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Research article

PHYTOCHEMICAL EVALUATION AND HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF FRUITS OF *CUCUMIS MELO* LINN AGAINST ISONIAZID AND RIFAMPICIN TOXICITY IN RATS.

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ABSTRACT: The great efficacy of isoniazid (INH) and rifampicin (RMP) combination, in the treatment and chemoprophylaxis of tuberculosis, at the same time hepatotoxicity is the most common serious complication. The fruits of *cucumis melo* linn(CM) was analysed for the hepatoprotective activity against albino rats with liver damage induced by rifampicin-isoniazid. Rifampicin-isoniazid treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury. The methanolic extract of the fruits of *cucumis melo* linn showed normalization of body weight, biochemical parameters like Serum alanine aminotransferase (ALT), Serum aspartate aminotransferase (AST), Serum alkaline phosphatase (ALP), Serum total bilirubin (BILT), and Serum total proteins (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 methanolic extract of the fruits of *cucumis melo* (100 mg/kgbw ip 250 mg/kgbw ip & 500 mg/kgbw ip) was compared with that of the standard drug silymarin. The methanolic extract showed significant hepatoprotective activity in 500 mg/kg ip dose. The hepatoprotective activity has also been supported by histopathological studies of liver tissue.

Key words: Isoniazid and rifampicin, *Cucumis melo*, Histopathology, liver homogenate, biochemical.

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INTRODUCTION`

Liver is the most important glandular organ, which plays a pivotal role in regulating, synthesizing, and restoring the various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles. Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages. In general, liver is the organ, which has the capability to regenerate itself. Inspite of tremendous advances in modern medicine, there are no effective drugs available that stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells. In absence of reliable liver protecting drugs in modern medicine, there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders and their usage is in vogue since centuries and are quite often claimed to offer significant relief (Shanmugasundaram P and Venkataraman S, 2006).

Cucumis melo Linn. (Cucurbitaceae) also called as musk melon fruits have been used, traditionally in Indian traditional system of medicine, for the treatment of various disorders such as liver tonic, cardio protective, antidiabetic, antiobesity, etc. (Jayant S. Bidkar *et al.*, 2012). It is an annual plant, herbaceous, prostrates, creeping and its fruit is as large as 2-40×8-24 mm, it is ellipsoid and yellow and dark green veined with bitter pulps (Nunez-Palentus HG *et al.,* 2008). The plant is a long trailing annual vine; it grows in sandy areas and also near river banks. It is a good source for appetite, weight loss, urinary tract infections, constipation, acidity, and ulcers (Desai B.B *et al.,* 2004). It is spreading, annual, more or less hairy vine. Its sprawling branches produce broad green leaves, bright yellow flowers and tendrils. Seeds are whitish of buff, flat, smooth, 5-15 mm long. Seeds have a unique aroma composed of fatty acids, carotenoids, amino acids and terpenes, steroidal glycosides, flavonoids. Musk melon is recommended for treatment of cardiovascular diseases, anti-tussive, stomachic, as a vermifuge, as analgesic and anti-inflammatory (Parle Milind and Singh Kulwant, 2011).It is an important vegetable crop that is widely cultivated in South East Asia, China, East Africa and throughout the tropical and subtropical regions (Yadav RC *et al.,*1996). The flesh fruit is a significant source of carbohydrates and water and the seeds are rich in oil and protein (Martyn RD, Miller ME, 1996). Melons are also considered an important source of ascorbic acid, folic acid, and potassium (Richter H, 2000).

MATERIALS AND METHODS

Plant material

The fresh trailing herb with fruits of *C.melo* Linn was collected from Ranga reddy district (T.S.) in the months of July-August 2015. The trailing herb was authenticated by Dr. K. Madhava Chetty (Assistant Professor, S.V. University, Tirupati, India) through comparing morphological features. The herbarium of the plant specimen (Voucher specimen number-997) was deposited at KVK College of pharmacy. They were peeled off mechanically and air dried under shadow and then grounded into coarsely powdered in grinder and powder material was passed through 120 mesh to remove fine powders and coarse powder was used for extraction.

