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Research Article

Thioacetamide Induced in Albino Wistar Rats: Importance of *Coleus aromaticus* Aqueous Leaf Extract for Hepatoprotective Activity

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Abstract

This study was conducted to hepatoprotective effects of *Coleus aromaticus* aqueous leaf extract (*C. aromaticus*-ALE) of on Thioacetamide and Silymarin induced liver poisonousness in wistar rats. Phytochemical screenings of *C. aromaticus*-ALE were used to find out the preliminary constituents. Adult male wistar rats (weight range: 200-220g) were divided into 6 groups (n= 6). Thioacetamide and Silymarin stayed managed intraperitoneally arranged the 5th day to rats in all groups but the normal control. Phytochemical screen of *C. aromaticus*-ALE contains flavonoids, saponins, tannins, phenol, tri-terpenoids, carbohydrate, anthraquinones, and phytosteroids. The biochemical markers of hepatic damage like AST, ALT, APT, Bilirubin and cholesterol. Furthermore, a significant hepatoprotective of the aqueous extract leaf and oral dose of Thioacetamide and Silymarin. *C. aromaticus*-ALE did not mortality or significant changes in the body weight. Process of hepatotoxicity induced by Thioacetamide in rats was interceded by control and these effects were comparable to those administered with Silymarin. This is the first report on hepatoprotective effect of *C. aromaticus*-ALE against Thioacetamide-induced hepatotoxicity.

Keywords: Thioacetamide, Silymarin, *Coleus aromaticus*, biochemical-hematological parameters, aqueous leaf extract.

INTRODUCTION

Liver disease strong position as one of the chief health troubles in the global, with it cirrhosis was drug stimulated liver injury according 9thsupremacy cause of death in modern and developing countries.[1] However, it caused by infectious agents or ingestion of toxic foods, chemical, overdose of drugs, and chemicals that cause liver damage are called hepato toxins.[2] It may be possible side effect of chronic medications or can be caused by chemicals, such as microcystins, as well as artificial chemicals, like contain antibiotics, tetrachloride, chemotherapeutic agents, dimethylnitrosamine, aflatoxin, carbon tetrachloride, pyrrolizidine alkaloids, allyl alcohol, thioacetamide, biomobenzene.[3] Susceptibility of the liver to chemical attacks, which comes in close contact with many harmful substances, environmental pollutants, xenobiotics, and chemotherapeutic agents could repress. However, maintaining a healthy liver is a challenge for overall health and well human being, and the treatment of such diseases is taken away standard using artificial pharmaceuticals, or secondary, by using separated main compounds or importance parts of indigenous medicinal plants utilized in popular medicine.[4] In spite of, there are nevertheless few drugs utilized to treat liver diseases, with possible effects on human.[5] Thus, important medicinal plants with hapatoprotective or curative process utilized for the therapy of hepatic disorders become important mostly important subjects of studies to explain their mechanism of action and characterize compounds that can be utilized for the increased of new hepatoprotective drugs.[6] Some experimental models are utilized to show the hepatoprotective action of certain medicinal plants, especially against thioacetamide stimulated liver damage.[7]

Thioacetamide (TAA) was particularly utilized as a fungicide to maintains agricultural citrus materials after later it was denied that it was a strong potent hepatotoxin and carcinogen due to organo-sulfur-containing compound enriched with liver damaging and carcinogenic activities.[8] Currently, is focused as a carcinogen, and very speedily metabolized into free bieradical derivatives such as TAA sulfoxide, TAA-S-S-dioxide, even though it leads to lipid peroxidation, thus eventually culminates in centrilobular damages and liver injuries.[7] Earlier

studies have also demonstrated that, rodents intoxicated with TAA was caused such as fibrosis, liver injury, cirrhosis, and steatosis in test animals of this disease with etiology, and pathology comparable equal to the one seen in humans.[9,10] However, TAA was recognized as an exemplary of liver fibrosis in rats. Though currently, the broadly utilized treatment of liver fibrosis and cirrhosis is inadequate; thus there is no effectively broadly utilized therapy that can prevent the improvement of hepatic diseases. Despite, newly improved drugs have been utilized to heal liver diseases; presently these drugs have abundant side effects. There is an urgent need for alternative deputing remedies or drugs, to the treatment of chronic liver disorders to change currently drugs of uncertain safety and non-effectiveness.[11] The liver markers are found of AST, Transaminases, APT, γ-glutamyl transferase, ALT, lipids, Bilirubin, cholesterol, and proteins are discharged in the blood. As a result of cell leakage, and the measurement of the particularly serum markers of the liver could be utilized for diagnosis of injuries.[12] Many products available commercially are from herbal origin, and herbal elements and dietary supplements have power as possible choice medicines for the therapy of chronic liver diseases and associated metabolic derailments.[13] Silymarin is an important component of *Silybum marianum*. Thus, it has been evidenced to be mostly hepatoprotective, and has been utilized for the therapy of abundant liver disorders such as cirrhosis, fatty acid infiltration due to alcohol and toxic chemicals, and hepatitis, it's specifically characterized by functional impairment or deterioration of necrosis.[14] However, it's mechanisms of the process is not entirely understood, it appears that it acts in various ways, including anti-inflammatory activities and antioxidant, membrane stabilizer, cell permeability regulator, inhibiting the deposition of collagen fibers and stimulating liver regeneration, which may be lead to cirrhosis.[15] The medicinal herb, *Coleus aromaticus* is a large succulent aromatic perennial herb with approximately 30-90 cm in highest and branched, fleshy highly aromatic pubescent herb with distinctive smelling leaves. It is folkloric medicinal plant used to treat hepatopathy, malarial fever, vesical calculi, convulsions, renal, cough, helminthiasis, hiccough, epilepsy, and bronchitis.[16] Therefore, in the current investigation, we make effort perhaps for the first time, to this work out the possible toxicology activity of *C. aromaticus*-ALE for hepatoprotective effect against TAA-induced hepatotoxicity.

MATERIALS AND METHODS

Chemicals

Aqueous, distilled water, 10% formalin, silymarin, thioacetamide, hematoxylin, eosin, acetaminophen were supplied by Merk (Germany).

