ejpmr, 2015,2(6), 141-146

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

HEPATOPROTECTIVE ACTIVITY STUDIES OF *CUCUMIS TRIGONUS* ROXB. AGAINST RIFAMPICIN-ISONIAZID-INDUCED TOXICITY IN RATS.

Subarayan Bothi Gopalakrishnan[a]*, Thangaraj Kalaiarasi[b]

[a]*Senior Professor of Chemistry and Controller of Examination, Noorul Islam University, Kumaracoil, Kanyakumari-629 180, Tamil Nadu, India.

[b]Assistant Professor of Chemistry, Kalasalingam University, Kalasalingam Academy of Research and Education, Anand Nagar, Krishnankoil-626126, Tamil Nadu, India.

***Correspondence for Author: Subarayan Bothi Gopalakrishnan**

Senior Professor of Chemistry and Controller of Examination, Noorul Islam University, Kumaracoil, Kanyakumari-629 180, Tamil Nadu, India.

Article Received on 22/08/2015 **Article Revised on 15/09/2015** **Article Accepted on 07/10/2015**

ABSTRACT

The fruits of *Cucumis trigonus* Roxb. was analysed for the hepatoprotective activity against albino rats with liver damage induced by rifampicin-isoniazid. Rifampicin (RIF) plus isoniazid (INH) treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury. The ethanolic extract of the fruits of *Cucumis trigonus* showed normalization of body weight, biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP), γ-glutamyl transpeptidase (GGTP), total bilirubin (TB), total protein (TP) as well as the levels of liver homogenates, Lipid peroxidase (LPO), glutathione peroxidase (GPx), glutathione reductase (GRD), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH). The effects of ethanolic extract of the fruits of *Cucumis trigonus* (100 mg/kgbw ip 250 mg/kgbw ip & 500 mg/kgbw ip) was compared with that of the standard drug, silymarin. The ethanolic extract showed significant hepatoprotective activity in 500 mg/kg ip dose. The hepatoprotective activity has also been supported by histopathological studies of liver tissue.

KEYWORDS: Hepatoprotective, *Cucumis trigonus*, Histopathology.

INTRODUCTION

Rifampicin (RIF) and isoniazid (INH), the two front-line drugs have been used in the treatment of tuberculosis, that is known to be potentially hepatotoxic. Rifampicin, which is generally co-administered with isoniazid in the treatment of tuberculosis, is toxic to hepatocytes. A meta analysis of studies involving the use of a multiplicity of antituberculosis drug regimens predominantly in adults have shown an incidence of liver injury of 1.1 % in patients with RIF alone, 1.6 % in patients with INH alone, and 2.55 % in patients with RIF plus INH.[1] Reactive oxygen species play a key role in RIF-INH-induced hepatotoxicity.[2] Since oxidative stress has been regarded as the major mechanism of antituberculosis drug-induced hepatotoxicity[3], antioxidants might be used as potential antihepatotoxic drugs against RIF-INH caused liver injury.[4]

A major defense mechanism involves the antioxidant enzymes, including SOD, CAT and GSH-Px, which convert active oxygen molecules into non-toxic compounds.[5] The pathogenesis of the hepatotoxicity is involved in all the hepatic cell types via death and regeneration processes, and liver diseases often progress from subclinical icteric hepatitis to hepatic fibrosis, cirrhosis and hepatocellular carcinoma.[6]

Cucumis trigonus (Fam. Cucurbitaceae) is commonly known as "Thummittikai" in Tamil, "Bitter gourd" in English, and "Vishala" in Sanskrit. The fruits of *Cucumis trigonus* are reported to be useful in treating leprosy, jaundice, diabetes, and other abdominal disorders. *Cucumis trigonus* fruit is shown to possess various activities such as antidiabetic activity[7], anabolic activity[8], cardioprotective activity[9], analgesic and anti-inflammatory[10], and diuretic activity.[11]

Hepatoprotective activity of the ethanolic extracts of the fruits of *Cucumis trigonus* and *Cucumis sativus* against paracetamol-induced toxicity in albino rats have been already performed by our group.[12, 13] In the present study hepatoprotective activity studies of the ethanolic extract of the fruits of *Cucumis trigonus* on RIF-INH-induced liver toxicity in albino rats have been carried out.