Extraction of the Plant Material

The collected fruits were cut into pieces, shade-dried at room temperature and powdered. The coarse powder of CMF (100 gm) was extracted by using successive soxhlet extraction using solvents of varying polarity such as petroleum ether (60-80ºc), chloroform, methanol, distilled water (8.2, 10.1, 4.2, 8.5g respectively) for 72 hrs. After completion of extraction, solvent was distilled off and concentrated extract was air-dried (Mukherjee PK, 2002).

Phytochemical screening:

The crude extract obtained by using various solvents were analysed for alkaloids, tannins, saponins, steroids, flavonoids, and phenolic compound using standard procedure of analysis (Khandelwal KR- Kokate CK, 1996).

Acute Oral Toxicity:

The acute toxicity of methanolic extract and fractions B. lanzan fruits were determined as per OECD guideline no. 423 (OECD, 2001). Based on the cut-off Value of the median lethal dose (LD^{50}), the therapeutically effective dose was derived.

Induction of experimental hepatotoxicity

The experiment has been conducted at nishka scientific and reference laboratory, Uppal, Hyderabad. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Reg. no. 1292/PO/Re/S/09/CPCSEA).Vijaya College of Pharmacy Munaganoor (V), Via Sanghinagar (Post) Ranga Reddy (Dist.)-501511.

Rats were divided into six groups, each group consisting of six animals. (Y. Jiang, R.X. Peng *et al.,* 2004) Each 50 mg/kgbw ip of RIF + INH solutions were prepared separately in sterile distilled water 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 methanolic extract of the fruits of *Cucumis melo* 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 methanolic extract of the fruits of *Cucumis melo* 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 melo*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.

Table-1: Effect of the methanolic extract of the fruits of *Cucumis melo* on the body weight on rifampicin-isoniazid-induced hepatotoxicity in rats.

S.No.	Groups	Dose (mg/kg ip)	Body weight		
			Initial weight	Final weight	Weight gain(↑)/loss(↓)
1	Control	2 mL saline	192.54±4.31	194.99±5.34	2.45↑
2	RIF-INH	50	198.33±5.39	174.14±5.85**	24.19↓
3	Silymarin	2.5	214.68±5.85	227.56±6.83##	12.88↑
4	CMFE	100	185.54±6.34	192.39±4.68#	6.85↑
5	CMFE	250	195.15±6.34	204.64±5.88#	9.49↑
6	CMFE	500	212.63±6.84	228.33±5.92##	15.7↑

Mean ± SD (n=6). *P < 0.05; **P < 0.01, #P<0.05; ## P<0.01, ns: not significant.
Statistical analysis ANOVA followed by Dunnett's t-test.

Table 2: Effect of the methanolic extract of the fruits of *Cucumis melo* on the biochemical parameters on rifampicin-isoniazid-induced hepatotoxicity in rats.

SN	Groups	ALT(U/L)	AST(U/L)	ALP(U/L)	BILT(mg/dL)	BILD(mg/dL)	TP(g/dL)	ALB (g/dl)	Globulin (g/dl)
1	Control	35.41±0.83	26.28±0.63	190.24± 11.02	0.64±0.12	0.41±0.021	7.56±0.52	4.12±0.25	3.85±0.12
2	Silymarin 2.5 mg/kg	29.63± 1.84##	32.63± 1.52#	181.70± 2.89#	0.97± 0.02#	0.60±0.17#	7.96±0.53 ns	4.03±0.62	3.56± 0.15#
3	RIF-INH 50 mg/kg	162.40± 3.70**	145.52± 3.65**	264.15± 10.63**	3.84± 0.84**	1.96± 0.038**	6.12± 0.35**	3.65±0.56	2.36± 0.12*
4	CMFE 100mg/kg	68.36± 4.56#	82.15± 1.42#	193.52± 8.29#	1.96± 0.89#	0.85±0.021	6.58±0.13	4.29±0.35	3.45±0.36
5	CMFE 250mg/kg	27.23± 4.63##	40.26± 1.89#	162.86± 2.96#	1.03±0.56	0.70±0.011	7.13± 0.72#	4.21±0.53	3.42±0.25
6	CMFE 500mg/kg	21.25± 2.12##	28.96± 1.96##	152.52± 8.14#	0.69± 0.031##	0.39± 0.032#	8.33± 0.52#	4.87±0.34	3.25± 0.65#

Values are Mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett's 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: Effect of the methanolic extract of the fruits of *Cucumis melo* 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	2mLsaline	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	50	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 melo	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±0.019ns	2.16±0.014	0.493±0.016ns	0.129±0.054#	2.671±0.016ns	19.14± 0.53ns
	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	2.51.13± 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.