Plant material and extract preparation

Fully developed leaves of *C. aromaticus* were collected from Kollihills (11°24'N to 11°13'N latitude, and 78°33'E to 78°09'E longitude), Namakkal District, Tamil Nadu, India, and washed methodically, blotted and shade dried. It was authenticated by plant taxonomist from the Department of Botany, Annamalai University. Coarsely powdered dried leaves, 500 g each of *C. aromaticus* are separately extracted to collapse with aqueous using a Soxhlet apparatus successively. The ALE thus gathered was drained beneath reduced pressure yielding 20.4 g of extract.

Phytochemical screening

We showed the bioactive chemical components detecting the occurs of secondary metabolites such as protein, flavonoids, alkaloids, steroids, terpenoids, saponins, anthraquinines, tannins, tri-terpenoids, phenol, carbohydrate, phytosteroids, amino acid and glycosides in the dissimilar extract fractions of *C. aromaticus*-ALE.[17]

Animals

The study was initiated after getting approval from the Institutional Animal Ethic Committee, Approval letter Reg No: 160/1999/CPCSEA. Proposal No:1096/Dt.9/10/14. Animal were handled according to the CPCSEA guidelines, India. Wistar albino rats (200-220g) of either sex bred (n=6 animals/group) in animal house, Annamalai University, Chidambaram, Tamil Nadu, were used. They were stored under still environmental states of temperature, humidity 12:12 light/dark. We gave a standard pellet diet and supplied it with water to animals.

Preparation of Silymarin

SY with 80% the state of being pure, (Pondicherry Scientific Chemicals, Pondicherry, India) as a standard drug, and dissolved in Tween 20 (10% w/v) and orally manage an organization or effort to rats at a dose of 50 mg/kg body weight (Fig. 1).[18]

Acute toxicity assay

The experiment followed the guideline of OECD 425 for the testing of chemicals (OECD, 2022). All animals fasted overnight before treatment. The rats were weighted and given the doses according to their body weight. Twenty five male rats were randomly divided into 6 groups (n=6/group). Group 1 was provided as the control, and TAA was treated in the rats (Group 2), TAA+ALE of *C. aromaticus* was treated at 250 and 500 mg/kg doses body weight (Group 3 and Group 4), TAA+SY was treated at 25 mg/kg doses body weight (Group 5) and *C. aromaticus* alone was treated at 500 mg/kg doses body weight (Group 6). The observation of animals was done every 30 min, 4h after treatment, and every 24h up to 14 days.

Those observations evaluate the mortality case, clinical toxicities signs, abnormalities behavior and body weight. During this study period, clinical observations were made for mortality, behavior and any other abnormalities, and their weight was measured every 3 days. In the days of the 15th, the animal was anesthetized with 50 mg/kg body weight of ketamine. The sacrificed organs (Liver, lungs, heart, gut, kidney left, right and ovaries) were collected and the weight was measured.

Thioacetamide induced hepatotoxicity

TAA and all other chemicals utilized were of analytical evaluated grade and obtained almost from Pondicherry Scientific Chemicals. TAA stock solution was arranged by dissolving 100 mg pure TAA (Fig. 2) which is in crystal form in 100 ml distilled water (10.0 % w/v) until all the crystals were dissolved. It was mixed by drinking water and given daily to rats. Perpetual exposure in rat to the amount of TAA induces changes in its liver diseases for both biochemical and morphological aspects comparison to that of human liver cirrhosis.[19]

Animals of all the groups were killed by light ether anesthesia on 6th day. The blood model of each one animal was gathered individually by carotid artery into completely clean and moistureless centrifuge tubes and allowed to coagulate for 30 mm. Serum transaminase viz., ALT, AST and AP were approximately evaluated by the standard automatically techniques according to the procedures prescribed by the manufacturers.

Histopathological studies

The rats were sacrificed, and the organ (Liver) of all groups was separated for histopathological studies. A complete

necropsy implemented. After, a comprehensive gross observation was implemented on the internal organs namely liver. They were shown for any signs of lesions. Further, the organs were more carefully take apart out, very cleanse of all fats and was weighed. Despite liver is organs of metabolism and waste materials excretion, possibly toxic products are likely to be affected them, so parts of these organs were very carefully fixed in buffered 10% formalin and 5 µm thick paraffin sections were made and stained with haemotoxylin and eosin for Histopathological study.[20]

Statistical analysis

The results of all parameters tested were evaluated by statistical analysis using the GraphPad Prism 9.0 software (GraphPad Co. Ltd., San Diego, CA, USA). Groups were examined Durnett-comparison utilized to search the data into the one-way ANOVA test, with $p< 0.05$ being considered as the limit of significance.

RESULTS

Preliminary screening of C. aromaticus-ALE

Phytochemical screening of *C. aromaticus*-ALE tested showed that 14 phytochemical screening and the leaves were strongly positive group in alkaloids, flavonoids, saponins, tri-terpenoids, anthraquinones, phenol, carbohydrate and phytosteroids (Table 1). *C. aromaticus*-ALE was analyzed by using CC with different solvent system. Benzene: ethyl acetate (8:2) gave 5 fractions; two fractions have had available in benzene: ethyl acetate (1.5:8.5). Three fractions have available in benzene: ethyl acetate (1:10).

Acute toxicity assay

The oral administration of *C. aromaticus*-ALE had not recorded the toxic sign of behavioral responses up to 14 days of observation (Table 2). There were no changes in the eye color, sedation, skin color, fur and skin, convulsion, diarrhea, eraction of fur, urination, saliva, breathing, drownise and behavior patterns. There was no observed lethality case over the 14 days of observation. The statistical evaluation of relative organ weights showed no significant differences among groups (Table 3).

Thioacetamide induced hepatotoxicity

In rats, effect of *C. aromaticus*-ALE on SY and TAA induced liver damage, with reference to biochemical changes in serum are noticed in Table 4. At the end of the 5th day test, blood sample of SY and TAA treated control animals seen important improvements in the level of AST, ALT, ALP, Bilirubin and cholesterol compare to control. Pretreatment with *C. aromaticus*-ALE at 250 and 500 mg/kg noticed marked decline of ALT, ALP, AST, Bilirubin, and cholesterol as compared to the SY and TAA treated group. The highest protection was shown by *C. aromaticus*-ALE at the dose of 500 mg/kg body weight and SY and TAA induced hepatic damaged rats caused a marked reduction in the activities of these enzymes. Those indicated that the LD_{50} was estimated at more than 500 mg/kg. The body weight increase of the rat throughout the experiment was within the normal range. The average weight among the groups had no significant difference on all days (Fig. 3a,b).