MATERIAL AND METHODS

Collection of plant materials

The fruits of *Cucumis trigonus* was collected in the month of March from Alangulam, Tirunelveli District, Tamil Nadu and identified by Prof. P. Jayaraman, Plant Anatomy Research Centre, West Thambaram, Chennai-600 045, Tamil Nadu, India (Reg.No of the authentification certificate: PARC/2013/2048).

Experimental animals
Male wistar albino rats weighing 150-200 g were used for hepatoprotective studies. The animals were fed with standard pellet diet supplied by Hindustan Lever Ltd., Kolkata, India and fresh water *ad libitum*. They were housed in standard stainless-steel cages at a 12 h cycle of light and dark. Room temperature was kept at (25° ± 3°C), humidity maintained at 50 %.

Drugs and chemicals
Rifampicin and isoniazid were purchased from Micro Labs, India. Silymarin was obtained as gift sample from Ranbaxy (Devas, India), Standard kits of SGPT, SGOT, SALP, bilirubin and total protein were obtained from Jain Scientific Industries, Moradabad, India. All other reagents used for the study were of analytical grade.

Preparation of extract
The collected fruits were cut into pieces, shade-dried at room temperature and powdered. The dried fruit Powder(500g) was successively extracted using petroleum ether (40°- 60°C), benzene, chloroform, ethanol and water by using Soxhlet apparatus. The last trace of solvent was removed under reduced pressure distillation and then vacuum dried. The dried crude ethanolic extract was used for the study.

Acute toxicity
Acute toxicity study was performed for the ethanolic extract of the fruits of *Cucumis trigonus* as per OECD guidelines.[14] Albino rats received 2000 mg/kgbw ip of the ethanol extract. The animals were observed for toxic symptoms continuously for the first 4 h after dosing. The rats were continuously observed for their mortality and behavioural response for 48 h and thereafter once in a day for 14 days. There was no mortality recorded. Therefore the drug should be free from toxicity.

Induction of experimental hepatotoxicity
Each 50 mg/kgbw ip of RIF + INH solutions were prepared separately in sterile distilled water. Rats were divided into nine groups, each group consisting of six animals.[15]
Group I: Control received the vehicle *viz*. normal saline (2 mL/kgbw ip).
Group II: Received 50 mg/kgbw ip per day of RIF + INH each by ip route for 21 days.
Group III: Received 100 mg/kgbw ip of the ethanolic extract of the fruits of *Cucumis trigonus* and simultaneously received 50 mg/kgbw ip per day of RIF + INH each by ip route for 21 days. (Low dose).
Group IV: Received 250 mg/kgbw ip of the ethanolic extract of the fruits of *Cucumis trigonus* and simultaneously received 50 mg/kgbw ip per day of RIF + INH each by ip route for 21 days. (Moderate dose).
Group V: Received 500 mg/kgbw ip of the ethanolic extract of the fruits of *Cucumis trigonus* and simultaneously received 50 mg/kg ip per day of RIF + INH each by ip route for 21 days. (High dose).
Group VI: Received 2.5 mg/kgbw ip of silymarin (Standard drug) and simultaneously received 50 mg/kgbw ip per day of RIF + INH each by ip route for 21 days.

At the end of 21 days, all the animals were sacrificed by cervical decapitation. Blood samples were collected, and the serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for the various biochemical parameters. Body weights of the rats were measured daily for 21 days. Daily changes in body weights were recorded.

Assessment of liver damage
Liver damage was assessed by the estimation of serum activities of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP), γ- glutamyl transpeptidase (GGTP), total bilirubin (TB), conjugated bilirubin, unconjugated bilirubin, total protein (TP), albumin, and globulin according to the method by using commercially available test kit.[16-22] Lipid peroxidase (LPO)[23] glutathione peroxidase (GPx)[24] glutathione reductase (GRD)[25] superoxide dismutase (SOD)[26] catalase (CAT)[27] and reduced glutathione (GSH)[28] were estimated in liver homogenate.

Histopathological studies
The livers were removed from the animals and the tissues were fixed in 10 % formalin for at least 24 h. Then, the paraffin sections were prepared (Automatic tissue processor, Autotechnique) and cut into 5 µm thick sections using a rotary microtome. The sections were then stained with Haematoxylin-Eosin dye and studied for histopathological changes, such as fatty changes, necrosis, vacuole, space formation, loss of cell boundaries for microscopic observations.

