International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 12; December 2017; Page No. 8237-8241
DOI: http://dx.doi.org/10.24327/ijcar.2017.8241.1317

Research Article

SCREENING OF HEPATO-PROTECTIVE POTENTIAL OF ETHANOLIC FLOWER EXTRACT OF TAGETES ERECTA IN ETHANOL INDUCED HEPATOTOXICITY IN RATS

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A R T I C L E I N F O

Article History:
Received 8th September, 2017
Received in revised form 25th October, 2017
Accepted 4th November, 2017
Published online 28th December, 2017

Key words:

Tagetes erecta, Thiobarbituric acid (TBA), Nitro-blue Tetrazolium (NBT) & Silymarin

A B S T R A C T

The present study was designed to evaluate the hepatoprotective effect of ethanolic flowers extract of *Tagetes erecta* on ethanol induced hepatotoxicity in rats. Liver functioning and antioxidant levels of the liver were also studied to find out hepatoprotective action of *Tagetes erecta* and compared with standard Silymarin.Group I: administered distilled water served as control group. Group II: The ethanol treatment induced hepatotoxicity in rats at the dose 3.76g/kg, *p. o.*when administered twice daily for twenty five days. Group III: Silymarin was administered at dose of 200 mg/kg; served as standard group. Group IV: *Tagetes erecta* was given to rats at the dose 200 mg/kg; Group V: *Tagetes erecta* was administered at dose 400 mg/kg; both served as test groups. It produced statistically significant hepatoprotective effect on every parameter tested, in dose dependent manner. In conclusion, further research is suggested to identify and isolate the active moiety responsible for this activity. In conclusion, it can be used in the treatment of hepatotoxicity induced by ethanol.

INTRODUCTION

The liver is one of the largest organs in the human body. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification of drugs and secretion of bile and storage of vitamins. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits, alcohol which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease [1]. Liver cell injury may be caused by various toxic chemicals like certain antibiotics, chemotherapeutic agents, carbon tetrachloride, thio-acetamide, excessive alcohol consumption and microbes. There are various herbal medicines which have been applied for the treatment of liver disorder from a long period of time (Roy *et al*., 2014). It is involved with almost biochemical pathways of growth, fight against disease, nutrient supply, energy provision and reproduction [2]. It is very popular as a garden plant and yields a strongly aromatic essential oil (*Tagetes* oil), which is mainly used for the compounding of high-grade perfumes. Different parts of this plant including flowers are used in folk medicine to cure various diseases. Leaves are used as antiseptic and in kidney troubles, muscular pain, piles and applied to boils and carbuncles.

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The flower is useful in fevers, epileptic fits (Ayurveda), astringent, carminative, stomachic, scabies and liver complaints and is also employed in diseases of the eyes [3]. Herbal drugs have a great growth potential in global market. Research work on chemistry of natural products, pharmacognosy, pharmaceutics, pharmacology and clinical therapeutics have been carried out on herbal drugs and most of the leading Pharmaceutical corporations have revised their strategies in favour of natural products**.** Many herbal remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases. *Tagetes erecta,* commonly known as Marigold has been recognized in different systems of traditional medicine for the treatment of diseases and human ailments [4].

Amaranthuscaudatus (Lalcholai - whole plant), *Asparagus racemosus* (Shatavari - root), *Azimatetracantha* (Kanda-gar-kamay - leaves), *Cucumistrigonus* (kharbooza - fruit), *Ficusreligiosa* (Peepal- stem bark), *Solanumnigram* (Mokoi - fruit) are some drugs that show a promising effect as hepatoprotective agents [5]. Antioxidant activity was carried outby DPPH radical scavenging activity assay, superoxide free radical scavenging (SO) assay, ABTS radicalcation scavenging activity, ferric reducing antioxidant power (FRAP), Reducing capacity assessment. Ethyl acetate extract was noted with the maximum flavonoids content and anti-oxidant potential [6]. Hepatoprotective activity of aqueous root extract of *Tagetes erecta* was studied on ethanol induced hepatotoxicity in rats

[10]. It demonstrated statistically significant hepatoprotective potential [7].