Histopathological changes in liver

The control rat's liver sees that the hepatic cells are radically placed and each cell has a broad spherical nucleus, granular cytoplasm, and not any injury (G1). TAA treated rats showed vacuolization, necrotic changes and deficit of cell over limit of liver damage were noticed (G2). Treatment of rats with *C. aromaticus*-ALE 250 mg/kg and 500 mg/kg to TAA treated rats focused markedly decreased the size of necrosis, fatty accumulation around the central vein and degree of deterioration (G3 and G4). In the mentioned group, i.e. TAA and SY, the liver construct was comparable to that focused on the control group (G5). *C. aromaticus*-ALE separately treated group rats had shown ordinary architecture of liver (G6) are showed in Fig. 4.

DISCUSSION

One of the main processes, liver is detoxification of xenobiotics. TAA is a power hepatoxic agent that is metabolized by cytochrome 450 enzyme existing in liver and is oxidative chains to toxic materials, which is called TAA-dioxide[21] and it is focused to stimulate centrilobular hepatic necrosis, liver cirrhosis, hepatocellular carcinoma and bile duct procreation.[22] Furthermore, TAA stimulated liver fibrosis and is provoked by free radical-mediated lipid peroxidation.[23] The higher production of reactive oxygen species (ROS) induces lipid peroxidation, that manages to a developed of malondialdehyde concentration in liver tissue.[24] In this study, as shown on Table 1, primary screening of CAALE tested showed that 14 phytochemicals and the leaves were strongly positive group in alkaloids, flavonoids, saponins, tri-terpenoids, anthraquinones, phenol, carbohydrate and phytosteroids. Present studies are comparable with earlier reports that the alkaline phosphatase is a transpeptidase that develops in bone andliver disorder. Most studies have focused that phenolic compounds existing in medicinal plants can avoid the toxic effects on the liver and result in decrease volume of glutamic pyruvic transaminase and alkaline phosphatase freed into blood[25] and *Eucalyptus camaladulensis* LCE shows the presence of glycoside, flavonoids and steroids.[26]

Acute toxicity study showed that *C. aromaticus*-ALE had not recorded the toxic sign of behavioral responses up to 14 days of observation. There were no changes in the eye color, sedation, skin color, fur and skin, convulsion, diarrhea, eraction of fur, urination, saliva, breathing, drownise and behavior patterns. There was no observed lethality case over the 14 days of observation. The statistical evaluation of relative organ weights showed no significant differences among groups. Continuously researched, the reductions in the body weights of rats during toxicity studies are possible due to the physiological adaptation responses, which may lead to low appetite and reduce the caloric intake by the animal.[27,28] Changes in the organ weight of the treatment group compared to the control give a preliminary sign for any damage or swelling of the organ caused by extract.[29]

Accordingly, the current test was undertaken to evaluation the effects of *C. aromaticus* in rat sample of chronic liver disease in order to a confirmation that this plant does truly have a deterrent advantage in liver disease. Results from Table 2 revealed that, animals treated with 500 mg/kg of *C. aromaticus*-ALE exposed a hepatoprotective effect comparison to treat with 50 mg/kg of SY in TAA stimulated liver injury. The rapied at 250 and 500 mg/kg body weight was rescued the biochemical parameters (AST, ALT, APT, bilirubin and cholesterol) towards ordinary. Similarly, management of many type of the extracts in compare with the usual control in histology of the animals focus glomerular expulsion and glomerular collapse with the currently results developed enlarge tubules, urinary space, vacuolations in some epithelial lining of very most of tubules in the medulla and inflammatory cellular penetration at some peritubulareareas.[30] Melatonin reduced the well development lipid peroxidation and transaminase evaluate in rats collected fluoxetine ($p<0.05$), and also focused the source of fluoxetine I inducing leucopenia, hypochromic, thrombocytopenia and macrocytic

anemia, which was blunted by melatonin. Both RBCs and platelets indexes were being corrected. Furthermore, rats accepted melatonin in join with fluoxetine have shown a decreased in the harshness severity of deterioration and inflammatory changes in different heart, tissues, brain, lungs, liver into the testes with kidneys as comparison to the fluoxetine group.[31]

However, in chronic TAA intoxication, it is substantial liver fibrosis and prominent regenerative nodule improvements had correlated, with portal hypertension and hyper dynamicis being circulation characteristics of liver cirrhosis.[32] Anticonvulsant effect of onopordia was blocked by applying a single dose of a non-nitro-L-arginine methyl ester hydrochloride and also a single dose of selective neuronal NOS (nNOS) inhibit 7-nitroindazole. Administration of ketamine as a N-Methyl-D-aspartic acid receptor antagonist with onopordia did not change the anticonvulsant effect of onopordia.[33] Plasma is proof the biochemical parameters (AST, ALT, APT, bilirubin and cholesterol) and histopathological analysis. However, the mechanism of oxidative pressure in TAA hepatotoxicity is still not clear. In presently, many studies have shown that various types of natural products have a wide range in biochemical, phyciological and pharmacological effects due to the properties of their constituents.[34,35] Treatment of *Schistosoma mansoni* infected mice with *Ozoroa pulcherrina*-MLE produced significant reduction of worm burden and ova count in the faces, liver and intestine.[36] Moreover, the induction of pre-diabetes outcome in development release of liver enzymes such as AST and ALT, is raised antioxidant enzymes. Despite, the administration of BA had raised sterol regulatory part binding protein (SREBP1c) and stress, and made better antioxidant enzymes such as SOD and GPx.[37]