Statistical analysis
The values were expressed as Mean ± SD. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison test and data on liver weight variations were analyzed using Student's 't' test. The levels of significance were mentioned as * $P \leq 0.05$, ** $P \leq 0.01$.

RESULTS AND DISCUSSION

Rifampicin is a first line drug used in the treatment of tuberculosis and leprosy. It possesses the ability to eliminate semi dormant or persisting organism. Short course chemotherapy containing rifampicin and isoniazid in combination has proved to be highly effective in the treatment of tuberculosis, but one of its adverse effects is hepatotoxicity. RIF-induces cytochrome P^{450} enzyme causing an increased production of toxic metabolites from acetyl hydrazine (AcHz). RIF can also increase the metabolism of INH to isonicotinic acid and hydrazine, both of which are hepatotoxic. The plasma half life of AcHz (metabolite of INH) is shortened by RIF and AcHz is quickly converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which

is related to the higher incidence of liver necrosis caused by INH and RIF in combination. Damaged hepatocytes or biliary epithelium may release cell constituents (e.g. enzymes) into blood resulting in increased levels of these analytes. The more commonly measured 'liver' enzymes are alanine aminotransferase (ALT, formerly SGPT), aspartate aminotransferase (AST, formerly known SGOT), serum alkaline phosphatase (SALP), and γ-glutamyl transpeptidase (GGTP).

Although there will be an increase of AST and ALT in heart and liver diseases, total bilirubin (TB), a byproduct of the breakdown of red blood cells in the liver is a good indicator of liver function. High levels will cause icterus and are indicative of damage to the liver and bile duct.[29] The estimation of GGTP level is a valuable screening test with high negative predictive value for liver disease.

Administration of RIF + INH combination only, showed a significant derangement of liver function as assessed by change in serum enzymes SGOT, SGPT, SALP, GGTP, TB, TP, albumin, globulin as well as the levels of liver homogenates, LPO, GPx, GRD, SOD, CAT, and GSH and also liver histopathology.

Table-1 shows the levels of SGOT, SGPT, SALP, GGTP in the serum and bodyweight. There was a significant increase in the levels of SGOT, SGPT, SALP, GGTP in serum of rats treated with RIF + INH when compared with that of the control rats. Whereas the levels of body weight in RIF + INH treated rats were decreased. There is a gain in body weight in all the drug treated groups. Pretreatment of rats with the ethanolic extract of the fruits of *Cucumis trigonus* caused a significant reduction in the levels of enzymes leading to a significant reversal of hepatotoxicity.

TABLE 1: Effect of the ethanolic extract of the fruits of *Cucumis trigonus* on the body weight and other biochemical parameters on rifampicin-isoniazid-induced hepatotoxicity in rats.

Groups	Dose (mg/kg ip)	Body weight			Parameters			
		Initial weight (g)	Final weight (g)	Weight gain(↑) / loss (↓) (g)	SGOT (U/L)	SGPT (U/L)	SALP (U/L)	GGTP (U/L)
Control	2 mL saline	192.54±4.31	194.66±5.34	02.12 ↑	27.27±0.94	36.59±0.93	191.26±10.11	8.59±1.21
RIF + INH	100	198.33±5.39	174.14±5.84**	24.19 ↓	144.51±4.56**	163.59±2.80**	263.16±11.36**	19.63±1.44**
Cucumis trigonus	100	185.54±6.34	192.39±4.69$^{\#}$	06.85 ↑	83.14±1.36$^{\#}$	69.36±4.54$^{\#}$	193.56±8.24$^{\#}$	17.66±0.94$^{\#}$
	250	195.15±6.34	204.65±5.89$^{\#}$	09.50 ↑	41.33±2.64$^{\#}$	28.24±5.64$^{\#\#}$	163.22±3.84$^{\#}$	16.08±0.48$^{\#}$
	500	212.63±6.84	228.33±5.93$^{\#\#}$	15.70 ↑	29.11±2.66$^{\#\#}$	20.26±1.87$^{\#\#}$	152.54±8.14$^{\#}$	11.27±0.73
Silymarin	2.5	214.68±5.84	227.55±6.84$^{\#\#}$	12.87 ↑	32.63±1.69$^{\#}$	29.59±1.94$^{\#\#}$	182.69±3.91$^{\#}$	11.84±0.17^{ns}

Values are Mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test.
*P < 0.05; **P < 0.01 as compared with normal control to liver damaged control;
$^{\#}$P<0.05; $^{\#\#}$ P<0.01 as compared with liver damaged control to drug treated animal.
ns: not significant.