The present study was designed to focus on hepatoprotective effect of ethanolic *Tagetes erecta* flowers in ethanol induced hepatotoxicity model. Liver functioning and antioxidant levels of the liver were studied to find out hepatoprotective action of *Tagetes erecta*.

MATERIALS AND METHODS

Plant collection and authentication

Fresh flowers of *Tagetes erecta* was collected from the local areas of Bareilly, UP, India during December-January, 2016 and identified taxonomically in Department of Botany, M.J.P. Rohilkhand University Bareilly (U.P) India. The flowers have been shade dried and pulverized.

Extraction of Tagetes erecta flower

Thus obtained flowers was dried under the shade for 15 days and well pulverized in an electric grinder. The powder material was soaked in 90% ethanol for four days. Stirring of the mixture was carried out twice daily. After the fourth day, the mixture was filtered and the marc pressed. This process repeated 3 times. All the alcoholic fractions were combined and the ethanol subjected for evaporation. The syrupy consistency material obtained was heated on the water bath until dry extract obtained. Thus obtained ethanolic extract of flowers of *Tagetes erecta* was labeled and stored in the desiccators for further usage [2].

Animals

For the study, Silymarin: Received as a gift sample from micro labs limited Hosur, Tamilnadu. Male Wister rats weighing 200-250g were used. The animals were housed in polypropylene cages. Paddy husk was provided as bedding material, which was changed every day. They were feed with standard pellet diet and purified water. They were kept in a well-aerated room and 12-hour light and dark cycle was maintained. The room temperature was maintained at 25 ± 2 ^{o}C. The study was conducted in the Department of Pharmacy, M.J.P.Rohilkh and University, Bareilly. Experiments were performed according to the guidelines for care and use of laboratory animals.

Drugs and chemicals

Ethanol, Thiobarbituric acid (TBA), nitro blue tetrazolium (NBT), hydroxylamine were purchased from Sigma–Aldrich (Bangalore, India), Silymarin.

Dose Selection

Doses of ethanol and silymarin were selected on thebasis of literature review. Silymarin was administered at a dose of 100 mg/kg/*p.o.* in rats as a standard hepatoprotective drug. Ethanol was administered at a dose of 3.76 g/kg, *p.o.* twice dailyin rats to induce the liver toxicity. Doses of *Tagetes erecta* were selected on the basis of literature review study, 200 mg/kg) and (400 mg/kg) [7].

Experimental Design

Rats were divided into 5 groups, each consisting of 6 animals. All groups were received drug for twenty five days as follows-

Table N. 1 Design of the experiment [10]

Group	Treatment
Group I	Distilled water only for twenty five days
Group II	40% ethanol, 3.76g/kg, *p.o.* twice daily for twenty five days
Group III	Silymarin suspended in 0.6% CMC, 100mg/kg orally and 40% ethanol, 3.76g/kg, *p.o.*twice daily for twenty five days.
Group IV	200 mg/kg of ethanolic extract of *Tagetes erecta* and 40% ethanol 3.76g/kg, *p.o.* twice daily for twenty five days.
Group V	400 mg/kg of ethanolic extract of *Tagetes erecta* and 40% ethanol 3.76g/kg, *p.o.* twice daily for twenty five days.

Albino rats of either sex weighing between 200-250g were selected and divided into five groups of six animals in each. The animals were fasted 24 hours prior to experiment. Group I was maintained as normal control, which was given with distilled water only. Group II received ethanol 3.76 gm/kg, twice daily, *p.o.* and animals in Group III were treated with Silymarin200 mg/kg, *p. o.*which served as standard. Animals in Groups IV and V were treated with two different doses extract of *Tagetes erecta* 200mg/kg and 400mg/kg, *p. o*. with ethanol for 25 days.

At the end of experimental period, all the animals were sacrificed by cervical dislocation. Blood samples were collected, allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters.

Parameters

Assessment of liver function

Biochemical parameters i.e., SGOT, SGPT and serum bilirubin were analyzed according to the reported methods. A 10% of liver homogenate was used for antioxidant studies such as lipid peroxidation (LPO/MDA) [8].