Result comparable for TAA is a classic hepatotoxic reagent used for liver cirrhosis induction.[38,39] Further, the acute toxicity research were determined the LD_{50} values which helps in determining protect dose ranges at which the drug could be utilized such that there is not harmful side effect on the animal (Vishnu et al., 2015).[40] TAA is hepatotoxic and effects DNA, RNA, protein synthesis and glutathione content, which in turn, induces intra hepatic metabolic changes[41], and known hepatotoxin that induces liver nectosis by producing free radicals.[42] In a short period of time, TAA induces liver damages[43] and toxin is metabolized by detoxification enzymes of P450 cytochrome system.[18] Studies have demonstrated that, immune system will fix this condition by exaggerating the anti-inflammatory genes and natural antioxidant will decrease the existence of the free radical in the body and existences of intracellular ion homeostasis also are essential for the physiological function of cells.[44] Particularly, *Artemisia* plant extract is fully replete with cholinergic acid which has anti-free radical action that devastates oxygen free radicals,[45] with it is also prohibits hemeoxygenase; thus, it is decreases the production of free radicals[46] and quercetin inhibits lipid peroxidation.[47] Our findings presented on Fig. 2 revealed *C. aromaticus*-ALE did not mortality or significant changes in the body weight. The previous studies focused, *in vivo* that the antioxidant enzymes were reduced by a chronic ethanol administration[48,49] and antioxidant enzyme actions in the liver tissue, including those of catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) were significantly reduced by a chronic ethanol administration, whereas the hepatic lipid-peroxidase level was development.[50] The current investigation showed Fig. 3 and 4 that, process of hepatotoxicity induced liver and kidney by TAA in rats was interceded by *C. aromaticus*-ALE control and these effects were comparable to those administered with SY. Similarly, significant develops in chloride level in group five compared with ordinary control and though these changes were not statistically significant in almost all the groups administered the disparate extracts, but it is possibility that its physiological effects could be adverse. Bicarbonate, chloride, sodium and potassium stable in the blood are observed to be a best indicator of usefully functioning of the heart. Significant change in the concentration of these body electrolytes is particularly of lowest renal functions or renal impairment.[51]

CONCLUSION

The very strong preliminary evidence that *C. aromaticus*-ALE has hepatoprotective effective against liver toxicity stimulated by TAA as confirmed by macroscopical, microscopical and biochemical tests. Further, effect of *C. aromaticus*-ALE is comparison to that of SY, the very standard hepatoprotective drug. Accordingly, ALE could be utilized as a useful herbal product for the blockage of chemical-induced hepatic damage. In the near future, a more research is warranted to isolate and characterize the bioactive components of *C. aromaticus*-ALE that have the hepatoprotective.

Table 1: Phytochemical screening of *C. aromaticus* ALE

Chemical constituents	Methanol	Ethyl acetate	Acetone	Benzene
Alkaloids	+	+++	+	--
Flavonoids	+++	++	--	+
Saponins	++	--	--	+
Steroids	+	++	++	+
Tannins	++	+++	+	--
Terpenoids	--	+	--	+
Tri-terpenoids	++	+++	++	+
Anthraquinones	++	+	--	+
Amino acid	--	--	--	--
Phenol	++	+	++	--
Glycosides	--	--	--	--
Carbohydrate	+++	+	+	--
Protein	--	+	--	+
Phytosteroids	++	+++	+	+

"+++" Strongly positive phytochemical group, "++"Positive phytochemical group, "+"Trace phytochemical group, "-" Absence of phytochemical group

Table 2: The behavioural pattern of rats in all groups

Organs	Observation of control and treatment groups																							
	4 h						24 h						72 h						14 days					
	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6
Eye color	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Sedation	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Skin color	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Fur and skin	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Convulsion	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Diarrhea	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Erection of fur	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Mortality	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Urination	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Saliva	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Breathing	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Behavior	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Drownise	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr

G- group; N- normal; Nil- no mortality observed.

Table 3: Effect of *C. aromaticus* ALE on relative organ weight (g) of rats during oral toxicity study

Organs	G1	G2	G3	G4	G5	G6
Liver	4.264±0.013^{c}	4.458±0.014^{c}	4.42±0.02^{c}	4.356±0.016^{c}	4.332±0.016^{c}	4.274±0.015^{c}
Lungs	0.822±0.014^{b}	0.922±0.017^{ab}	0.854±0.015^{b}	0.822±0.014^{b}	0.804±0.015^{b}	0.834±0.018^{b}
Heart	0.362±0.016^{a}	0.482±0.023^{a}	0.378±0.019^{a}	0.452±0.014^{a}	0.44±0.018^{a}	0.36±0.018^{a}
Gut	12.99±0.424^{d}	12.04±0.637^{d}	11.99±0.494^{d}	13.01±0.219^{d}	12.81±0.259^{d}	12.30±0.269^{d}
Kidney left	0.406±0.015^{a}	0.52±0.015^{a}	0.41±0.014^{a}	0.432±0.008^{a}	0.414±0.018^{a}	0.372±0.014^{a}
Kidney right	0.396±0.008^{a}	0.502±0.008^{a}	0.422±0.010^{a}	0.43±0.014^{a}	0.402±0.008^{a}	0.342±0.010^{a}
Ovaries	0.65±0.014^{ab}	0.946±0.008^{b}	00.8±0.007^{a}	0.694±0.011^{ab}	0.652±0.016^{ab}	0.688±0.016^{ab}

Values were represented as mean ± SEM (n=6). The data were evaluated by ANOVA followed by Dunnett's multiple comparison test. G- group, respectively.

Table 4: Effect of TAA, SY and *C. aromaticus* ALE on biochemical parameters

Groups	AST (U/L)	ALT (U/L)	ALP(U/L)	Bilirubin (mg/dl)	Cholesterol (mg/dl)
G1	64.60±8.23[a]	40.68±3.24[a]	145.00±7.44[a]	0.80 ± 0.03[a]	82.67 ± 4.18[a]
G2	183.45±11.65[d]	135.23±4.46[d]	449.33±14.52[d]	3.42 ± 0.13[d]	148.00 ± 7.42[d]
G3	82.98±5.74[c]	59.83±2.92[c]	208.00±9.16[c]	1.38 ± 0.08[bc]	112.33 ± 9.28[c]
G4	74.25±6.12[ab]	47.22±4.12[b]	185.83±8.38[ab]	1.20 ± 0.04[ab]	92.00 ± 5.13[ab]
G5	77.53±8.04[b]	49.50±3.38[b]	198.66±4.64[b]	1.28 ± 0.08[b]	97.33 ± 4.95[b]
G6	64.35±7.95[a]	40.00±2.96[a]	142.50±6.98[a]	1.79 ± 0.06[c]	82.66 ± 5.36[a]

Data represent the mean ± SEM (n=6 in each group). The data were evaluated by ANOVA followed by Dunnett's multiple comparison test. Figures in parenthesis indicate percent reduction in individual biochemical parameters from their elevated value caused by the hepatotoxin. All values were significantly different p <0.05.