Table-2 shows the changes in the levels of total bilirubin, conjugated bilirubin, unconjugated bilirubin, total protein, albumin, and globulin in the serum of different experimental rats. In comparison with the control group, in the RIF + INH treated rats, significant increase in the levels of total bilirubin, conjugated bilirubin, unconjugated bilirubin (p < 0.01) were noticed. There was a significant reduction in the levels of total protein, albumin, and globulin (p < 0.05). Interestingly, in the RIF + INH-induced rats, the levels of total bilirubin, conjugated bilirubin, unconjugated bilirubin, total protein, albumin, and globulin in the liver could be normalized by the pretreatment with the ethanolic extract of the fruits of *Cucumis trigonus*. 500 mg/kgbw ip of *Cucumis trigonus* showed better activity.

TABLE 2: Effect of the ethanolic extract of the fruits of *Cucumis trigonus* on the biochemical parameters on rifampicin-isoniazid-induced hepatotoxicity in rats.

Groups	Dose (mg/kg ip)	Parameters						
		Total bilirubin (mg/dL)	Conjugated bilirubin (mg/dL)	Unconjugated bilirubin (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A/G Ratio
Control	2 mL saline	0.63±0.04	0.22±0.012	0.41±0.021	7.94±0.51	4.11±0.26	3.83±0.12	1.0:1
RIF + INH	100	3.84±0.84**	1.80±0.014**	2.04±0.037**	6.24±0.36**	3.94±0.53	2.30±0.16*	1.4:1
Cucumis trigonus	100	2.04±0.92$^{\#}$	1.19±0.033	0.85±0.021	7.68±0.14	4.28±0.26	3.40±0.32	1.2:1
	250	1.02±0.76	0.32±0.016$^{\#\#}$	0.70±0.011	8.14±0.73$^{\#}$	4.21±0.53	3.46±0.11	1.2:1
	500	0.59±0.021$^{\#\#}$	0.21±0.040$^{\#\#}$	0.38±0.042$^{\#}$	8.33±0.51$^{\#}$	4.87±0.34	3.93±0.23$^{\#}$	1.2:1
Silymarin	2.5	0.96±0.02$^{\#}$	0.26±0.03$^{\#\#}$	0.70±0.16$^{\#}$	7.89±0.53^{ns}	4.02±0.62	3.87±0.16$^{\#}$	1.0:1

Values are Mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test.
*P < 0.05; **P < 0.01 as compared with normal control to liver damaged control;
$^{\#}$P<0.05; $^{\#\#}$ P<0.01 as compared with liver damaged control to drug treated animal. ns: not significant.

Table-3 shows the levels of LPO, GPx, GRD, SOD, CAT, and GSH in the liver homogenate. The level of lipid peroxide sharply increased (6.93±0.013 nm MDA/mg protein ($p < 0.01$) after RIF + INH intoxication. However, the levels of GPx, GRD, SOD, CAT, GSH decreased after RIF + INH intoxication. The administration of all the three doses, *viz*, the low dose, moderate dose, and high dose of *Cucumis trigonus* decreased the level of LPO and increased the levels of GPx, GRD, SOD, CAT, GSH ($p < 0.01$). Among the three different doses, 500 mg/kgbw ip dose showed better activity than the standard drug, silimarin, in the case of LPO and GRD. The protective effect was dose-dependent. The hepatoprotective role of the ethanolic extract of the fruits of *Cucumis trigonus* might be due to the antioxidant potential of the drugs.[30]

The ethanolic extract of the fruits of *Cucumis trigonus* improved liver function by decreasing the serum enzymes SGOT, SGPT, SALP, GGTP, TB, conjugated bilirubin, unconjugated bilirubin, LPO. However, the levels of total protein, albumin, globulin, GPx, GRD, SOD, CAT, and GSH are increased. This indicates the protective effect over liver and improvement in its functional efficiency.