Serum glutamate pyruvate transaminase level in liver tissue (SGPT) and Serum glutamate oxaloacetate transaminase level in liver tissue (SGOT)

Activities of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by the method of Reitman and Frankel. 0.05 ml of serum with 0.25 ml of substrate (aspartate and α-ketoglutarate for SGOT; alanine and α - ketoglutarate for SGPT, in phosphate buffer pH 7.4) was incubated for an hour in case of SGOT and 30 min. for SGPT. 0.25 ml of DNPH solution was added to arrest the reaction and kept for 20 min at room temperature. After incubation 1 ml of 0.4 N NaOH was added and absorbance was noted at 505 nm in *UV* Spectrophotometer. Activities were expressed as IU/dl.

Alkaline phosphatase level in liver tissue (ALP)

Based on the method of King and Armstrong alkaline phosphatase, activity was assayed using disodium phenyl phosphate as substrate. The colour developed was read at 510 nm in *UV* Spectrophotometer after 10 min. Activities of the ALP were expressed as IU/dl.

Serum bilirubin level in liver tissue

Serum total bilirubin level was estimated based on the method of Malloy and Evelyn. Diazotized sulphonilic acid (0.25 ml) reacts with bilirubin in diluted serum (0.1 ml serum + 0.9 ml distilled water) and forms purple colored azobilirubin, which was measured at 540 nm in *UV* Spectrophotometer. Activities of total bilirubin were expressed as mg/dl.

Malondialdehyde (MDA)

Determination of Thiobarbituric Acid Reactive Substances (TBARS)

Lipid peroxidations in liver tissues were estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa *et al*. To 0.2ml of sample, 0.2ml of 8.1% Sodium dodecyl sulfate, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA were added. The volume of the mixture was made up to 4 ml with distilled water and then heated at 950 °C in a water bath for 60 min. After incubation the tubes were cooled to room temperature and the final volume was made upto 5 ml in each tube. Then 5 ml of n-butanol: Pyridine mixture was added and the contents were mixed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min the upper organic layer was taken and its OD was read at 532 nm against an appropriate blank without the sample.

RESULTS AND DISCUSSION

The rats of all the two groups treated with alcoholic extract of *Tagetes erecta* at different dose levels showed significant reduction in SGOT, SGPT, ALP, total bilirubin and MDA compared to the ethanol treated group. The results obtained so are statistically significant and comparable to the silymarin treated group as shown in the table no 2,3,4,5 and 6.

Table N. 2 Effect of ethanolic extract of *Tagetes erecta* on SGPT on ethanol induced hepatotoxicity in rats

Groups	Treatment	SGPT (IU/dl)
Control	Vehicle	40.8±1.44[a]
Negative	Ethanol (3.76 g/kg)	115.19±1.84[b]
Standard	Silymarin (100mg/kg) + ethanol (3.76 g/kg)	63.09±0.46[c]
Test 1	Ethanolic extract of *tagetes erecta*(200 mg/kg) + ethanol (3.76 g/kg)	108.89±0.31[d]
Test 2	Ethanolic extract of *tagetes erecta*(400 mg/kg) + ethanol (3.76 g/kg)	101.15±0.14[e]

All values are represents mean ± SEM; n = 6 animals.
Note: [a]P< 0.05:control group; [b]P< 0.05: ethanol treated group; [C]P < 0.05 standard group; [d]P< 0.05 test 1 group and [e]P< 0.05 test 2 group. One-way ANOVA followed by XLSTAT test for multiple comparisons.

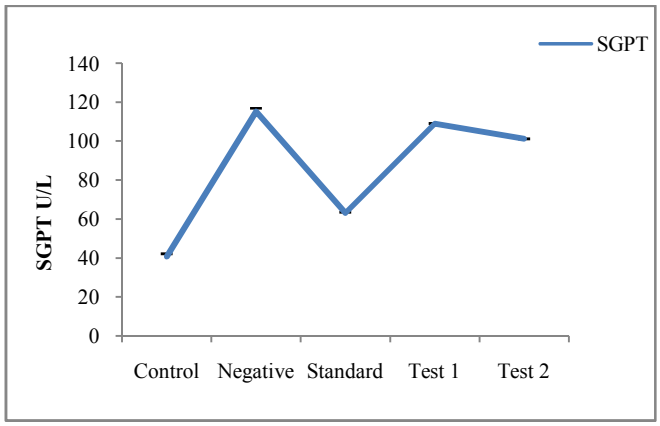

Fig. N. 1 Effect of ethanolic extract of *Tagetes erecta* on SGPT on ethanol induced hepatotoxicity in rats.