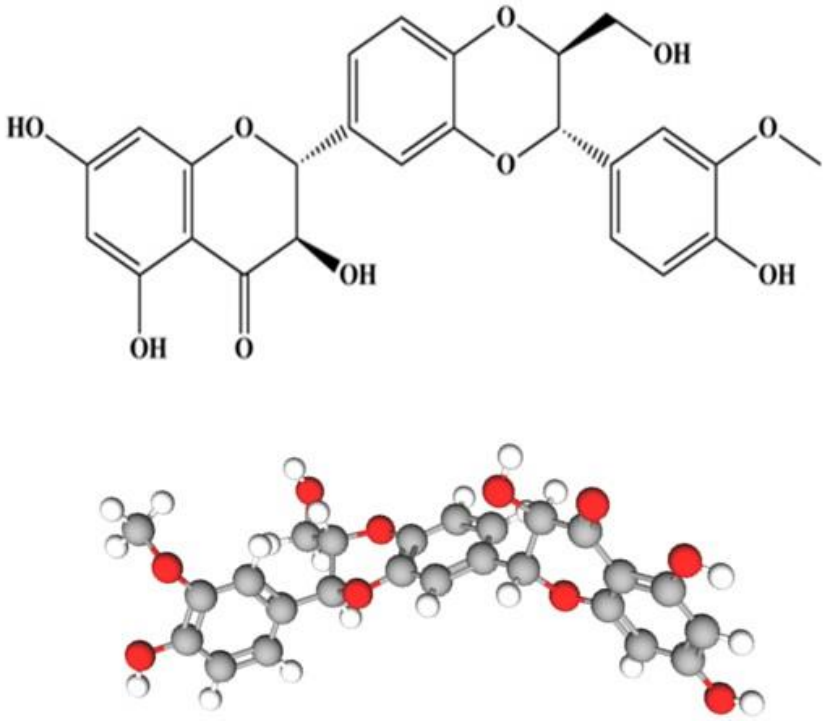

Figure 1: *2D* and *3D* structure of SY(Silymarin)

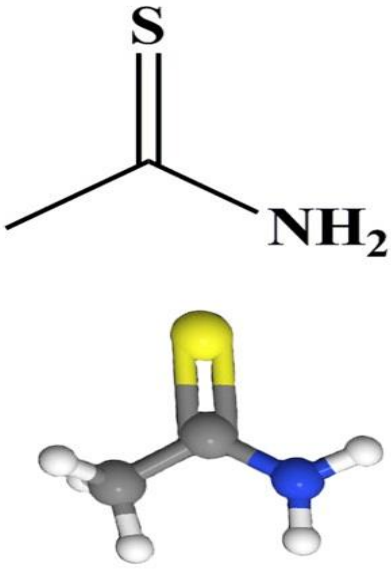

Figure 2: 2D and 3D structure of TAA (Thioacetamide)

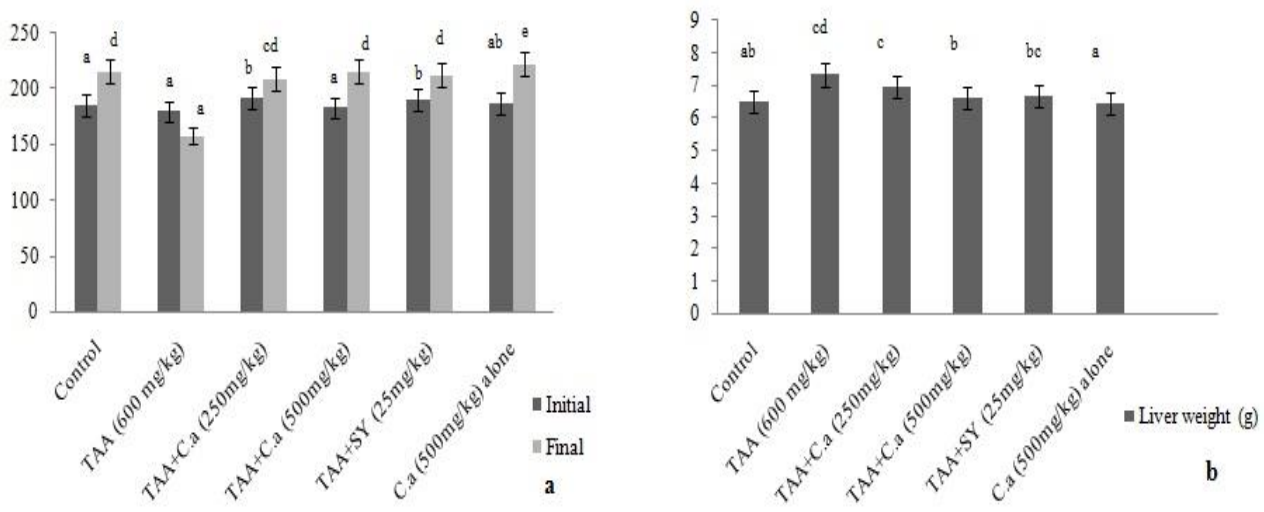

Figure 3: Weight changes in a) body weight; b) liver weight changes in control and experimental groups; C.a- *Coleus aromaticus*

Values are expressed as mean ± standard error, n= 6. Values were significantly different p <0.05. Within each row, different letters indicate significant differences (ANOVA, DMRT (Duncan's new multiple range method) test).