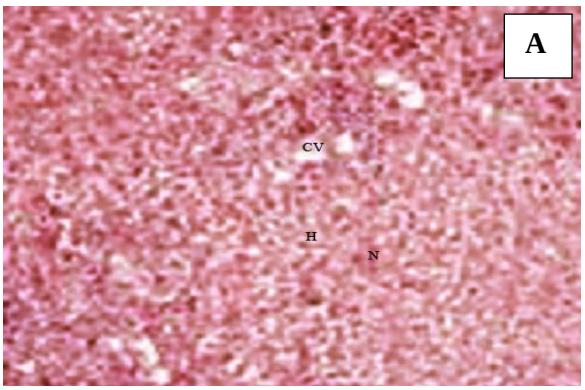
TABLE 3: Effect of the ethanolic extract of the fruits of *Cucumis trigonus* on the liver homogenate biochemical parameters on rifampicin-isoniazid-induced hepatotoxicity in rats.

Groups	Dose (mg/kg ip)	Parameters					
		LPO (nm MDA/mg protein)	GPX (U/mg protein)	GRD (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µg/mg protein)
Control	2 mL saline	1.89±0.024	2.936±0.112	0.684±0.031	0.193±0.046	3.931±0.018	31.59±0.93
RIF + INH	100	6.93±0.013**	0.914±0.114**	0.112±0.014*	0.073±0.011**	1.583±0.024**	11.46±0.73**
Cucumis trigonus	100	4.77±0.026$^{\#}$	1.126±0.080	0.431±0.054$^{\#}$	0.101±0.061$^{\#}$	1.893±0.014$^{\#}$	16.91±0.34$^{\#}$
	250	2.14±0.019^{ns}	2.16±0.014	0.493±0.016^{ns}	0.129±0.054$^{\#}$	2.671±0.016^{ns}	19.14±0.53^{ns}
	500	1.73±0.011$^{\#\#}$	2.816±0.162	0.691±0.014$^{\#\#}$	0.151±0.034$^{\#}$	3.738±0.014$^{\#}$	24.59±0.49$^{\#}$
Silymarin	2.5	1.13±0.054$^{\#}$	2.948±0.029$^{\#}$	0.656±0.054$^{\#}$	0.203±0.0123$^{\#}$	3.91±0.014$^{\#}$	30.11±0.73$^{\#\#}$

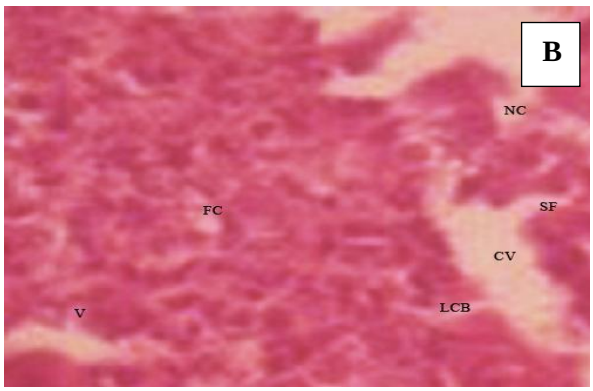
Values are Mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test.
*P < 0.05; **P < 0.01 as compared with normal control to liver damaged control;
$^{\#}$P<0.05; $^{\#\#}$ P<0.01 as compared with liver damaged control to drug treated animal; ns: not significant.