Table N. 3 Effect of ethanolic extract of *Tagetes erecta*o On SGOT on ethanol induced hepatotoxicity in rats

Groups	Treatment	SGOT (IU/dl)
Control	Vehicle	56.73±0.221[a]
Negative Control	Ethanol (3.76 g/kg)	125.38±0.320[b]
Standard	Silymarin (100mg/kg) + ethanol (3.76 g/kg)	72.83±0.260[c]
Test 1	Ethanolic extract of *tagetes erecta*(200 mg/kg) + ethanol (3.76 g/kg)	113.27±0.298[d]
Test 2	Ethanolic extract of *tagetes erecta*(400 mg/kg) + ethanol (3.76 g/kg)	109.53±0.328[e]

All values are represents mean ± SEM; n = 6 animals.
Note: [a]P< 0.05: control group; [b]P< 0.05: ethanol treated group; [C]P < 0.05 standard group; [d]P< 0.05 test 1 group and [e]P< 0.05 test 2 group. One-way ANOVA followed by XLSTAT test for multiple comparisons.

Fig. N. 2 Effect of ethanolic extract of *Tageteserecta*on SGOT on ethanol induced hepatotoxicity in rats

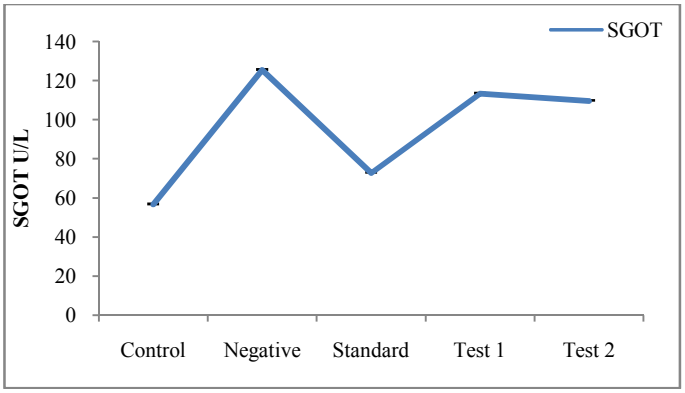

Table N. 4 Effect of ethanolic extract of *Tagetes erecta* on ALP on ethanol induced hepatotoxicity in rats

Groups	Treatment	ALP (IU/dl)
Control	Vehicle	104.5±0.84[a]
Negative Control	Ethanol (3.76 g/kg)	229.83±1.18[b]
Standard	Silymarin (100mg/kg) + ethanol (3.76 g/kg)	140.17±0.94[c]
Test 1	Ethanolic extract of *tagetes erecta*(200 mg/kg) + ethanol (3.76 g/kg)	214.33±0.97[d]
Test 2	Ethanolic extract of *tagetes erecta*(400 mg/kg) + ethanol (3.76 g/kg)	209.83±1.10[e]

All values are represents mean ± SEM; n = 6 animals.
Note: [a]P< 0.05: control group; [b]P< 0.05: ethanol treated group; [C]P < 0.05 standard group; [d]P< 0.05 test 1 group and [e]P< 0.05 test 2 group. One-way ANOVA followed by XLSTAT test for multiple comparisons.

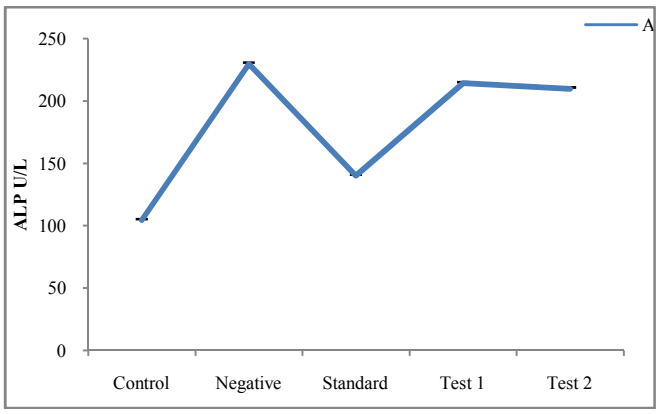

Fig. N. 3 Effect of ethanolic extract of *Tagetes erecta* on ALP on ethanol induced hepatotoxicity in rats.