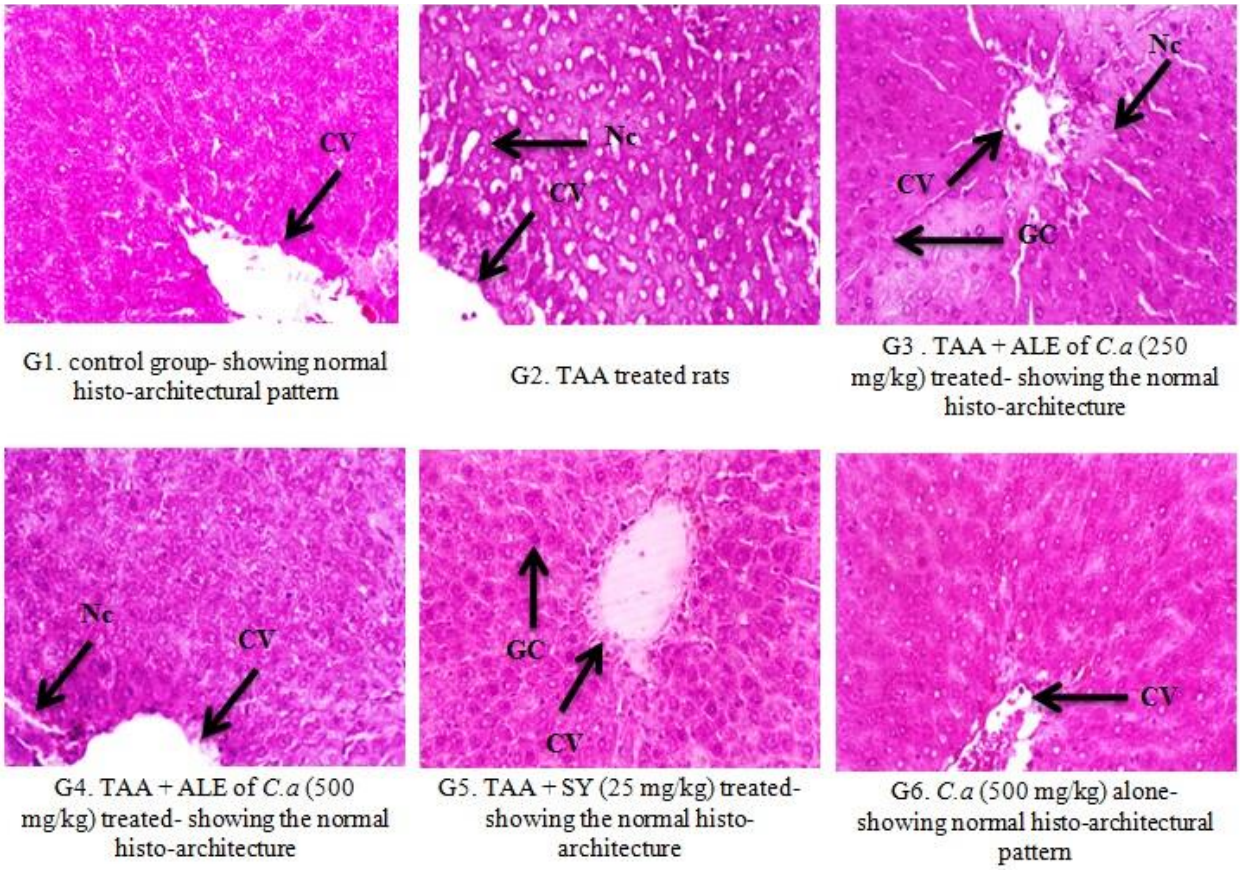

Figure 4. Histopathological images of liver from different experimental groups, CV- Central Vein; Nc- Necrosis; GC- Granular cytoplasm, C.a- *Coleus aromaticus.*

Conflict of Interest

The authors declare that there are no conflicts of interest.

REFERENCES

1. Al-Snafi AE, Thuwaini MM. Arabian medicinal plants with hepatoprotective activity. Res J Pharmaceut Biol Chem Scienc. 2018; 9:1469-1497. https://doi.org/10.33887/rjpbcs
2. Saibal Das S, Sanji B, Anand R, Somnath M. Evaluation of hepatoprotective activity of aqueous extracts of leaves of Basellaalba in albino rats. Nat Prod Res. 2014; 29: https://doi.org/10.1080/14786419.2014.971795
3. Domitrovic R, Potocnjak I. A comprehensive overview of hepatoprotective natural compounds: mechanism of action and clinical perspectives. Archiv Toxicol. 2015; 90: https://www.researchgate.net/publication/282044038

4. Dhiman Chawla D. Herbal Medicines for Liver Diseases Digestive. Digest Dis Sci. 2005; 50:1807. https://doi.org/10.1007/s10620-005-2942-9

5. Al-Attar AM, Al-Rethea HA. Chemoprotective effect of omega-3 fatty acids on thioacetamide-induced hepatic fibrosis in male rats. Saudi J Biol Sci. 2017; 24:956-965. doi: 10.1016/j.sjbs.2016.01.029

6. Ganesan K, Sukalingam K, Xu B. *Solanum trilobatum* L. ameliorate thioacetamide-induced oxidative stress and hepatic damage in albino rats. Antioxidant (Basel). 2017; 6:68. https://doi.org/10.3390/antiox6030068

7. Kumeshini Sukalingam, Kumar Ganesan, Baojun Xu. Protective effect of aqueous extract from the leaves of *Justicia tranquebariesis* against thioacetamide-induced oxidative stress and hepatic fibrosis in rats. Antioxidant (Basel). 2018; 7:1-10. doi: 10.3390/antiox7070078

8. Salunkhe AJ and Patil RN. Hepatoprotective effect of ethanolic extract of *Andrographis paniculata* against thioacetamide induced toxicity in the liver of albino rats. Res J Life Scie Bioinform Pharmaceut Chem Sci. 2018; 4:549-559. https://doi.org/10.26479/2018.0406.43

9. Kaur V, Kumar M, Kaur P, Kaur S, Singh AP, Kaur S. Hepatoprotective activity of *Butea monosperma* bark against thioacetamide-induced liver injury in rats. Biomed Pharmacother. 2017; 89:332-341. https://doi.org/10.1016/j.biopha.2017.01.165

10. Bashandy SA, Alaamer A, Moussa SA, Omara E. Role of zinc oxide nanoparticles in alleviating hepatic fibrosis and nephrotoxicity induced by thioacetamide in rats. Canadian J Physiol Pharmacol. 2017; 16:1-8. https://doi.org/10.1139/cjpp-2017-0247

11. Feng YM, Wang X, Wang L, Ma XW, Wu H, Bu HR, et al. Efficacy and safety of combination therapy of chemoembolization and radio frequency ablation with different time intervals for hepatocellular carcinoma patients. Surgic Oncol. 2017; 26:236-241. https://doi.org/10.1016/j.suronc.2017.04.006

12. Sunderman FW, Sunderman FW. Association of clinical scientists. Laboratory diagnosis of liver diseases. St Louis. 1968; 542.