Histopathological examination

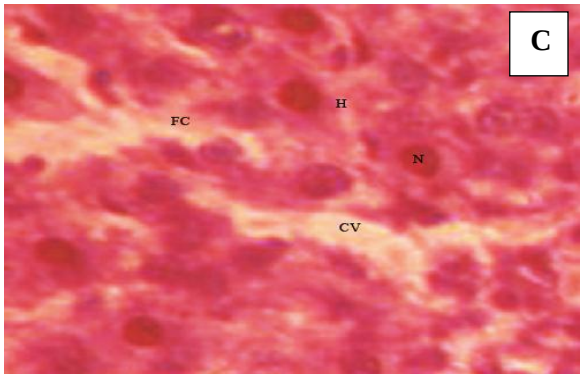
In histopathological studies of liver [Figure 1], the control showed normal gross appearance; dark maroon color of liver having smooth surfaces, microscopically normal lobular appearance having normal central vein, normal hepatic cells each with well-defined cytoplasm, prominent nucleus, well brought out central vein, normal architecture of liver, radiating cords of hepatocytes, and normal portal tract. RIF + INH treated rats showed moderate to severe liver damage characterized by disarrangement of normal hepatic cells, vacuolization, loss of cell boundaries, space formation, and crowding of central vein marked level of fatty changes or degeneration and centrilobular hepatic necrosis of the liver cells. RIF + INH and low dose (100 mg/kgbw ip) of the ethanolic extract of the fruits of *Cucumis trigonus* showed minimal necrosis, mild inflammation and less steatosis. RIF + INH and moderate dose (250 mg/kgbw ip) of the ethanolic extract of the fruits of *Cucumis trigonus* showed slight recovery and evidence of regeneration in some hepatocytes. RIF + INH and high dose (500 mg/kgbw ip) of the ethanolic extract of the fruits of *Cucumis trigonus* showed significant recovery showing absence of necrosis, space formation and vacuoles. RIF + INH and silymarin (2.5 mg/kg ip) showed normal liver architecture and occasional inflammatory cells with no traditis or necrosis.



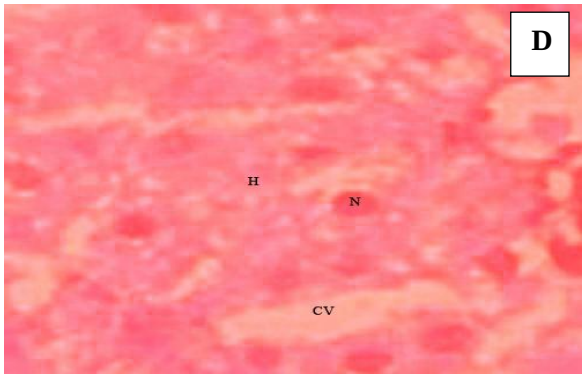
Control group



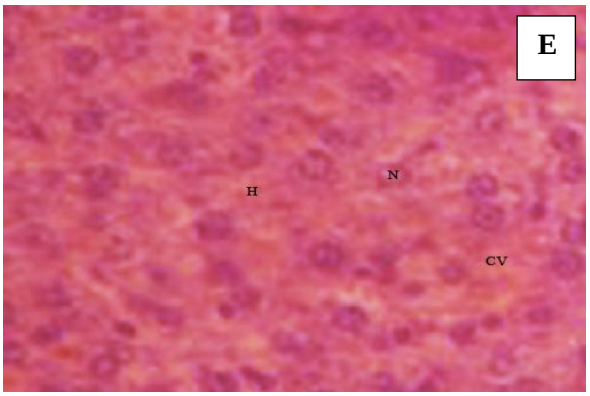
RIF + INH (100 mg/kg ip)



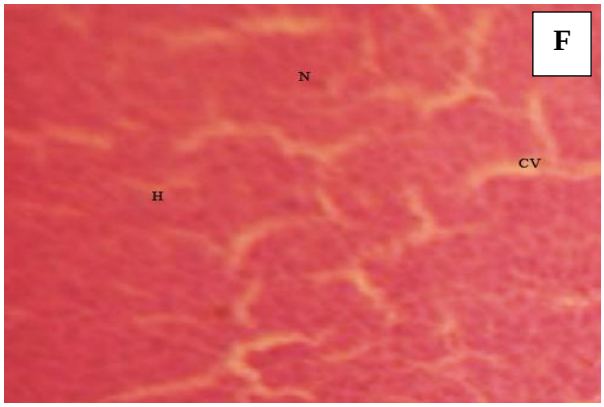
RIF + INH + EE of *CT* (100 mg/kg ip)



RIF + INH + EE of *CT* (250 mg/kg ip)



RIF + INH + EE of *CT* (500 mg/kg ip)



RIF + INH + silymarin (2.5 mg/kg ip).

Figure 1. Histopathology of the ethanolic extract of the fruits of *Cucumis trigonus* on rifampicin-isonizid-induced hepatotoxicity in albino rats.