Table-4: Phytochemical screening of different extract the fruits of *Cucumis melo*

S.No.	Chemical constituents	Chloroform extract	Methanolic extract	Aqueous extract
1	Carbohydrates	-	++	++
2	Tannins	+	++	++
3	Alkaloids	-	-	-
4	Saponins	-	++	++
5	Flavonoids	-	+	+
6	Glycosides	+	+	-
7	Steroids	++	++	+
8	Amino acids	-	+	+
9	Proteins	-	+	+

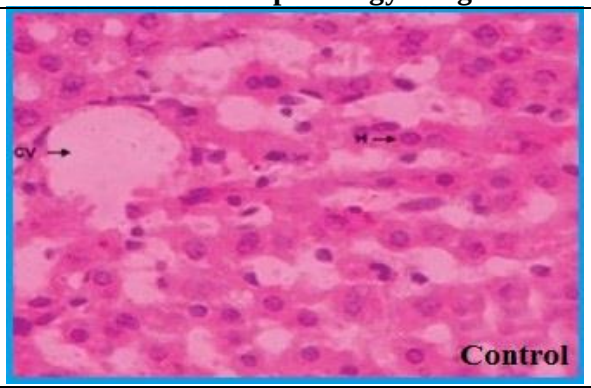
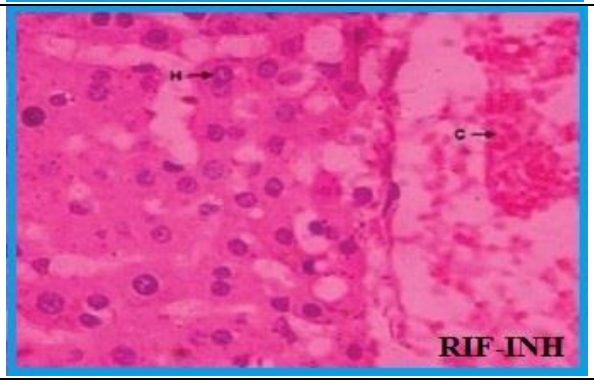
(+) Presence, (-) Absence, (++) Maximum presence of Chemical constituents.

