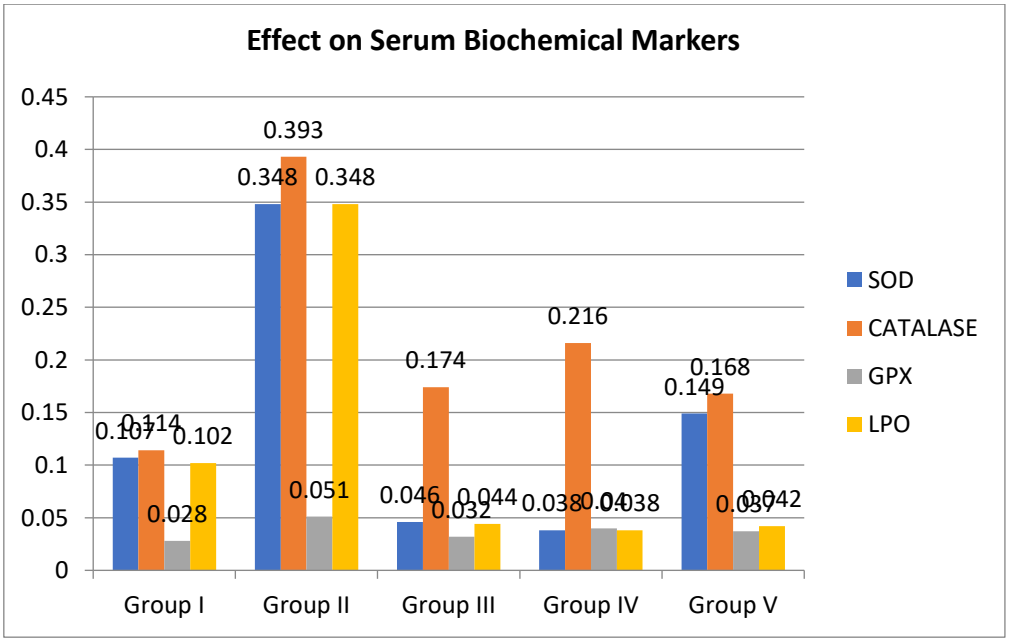
Paracetamol and silymarin group and both dosages of EECS (0.306±0.136), (0.293±0.131) lower the protein levels in homogenized liver of albino mice. The determination of cellular protein content is very advantageous over other markers due to early formation, greater stability and reliability and also their longer life-span.

Effect on Serum Biochemical Markers

Effect of ethanolic extract of *Crocus sativus*on serum biochemical markers in paracetamol induced hepatotoxicity

GROUP	SOD	CATALASE	GPX	LPO
Group I	0.107±0.047	0.114±0.053	0.028±0.013	0.102±0.047
Group II	0.348±0.155	0.393±0.160	0.051±0.020	0.348±0.155
Group III	0.046±0.019**	0.174±0.071*	0.032±0.015	0.044±0.019
Group IV	0.038±0.017**	0.216±0.088**	0.040±0.017	0.038±0.017**
Group V	0.149±0.068*	0.168±0.068**	0.037±0.016*	0.042±0.019**

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with CONTROL group.



The study shows that silymarin (0.046±0.019), 250mg/kg(0.038±0.017) and 500 mg/kg (0.149±0.068) extract lower the levels of Superoxide dismutase enzymes.SOD, a key enzyme in free radical protection, increases significantly in the livertissue of group 2 that received only paracetamol (0.348±0.155) suggesting thatproducts of free radical reactions are involved in pathogenesis. A significant decrease offered by the both doses of EECS shows that the hepatoprotective activityof *Crocus sativus* is comparable to standard drug Silymarin.

Catalase is a common enzyme found in nearly all living organisms exposedto oxygen. It catalyzes the decomposition of hydrogen peroxide to water andoxygen. It is a very important enzyme in protecting the cell from oxidative damageby reactive oxygen species (ROS). Both standard and test doses shows a decline in catalase levels (0.216±0.088, 0.168±0.068).

The main biological role of GPx is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water.The mice given paracetamol show marked elevation of GPx levels (0.051±0.020). The standard drug silymarin shows a marked reduction of GPx activity. The Low dose EECS (0.040±0.017) and high dose EECS reduce the level of glutathione peroxidase but not as well as the standard drug(0.037±0.016).

Lipid peroxidation is the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage (0.348±0.155). Paracetamol causes marked elevation of Lipid peroxidase in albino mice. The control and the test groups drastically reduce the LPO levels showing that the EECS in both low and high doses (250mg/kg and 500mg/kg) 0.038±0.017, 0.042±0.019 protect the liver as well as Silymarin in paracetamol induced hepatotoxicity.

SUMMARY AND CONCLUSION

The present study was designed to evaluate the possible protective effect of ethanolic extract of *Bauhinia tomentosa* Linn (EECS) against paracetamol induced hepatoxicity in animals.A literature survey revealed that more studies were needed for this plant to ascertain the hepatoprotective potential.The detailed preliminary phytochemical investigations rationalized its use as a drug of therapeutic importance. Theethanolic extract of the planthas phytoconstituents like flavonoids, terpenoids, sterods, alkaloids, saponins and tannins.The hepatoprotective effect was assessed using a battery of biochemical and histopathological tests. SGOT, SGPT, ALP, LDH, ACP were some of the biochemical tests done. In vivo tests for antioxidants (SOD, CAT, GSH, and LPO) were conducted on albino mice and wistar rats.In paracetamol and induced hepatotoxicity, a lower dose and a high dose of extract were used and compared with the hepatoprotective activity of standard drug silymarin. Control group and an only drug group were also used.The EECS showed marked hepatoprotective activity in lowered levels of body weight, positive effect on total bilirubin, total protein and on liver enzymes.

Histological sections of liver showed that centrilobular necrosis, the pathognomonic feature of hepatotoxicity, which appeared in paracetamol-intoxicated mice, was strikingly reduced in EECS treated mice. Furthermore, the congestion and inflammatory cell infiltration evoked by paracetamol was considerably decreased by EECS indicating its possible antihepatotoxic action. EECShas hepatoprotectiveeffects against liver toxicity induced by

The effects of EECS are comparable to that of Silymarin, the Standard hepatoprotective drug. Accordingly, EECS could beused as an effective herbal product for the prevention of chemical-induced hepatic damage.

In conclusion, we can say that *Crocus sativus* has the ability to protect the liver from the damaging effects of paracetamol and thioacetamidein toxic doses and stimulation of endogenous anti-oxidant defense system.

In the near future, a further study is warranted to isolate, characterize and screen the active components of *Crocus sativus* that have the hepatoprotective activity.

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