Table N 5 Effect of ethanolic extract of *Tagetes erecta* on total bilirubin on ethanol induced hepatotoxicity in rats.

Groups	Treatment	Total bilirubin (mg/dl)
Control	Vehicle	0.99±0.0083[a]
Negative Control	Ethanol (3.76 g/kg)	4.22±0.0152[b]
Standard	Silymarin (100mg/kg) + ethanol (3.76 g/kg)	1.22±0.0112[c]
Test 1	Ethanolic extract of *tagetes erecta*(200 mg/kg) + ethanol (3.76 g/kg)	2.9±0.0352[d]
Test 2	Ethanolic extract of *tagetes erecta*(400 mg/kg) + ethanol (3.76 g/kg)	2.071±0.0200[e]

All values are represents mean ± SEM; n = 6 animals.
Note: [a]P< 0.05: control group; [b]P< 0.05: ethanol treated group; [C]P < 0.05 standard group; [d]P< 0.05 test 1 group and [e]P< 0.05 test 2 group. One-way ANOVA followed by XLSTAT test for multiple comparisons.

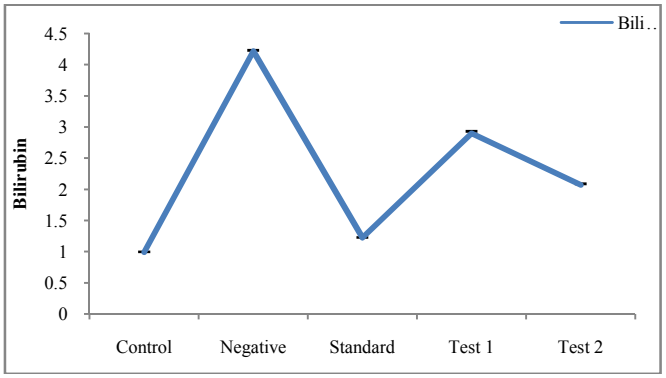

Fig. N. 4 Effect of ethanolic extract of *Tageteserecta*on total bilirubin on ethanol induced hepatotoxicity in rats

Table N. 6 Effect of ethanolic extract of *Tageteserecta*on MDA Level on ethanol induced hepatotoxicity in rats.

Groups	Treatment	MDA(nmols/mg protein)
Control	Vehicle	1.65±0.0082[a]
Negative Control	Ethanol (3.76 g/kg)	5.65±0.0120[b]
Standard	Silymarin (100mg/kg) + ethanol (3.76 g/kg)	1.88±0.402[c]
Test 1	Ethanolic extract of *tageteserecta*(200 mg/kg) + ethanol (3.76 g/kg)	5.21±0.0120[d]
Test 2	Ethanolic extract of *tageteserecta*(400 mg/kg) + ethanol (3.76 g/kg)	4.95±0.0120[e]

All values are represents mean ± SEM; n = 6 animals.
Note: [a]P< 0.05: control group; [b]P< 0.05: ethanol treated group; [C]P < 0.05 standard group; [d]P< 0.05 test 1 group and [e]P< 0.05 test 2 group. One-way ANOVA followed by XLSTAT test for multiple comparisons.

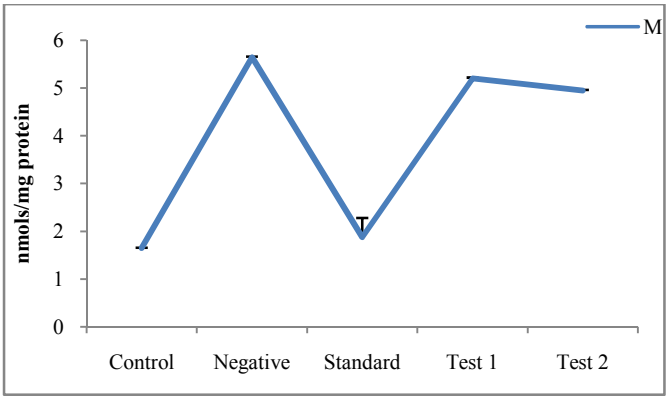

Fig N 5 Effect of ethanolic extract of *Tagetes erecta* on MDA Level on ethanol induced hepatotoxicity in rats.