EE - Ethanolic extract, CT - *Cucumis trigonus*, RIF- Rifampicin, INH – Isonizid, CV - Central vein, H - Hepatocyte, N - Nucleus, FC - Fatty changes, NC - Necrosis, V - Vacuole, SF - Space formation, LCB - Loss of cell boundaries.

CONCLUSION

Hepatotoxicity occurs significantly with anti-TB drugs. The present study proves that the ethanolic extract of the fruits of *Cucumin trigonus* shows significant protective action against the hepatotoxicity induced by the drugs used in the treatment of tuberculosis. However, treatment of these extract completely protected the liver cells. GC-MS analysis indicated the presence of bile acids, carotenoids, antibiotics, steroids and phorbol ester in *Cucumis trigonus*.[31] Hence the hepatoprotective effect of the extract may be due to the presence of one or more phytochemical constituents present in the *Cucumis trigonus* which scavenged the free radical offering hepatoprotection. Thus the ethanolic extract of the fruits of *Cucumis trigouns* which are useful in controlling hepatic injury in drug induced hepatotoxicity. Isolation and characterization of the active principles may yield good hepatoprotective drugs.

REFERENCES

1. M.A. Steele, R.F. Burk, R.M. DesPrez, "Toxic hepatitis with isoniazid and rifampicin. A meta-analysis", *Chest*, 1991; 99: 465–471.
2. A. Chowdhury, A. Santra, K. Bhattacharjee, S. Ghatak, D.R. Saha, G.K. Dhali, "Mitochondrial oxidative stress and permeability transition in Isoniazid and Rifampicin- induced liver injury in mice", *Journal of Hepatology*, 2006; 45: 117–126.
3. Xi Chen, Juan Xu, Cheng Zhang, Tao Yu, Hua Wang, Mei Zhao, Zi-Hao Duan, Ying Zhang, Jian-Ming Xu, De-Xiang Xu, "The protective effects of ursodeoxycholic acid on isoniazid plus rifampicin-induced liver injury in mice" *European Journal of Pharmacology*, 2011; 659: 53–60.
4. K. Sano, H. Tomioka, K. Sato, C. Sano, H. Kawauchi, S. Cai, T. Shimizu, "Interaction of antimycobacterial drugs with the anti-mycobacterium avium complex effects of antimicrobial effectors, reactive oxygen intermediates, reactive nitrogen intermediates, and free fatty acids produced by macrophages", *Antimicrobial Agents and Chemotherapy*, 2004; 48: 2132–2139.
5. Yen-Hung Yeh, You-Liang Hsieh, Ya-Ting Lee, Chao-Chin Hu, "Protective effects of *Geloina eros* extract against carbon tetrachloride-induced hepatotoxicity in rats", *Food Research International*, 2012; 48: 551–558.
6. Dongying Wang, Yan Zhao, Yanfei Sun, Xingbin Yang, "Protective effects of Ziyang tea polysaccharides on CCl_4-induced oxidative liver damage in mice", *Food Chemistry*, 2013; 143; 371–378.