13. Rehman J, Akhtar N, Asif HM, Sultana S, Ahmad M. Hepatoprotective evaluation of aqueous-ethanolic extract of *Capparis decidua* (Stems) in paracetamol-induced hepatotoxicity in experimental rabbits. Pakistan J Pharmaceut Sci. 2017; 30:507-511. https://pubmed.ncbi.nlm.nih.gov/28649077/

14. Ball KR, Kowdley KV. A review of *Silybum marianum* (milk thistle) as a treatment for alcoholic liver disease. J Clinc Gastroenterol. 2005; 39:520-528. DOI: 10.1097/01.mcg.0000165668.79530.a0

15. Jayaraj R, Deb U, Bhaskar AS, Prasad GB, Rao PV. Hepatoprotective efficacy of certain flavonoids against microcystin induced toxicity in mice. Environm Toxicol. 2007; 22:472-479. DOI: 10.1002/tox.20283

16. Dadasaheb DW and Prakash EP. *Coleus aromaticus*: a therapeutic herb with multiple potentials. J Food Sci Technol. 2016; 53:2895-2901.

17. Baranitharan M, Kandeel M, Shanmugavel G, Kaliyaperumal K, Subramanian K, Elumalai K et al. Fabrication of Silver Nanoparticles Using *Fimbristylis miliacea*: A Cheap and Effective Tool against Invasive Mosquito Vector, *Aedes albopictus*. J Nanomaterial. 2022; 9: https://doi.org/10.1155/2022/4083663

18. Alshawsh MA, Abdulla MA, Ismail S, Amin ZA. Hepatoprotective effects of *Orthosiphon stamineus* extract on thioacetamide-induced liver cirrhosis in rats. Evidence-Base Complemen Alternat Med. 2011; 103039: 6. DOI: 10.1155/2011/103039

19. Muller A, Machnik F, Zimmermann T, Schubert H. Thioacetamide-induced cirrhosis-like liver lesions in rats usefulness and reliability of this animal model. Journal of Experimental Pathology. 1988; 34:229-236. DOI: 10.1016/s0232-1513(88)80155-5

20. Yi-Chen, Wu MX, Jie-Liu, Ma XJ, Shi JL, Wang SN, et al. Acute and sub-acute oral toxicity studies of the aqueous extract from radix, radix with cortex and cortex of *Psammosilene tunicoides* in mice and rats. J Ethnopharmacol. 2018; 213:199–209. https://doi.org/10.1016/j.jep.2017.11.011

21. Kadir FA, Othman F, Abdulla MA, Hussan F, Hassandarvish P. Effect of *Tinospora crispa* on thioacetamide-induced liver cirrhosis in rats. Indian J Pharmacol. 2011; 43:64-68. doi: 10.4103/0253-7613.75673

22. Towseef H, Veerakumar D, Insha N, Anandhi N. Hepatoprotective activity of some medicinal plants: a review. Int Res J Pharm. 2019; 10:9-16. https://doi.org/10.7897/2230-8407.1005154

23. Bruck R, Aeed H, Shirin H, Matas Z, Zaidel L, Avni Y, et al. The hydroxyl radical scavengers dimethylsulfoxide and dimethylthiourea protectrats against thioacetamide-induced fulminant hepatic failure. J Hepatol. 1999; 31:27-38. DOI: 10.1016/s0168-8278(99)80160-3

24. Dkhil MA. Role of berberine in ameliorating *Schistosoma mansoni*-induced hepatic injury in mice. Biologic Res. 2014; 47:8. doi: 10.1186/0717-6287-47-8

25. Taheri S, Zarei A, Ashtiyani S, Rezaei A, Zaheiri S. Evaluation of the effects of hydroalcholic extract of *Berberis valgaris* root on the activity of liver enzymes in male hypercholestrolemic rats. Avicenna J Phytomed. 2012; 2:153-161. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4075670/

26. Upreti C, Konstantinov E, Kassabov SR, Bailey CH, Kandel ER. Serotonin induces structural plasticity of bothextrinsic modulating and intrinsic media tingcircuits in vitroin *Aplysia californica*. Cell Report. 2019; 28:2955-2965. DOI: 10.1016/j.celrep.2019.08.016

27. Vysakh A, Jayesh K, Helen LR, Jyothis M, Latha MS. Acute oral toxicity and anti-inflammatory evaluation of methanolic extract of *Rotula aquatica* roots in Wistar rats. J Ayurveda Integr Med. 2020; 11:45–52. https://doi.org/10.1016/j.jaim.2017.09.007

28. Wahyuni TS, Ilmi H, Kristiaji AS, Candra Dewi M, Nisa HK, Hafid AF, et al. Acute and repeated dose 28-day oral toxicity of *Ruta angustifolia* Pers. leaves ethanolic extract in Wistar rats. J Pharm Pharmacog Res. 2023; 11:437-447. https://doi.org/10.56499/jppres23.1609_11.3.437

29. Yam MF, Lim CP, Fung AL, Por LY, Wong ST, Asmawi MZ, et al. Antioxidant and toxicity studies of 50% methanolic extract of *Orthosiphon stamineus* Benth. Biomed Res Int 2013; 351602: https://doi.org/10.1155/2013/351602

30. Imo I, Kayode AA, Chukwuma SE, Ojochenemi EY, Chukwumaobim DN, Nkiruka CA, et al. Effects of ethanolic extracts of leaf, seed and fruit of *Datura metel* L. onkidney function of male albino rats. J Trad Complement Med. 2019; 9:271-277. doi: 10.1016/j.jtcme.2017.09.001

31. Khaksar M, Oryan A, Sayyari M, Rezabakhsh A, Rahbarghazi R. Protective effects of melatonin on long-term administration of fluoxetine inrats. Experim Toxicol Pathol. 2017; 69:564-574. DOI: 10.1016/j.etp.2017.05.002

32. Hori N, Okanoue T, Sawa Y, Mori T, Kashima K. Hemodynamic characterization in experimental liver cirrhosis induced by thioacetamide administration. Digest Disease Sci. 1993; 38:2195-2202. https://link.springer.com/article/10.1007/BF01299895