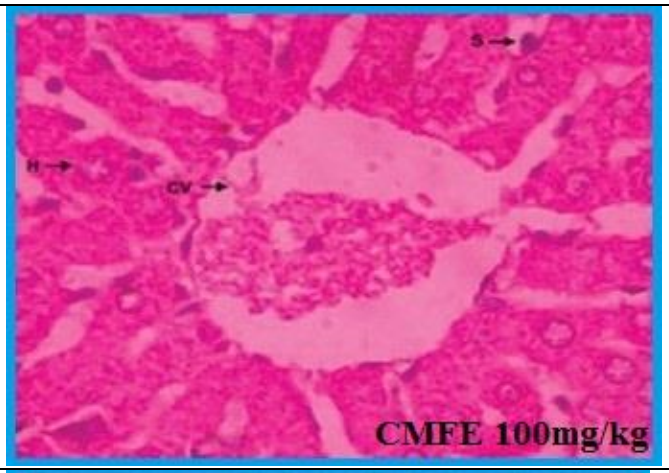
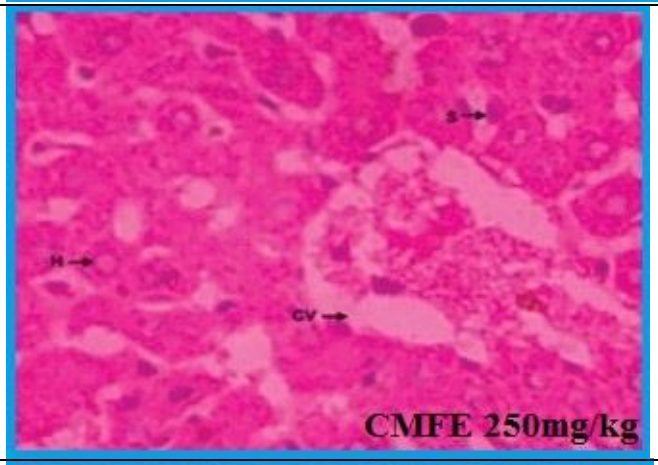
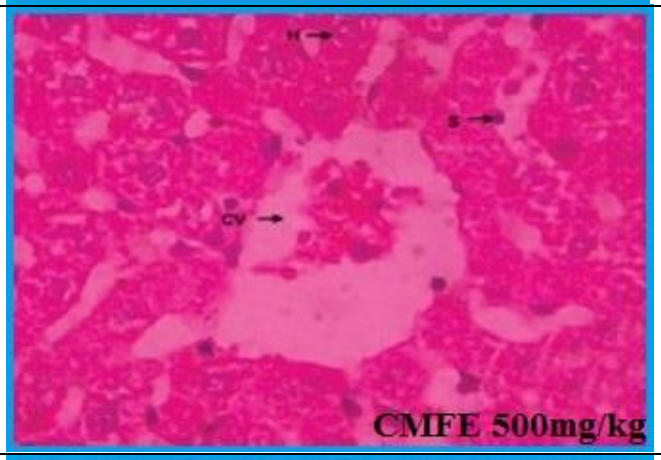
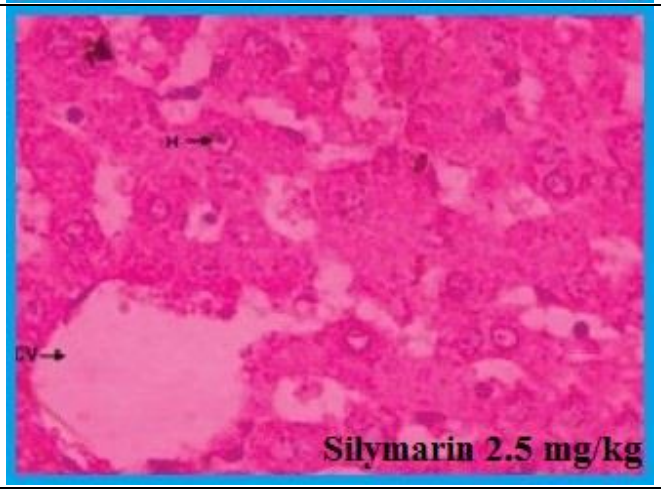
Assessment of liver damage

Liver damage was assessed by the estimation of serum activities of Serum alanine aminotransferase (ALT), Serum aspartate aminotransferase (AST), Serum alkaline phosphatase (ALP), Serum total bilirubin (BILT), and Serum total proteins (TP), albumin(ALB), and globulin (GLO)according to the method by using commercially available test kit.(S Reitman *et al.,* 1957- P.R. Kind *et al.,* 1954- J.P. Persijn *et al.,* 1976- L.Jendrassik, 1938- O.H. Lowry *et al.*, 1951- A.E. Pinnel *et al.*, 1978- E.H. Coles, 1974). Lipid peroxidase (LPO) (H. Ohkawa *et al.*, 1979) glutathione peroxidase (GPx) (J.T. Rotruck, 1973) glutathione reductase (GRD) (J. Mohandas *et al.*, 1984) superoxide dismutase (SOD) (S. Rai *et al.*, 2006) catalase (CAT) (H. Aebi , 1984) and reduced glutathione (GSH) (G.L. Ellman, 1959) were estimated in liver homogenate.

Histopathology studies

At the end of the treatment, rats were sacrificed and 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, Auto technique) 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. Results were expressed as mean±S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall P-value was found statistically significant ($P < 0.05$), further comparisons among groups were made according to post hoc Tukey's test. All statistical analyses and the diagrammatic representation of the data were performed by using Graph pad PRISMsoftware.

S.No	Groups	Histopathology Images
1	Control:- the control showed normal gross appearance; dark maroon colour 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	
2	RIF-INH :- 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.	

3	CMFE 100mg/kg:- RIF + INH and low dose (100 mg/kgbw ip) of the methanolic extract of the fruits of *Cucumis melo* showed minimal necrosis, mild inflammation and less steatosis	
4	CMFE 250mg/kg:- RIF + INH and moderate dose (250 mg/kgbw ip) of the methanolic extract of the fruits of *Cucumis melo* showed slight recovery and evidence of regeneration in some hepatocytes	
5	CMFE 500mg/kg:- RIF + INH and high dose (500 mg/kgbw ip) of the methanolic extract of the fruits of *Cucumis melo* showed significant recovery showing absence of necrosis, space formation and vacuoles.	
6	Silymarin 2.5 mg/kg:- RIF + INH and silymarin (2.5 mg/kg ip) showed normal liver architecture and occasional inflammatory cells with no traditis or necrosis.	