7. M.D. Salahuddin, S. Sunil Jalalpure, "Antidiabetic activity of aqueous fruit extract of *Cucumis trigonus* Roxb. in streptozotocin-induced-diabetic rats", *Journal of Ethnopharmacology*, 2010; 127: 565–567, 2010.
8. A.V. Mainkar, V.R. Naik., V.G. Dhume, N.V. Agshikar, "Anabolic activity of alcoholic extract of *Cucumis trigonus* Roxburghii", Indian Journal of Pharmacology, 1986; 18: 261–262.
9. B.S. Thippeswamy, S.P. Thakker, S. Tubachi, G.A. Kalyani, M.K. Netra, U. Patil, S. Desai, C.C. Gavimath, V.P. Veerapur, "Cardioprotective Effect of *Cucumis trigonus* Roxb on Isoproterenol-Induced Myocardial Infarction in Rat," *American Journal of Pharmacology and Toxicology*, 2009; 4: 29-37.
10. V.R. Naik, N.V. Agshika, G.J. Abraham, "Analgesic and anti inflammatory activity in alcoholic extracts of *Cucumis trigonus* Roxburghii", *A preliminary communication. Pharmacology*, 1980; 20: 52–56.
11. V.R. Naik, N.V. Agshika, G.J. Abraham, "*Cucumis trigonus* Roxb. II. Diuretic activity," *Journal of Ethnopharmacology*, 1981; 3: 15–19.
12. S. Gopalakrishnan, T. Kalaiarasi, "Hepatoprotective activity of the ethanolic extract of the fruits of *Cucumis trigonus* Roxb", *"International Journal of Pharmacy and Pharmaceutical Sciences,"* 2013; 5: 268-272.
13. S. Gopalakrishnan, T. Kalaiarasi, "Hepatoprotective activity of the fruits of *Cucumis sativus* (L.)", *International Journal Pharmaceutical Sciences Review and Research*, 2013; 20: 229-234.
14. S.K. Ashok, S.N. Somayaji, K.L. Bairy , "Hepatoprotective effects of *Ginkgo biloba* against carbon tetrachloride-induced hepatic injury in rats", *Indian Journal of Pharmacology*, 2001; 33: 260-6.
15. Y. Jiang, R.X. Peng, J. Yang, R. Kong, J. Liu, "CYP2E1 mediated isoniazid-induced hepatotoxicity in rats", *Acta Pharmacologica Sinica*, 2004; 25: 699-704.
16. S Reitman, A. Frankel, "Colorimetric method for determination of serum glutamate oxaloaectate and glutamic pyruvate transaminase", *American Journal of Clinical Pathology*, 1957; 28: 56-58.
17. P.R. Kind, E.J. King, "Estimation of plasma phosphates by determination of hydrolyzed phenol with antipyrin", *Journal of Clinical Pathology*, 1954; 7: 322-326.
18. J.P. Persijn, W. Van der Slik, "A New Method for the Determination of γ-Glutamyltransferase in Serum", *Journal of Clinical Chemistry & Clinical Biochemistry*,, 1976; 14: 421-427.
19. L.Jendrassik, "Colorimetric determination of bilirubin", *Biochemistry*, 1938; 97: 72-81.
20. O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J. Randall, *J Biol Chem*, 1951; 193: 265–275.
21. A.E. Pinnel, B.E. Northam, "New automated dye-binding method for serum albumindetermination with bromocresol purple", *Clinical Chemistry*, 1978; 24: 80.
22. E.H. Coles, Veterinary Clinical Pathology. Saunders Company, Philadelphia and London., 1974.
23. H. Ohkawa, N. Ohishi, K. Yagi, "Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction", *Analytical Biochemistry*, 1979; 95: 351-358.
24. J.T. Rotruck, A.L. Pope, H.E. Ganther, D.G. Hafeman, W.G. Hoekstra, *Science*, 1973; 179: 588-590.
25. J. Mohandas, J.J. Marshall, G.G. Duggin, J.S. Horvath, D, Tiller, "Differential distribution of glutathione and glutathione related enzymes in rabbit kidney, Possible interactions in analgesic neuropathy". *Cancer Research*, 1984; 44: 5086–91.
26. S. Rai, A. Wahile, K. Mukherjee, B.P. Saha, P.K. Mukherjee, "Antioxidant activity of *Nelumbo nucifera* (sacred lotus) seeds", *Journal of Ethnopharmacology*, 2006; 104: 322–7.
27. H. Aebi, "Catalase *in vitro*", *Methods in Enzymology*, 1984; 105; 121–126.
28. G.L. Ellman, "Tissue sulfhydryl groups", *Archives of Biochemistry and Biophysics*, 1959; 82: 70–7.
29. K.G. Rajesh, N.K. Achyut, W. Geeta, P.S. Murthy, C. Ramesh, T. Vibha, "Nutritional and Hypoglycemic Effect of Fruit Pulp of *Annona squamosa* in Normal Healthy and Alloxan-Induced Diabetic Rabbits", *Annals of Nutrition and Metabolism*, 2005; 49: 407–413.
30. A. Balakrishnan, R. Kokilavani, "*In vitro* Free Radical Scavenging Activity of Ethanolic Extract of *Cucumis trigonus* Roxburxii fruit" *International Journal of Pharmaceutical & Biological Archives*, 2011; 2: 1439-1443.
31. S. Gopalakrishnan, T. Kalaiarasi, "Identification of chemical compounds from the fruits of *Cucumis trigonus* Roxb. by GC-MS analysis", *International Journal of Phytopharmacy*, 2012; 2: 122-128.