33. Malihe Hassanzadeh, Niusha Sharifi, Shabnam Mahernia, Nastaran Rahimi, Ahmad Reza Dehpour, Massoud Amanlou. Effects of onopordia, a novel isolated compound from *Onopordon acanthium*, on pentylenetetrazole-induced seizures in mice: Possible involvement of nitric oxide pathway. *Journal* of Traditional Complementary Medicine. 2019; https://doi.org/10.1016/j.jtcme.2019.11.005

34. Kuzu N, Metin K, Dagli AF, Akdemir F, Orhan C, Yalniz M, et al. Protective role of genistein in acute liver damage induced by carbon tetrachloride. Mediat Inflammat. 2007; 36381:6. doi: 10.1155/2007/36381

35. Parveen R, Baboota S, Ali J, Ahuja A, Vasudev SS, Ahmad S. Effects of *Silymarin nanoemulsion* against carbontetrachloride-induced hepatic damage. Archive Pharm Res. 2011; 34:767-774. DOI: 10.1007/s12272-011-0510-8

36. Jatsa HB, Femoe UM, Njiaza J, Tombe Tombe DS, Mbolang LN, Nkondo ET. et al. (2018). Efficacy of *Sida pilosa* Retz aqueous extract against *Schistosoma mansoni* - induced granulomatous inflammation in the liver and the intestine of mice: histomorphometry and gastrointestinal motility evaluation. BMC. Complement. BMC Complem Alternat Med. 2018; 18:247. DOI: 10.1186/s12906-018-2318-2

37. Akinjide Moses Akinnuga, Angezwa Siboto, Bongiwe Khumalo, Ntethelelo Hopewell Sibiya, Phikelelani Ngubane, Andile Khathi. Bredemolic acid ameliorates selected liver functionbiomarkers in a diet-induced prediabetic rat model. Canadian J Gastroenterol Hepatol. 2020; 2475301:1-9. doi: 10.1155/2020/2475301

38. Nozu FN, Takeyama N, Tanaka T. Changes of hepatic fatty acid metabolism produced by chronic thioacetamide administration in rats. J Hepatol. 1992; 15:1099-1106. DOI: 10.1002/hep.1840150621

39. Najmeh K, Mahbubeh S, Mahboobeh AD. Protective effects of *Kombucha tea* and silimarin against thioacetamide induced hepatic injuries in wistar rats. World Appl Scien J. 2013; 27:524-532. https://www.idosi.org/wasj/wasj27(4)13/18.pdf

40. Vishnu SN, Sunil K, Ramdas P. Acute toxicity study of *Hygrophila auriculata* L. leaves methanolic extract in albino rats. J Pharmaceut Chem Biol Sci. 2015; 3:388-395. https://www.semanticscholar.org/paper/Acute-Toxicity-Study-of-Hygrophila-auriculata-L.-in-Neharkar-Kshirsagar/9560d516d146987ef7e40cf6df203c8a8b22a6d1

41. Dashti H, Jeppson B, Haggerstrand I, Hultberg B, Srinivas U, Abdulla M. Thioacetamide- and carbon tetrachloride-induced liver cirrhosis. European Surg Res. 1989; 21:83-91. DOI: 10.1159/000129007

42. Azam R, Shahnaz SF, Saeed CA, Hydar A, Ali Z, Maryam A, et al. The effects of *Artemisia aucheri* extract on hepatotoxicity induced by thioacetamide in male rats. Avicenna J Phytomed. 2013; 3:293-301. https://pubmed.ncbi.nlm.nih.gov/25050286/

43. Muthulingam M, Savio PD, Sathanantha Seeli T, Sethupathy S. Therapeutic Role of edible Mushroom *Pleurotus florida* on Thioacetamide induced hepatotoxicity in rats. Int J Curr Res. 2010; 5:041-046. http://journalcra.com/sites/default/files/issue-pdf/MSO%20094%20ls.pdf

44. Yu X, Hong F, Zhang YQ. Cardiac inflammation involving in PKCε or ERK1/2-activated NF-κB signalling pathway in mice following exposure to titanium dioxide nanoparticles. J Hazard Mat. 2016; 313:68-77. DOI: 10.1016/j.jhazmat.2016.03.088

45. Mirzaei A, Akbartabar M, Sadeghi H, Sharifi B. The antioxidant activities and total phenolic of *Artemisia martima*, *Achillea millefolium* and *Matricaria recutica*. J Yasuj University Med Sci. 2010; 15:243-252. http://armaghanj.yums.ac.ir/browse.php?a_code=A-10-1-334&slc_lang=en&sid=1

46. Jafari Dinani N, Asgary S, Madani H, Mahzoni P. Effect of *Artemisia aucheri* extract plants on atherogenic lipids and atherogenesis in hypercholesterolemic rabbits. J Med Chem. 2007; 6:20-28. http://jmp.ir/article-1-584-en.html

47. Romaiana PP, Roselei F, Alessandro de SP, Robson LP, Gloria Narjara SS, Berta Maria H, et al. Antioxidant effects of different extracts from *Melissa officinalis, Matricaria recutita* and *Cymbopogon citratas.* Neurochem Res. 2009; 34:973-983. DOI: 10.1007/s11064-008-9861-z

48. Ahmed MM. Rosmarinic acid attenuates the hepatotoxicity induced by ethanol in rats. American J Biochem. 2016; 6:82-90. http://article.sapub.org/10.5923.j.ajb.20160603.03.html

49. Livero FA, Martins GG, Queiroz Telles JE, Beltrame OC, Petris Biscaia SM, Cavicchiolo Franco CR, et al. Hydroethanolic extract of *Baccharis trimera* ameliorates alcoholic fatty liver disease in mice. Chemico-Biol Interact. 2016; 260:22-32. DOI: 10.1016/j.cbi.2016.10.003

50. Park SY, Ahn G, Um JH, Han EJ, Ahn CB, Yoon NY, et al. Hepatoprotective effect of chitosan-caffeic acid conjugate against ethanol treated mice. Experimental and Toxicologic Pathology. 2017; 69: 618-624. https://doi.org/10.1016/j.etp.2017.05.009

51. Imo C, Uhegbu FO. Renal protective effect of ethanolic leaf extract of *Gongronema latifolium* benth in acetaminophen-induced renal toxicity in male albino rats. American Chemical Science International Journal. 2015; 8:1-10. DOI: 10.9734/ACSJ/2015/18881