The rats of all the three groups treated with alcoholic extract of *Tagetes erecta* at different dose levels showed significant reduction in SGOT, SGPT, ALP and total bilirubin, compared to the ethanol treated group. The results obtained so are statistically significant and comparable to the silymarin treated group as shown in the table no. 2,3,4,5 & 6. and fig no. 1,2,3,4 & 5. SGOT in ethanol treated group have significantly increased compared to control group ($p<0.05$). SGOT were decreased significantly in test group up to ($p<0.05$) at doses of 200 and 400mg/kg body wt. respectively as compared to the only ethanol treated group. Silymarin also have decreased SGOT level to ($p<0.05$) compare to ethanol treated group. Serum enzyme SGPT levels were increased significantly in ethanoltreated group as compared to the control rats. The values were increased up to ($p<0.05$), compared to control group, which was the serum SGPT were decreased significantly in test group up to ($p<0.05$) at doses of 200 and 400 mg/kg body wt. respectively as compared to the only ethanol treated group. Silymarin also have decreased the serum SGPT level to ($p<0.05$). Serum ALP levels were increased significantly in ethanol treated group as compared to the control rats. The values were increased up to ($p<0.05$), compared to control group. The serum ALP were decreased significantly in test group up to ($p<0.05$) at doses of 200 and 400 mg/kg body wt. respectively as compared to the ethanol treated group. Silymarin also have decreased the serum ALP level to ($p<0.05$). Total Serum Bilirubin levels in ethanoltreated group have significantly increased compared to control group. The values were increased up to ($p<0.05$), compared to control group. The serum total bilirubin values were reduced significantly in test group up to ($p<0.05$) at doses of 200 and 400mg/kg body wt. respectively as compared to the only ethanol treated group. Silymarin also have reduced serum total bilirubin level to ($p<0.05$). compared with the standard group significantly ($p<0.05$) decrease in MDA levels in the livers of ethanol treated rats suggests that main target of oxidative stress is the polyunsaturated fatty acids in cell membranes causing lipid peroxidation and excessive formation of MDA which may lead to damage of the cell structure. Enhanced peroxidation leads to tissue damage and failure of the antioxidant defense mechanisms to prevent the formation of excessive free radicals.

Whereas the extract treated animals were significantly reduced SGPT, SGOT and ALP levels indicating their hepatoprotective effect against alcohol-induced liver damage. The elevated activities of these marker enzymes in sera of the ethanol treated rats in the present study were due to the extensive liver damage caused by the toxin. Treatment with the test drug as well as the reference drug silymarin significantly reduced the ethanol induced elevation in the activities of these enzymes.

CONCLUSION

In conclusion, our results of this study reported that ethanolic extract of *Tagetes erecta* was effective treatment for the control of hepatotoxicity induced by ethanol. The degree of protection was measured by using biochemical parameters like alkaline phosphatase, bilirubin, SGPT, SGOT and MDA characters. The ethanolic extract *Tagetes erecta* showed the significant hepatoprotective activity comparable with standard drug silymarin. From this investigation, compounds of *Tagetes erecta* flower extract may be better responsible for the hepatoprotective activity. Our results demonstrated that the plant derived drugs are the good alternative drugs for synthetic

or chemical drug.Further research is suggested to identify and isolate the active moiety responsible for this activity. In conclusion, it can be used in the treatment of hepatotoxicity induced by ethanol.

Acknowledgement

I would like to thank Mr. Mohd Ruman Khan, Ms. Huma Noor and Dr. S. Ramasamy without whom this work has never been completed. I am also highly grateful to Mr. Mohd Asif Khan who encouraged and helped me in the writing of this paper.

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How to cite this article:

Yogendra Singh *et al* (2017) 'Screening of Hepato-Protective Potential of Ethanolic Flower Extract of Tagetes erecta In Ethanol Induced Hepatotoxicity in Rats', *International Journal of Current Advanced Research,* 06(12), pp. 8237-8241. DOI: http://dx.doi.org/10.24327/ijcar.2017.8241.1317

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