CM - *Cucumis melo*, RIF-Rifampicin, INH - Isoniazid, CV - Central vein, H - Hepatocyte, S - Space formation.

Figure 1:-Histopathological studies of liver

RESULTS AND DISCUSSION

The methanolic extract contains flavonoid was proved by chemical test and TLC. In UV chamber the compound shows pink fluorescent indicating the presence of flavonoids rich in the extract. During the metabolism of INH, hydrazine is produced directly (from INH) or indirectly (from acetyl hydrazine). From earlier study (Garner P *et al.*, 2004) it is evident that hydrazine play a role in INH induced liver damage in rats, which is consistent with the report by Sarich *et al*. (Sarich TC *et al.*, 1996) The combination of INH and RIF was reported to result in higher rate of inhibition of biliary secretion and an increase in liver cell lipid peroxidation, and cytochrome P450 was thought to be involved the synergistic effect of RIF on INH (Skakun NP *et al.*, 1985). However, its role in INH induced hepatotoxicity is not clear, because, INH itself is an inducer of CYP2E1 (Ramaiah SK *et al.*, 2001). INH itself does not produce complete damage to the liver (Yasuda K *et al.*, 1990- Wu JC *et al.*, 1990). INH is metabolized in the liver primarily by acetylation and hydrolysis, and these acetylated metabolites are thought to be hepatotoxins (Steele MA *et al.*, 1991- Peretti E *et al.*, 1987). Previous report in rats suggest that the hydrazine metabolite of INH and is subsequent effect on CYP2E1 induction is involved in the development of INH-induced hepatotoxicity and also oxidative stress as one of the mechanism for INH+RIF induced hepatic injury (Peretti E *et al.*, 1987).

The more commonly measured enzymes are Serum alanine aminotransferase (ALT), Serum aspartate aminotransferase (AST), Serum alkaline phosphatase (ALP), Serum total bilirubin (BILT), Serum total proteins (TP)albumin(ALB), and globulin (GLO). Although there will be an increase of AST and ALT in heart and liver diseases; total bilirubin a by-product 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. (K.G. Rajesh *et al.*, 2005). 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 ALT, AST, ALP, BILT, TP, ALB and GLO as well as the levels of liver homogenates, LPO, GPx, GRD, SOD, CAT, and GSH and also liver histopathology. Table-1 & 2 shows the levels of ALT, AST, ALP, BILT, TP, ALB and GLOin the serum and bodyweight. There was a significant increase in the levels of ALT, AST, ALP, BILT, TP, ALB and GLO 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 methanolic extract of the fruits of *Cucumis melo* caused a significant reduction in the levels of enzymes leading to a significant reversal of hepatotoxicity.

Table-3 shows the levels of LPO, GPx, GRD, SOD, CAT, and GSH in the liver homogenate. The level of lipid peroxide sharply increased. 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 melo* 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, silymarin, in the case of LPO and GRD. The protective effect was dosedependent. The hepatoprotective role of the methanolic extract of the fruits of *Cucumis melo* might be due to the antioxidant potential of the drugs (A. Balakrishnan, *et al.*, 2011).

The methanolic extract of the fruits of *Cucumis melo* improved liver function by decreasing the serum enzymes ALT, AST, ALP, BILT, TP, ALB and GLO. However, the levels of GPx, GRD, SOD, CAT, and GSH are increased. This indicates the protective effect over liver and improvement in its functional efficiency.

CONCLUSION

Anti-TB drugs cause significant Hepatotoxicity. The present study proves that the methanolic extract of the fruits of *Cucumis melo*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. Hence the hepatoprotective effect of the extract may be due to the presence of one or more phytochemical constituents present in the *Cucumis melo* which scavenged the free radical offering hepatoprotection. Thus the methanolic extract of the fruits of *Cucumis melo*which are useful in controlling hepatic injury in drug induced hepatotoxicity. Isolation and characterization of the active principles may yield good hepatoprotective drugs.

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