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Research Article

Protective Effect of *Momordica Charantia* Against Hepatic Ischemic Reperfusion Injury Model in Rats

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Abstract

Protection from the effects of ischemia and reperfusion injury (I/R) posesa serious problem in liver transplantation and resection surgery. Liver ischemia is a common cause of frequent clinical complications i.e.hepatocytes death, liver failure, and liver graft rejection. Oxidative stress playsa key role in hepatic I/R. *Momordica charantia* L. (Cucurbitaceae) is a strong antioxidant countering oxidative stress. This study was carried out to evaluate the effect of *Momordica Charantia* (MC) fruit extract on hepatic ischemic reperfusion injury in rats.Adult Wistar albino rats underwent 45 min of ischemia followed by 3 h of reperfusion by ligation of hepatic artery and left portal vein. *Momodica charantia* fruit extract was administered once daily for 4 days at a dose of 200mg/kg. Allopurinol (50mg/kg) was used as standard drug. After four days of drug administration, surgery was done on the fifth day and serum and liver samples were collected from all experimental animals after surgery. Biochemical parameters like SGOT (serum glutamic oxaloacetic transaminase), SGPT (serum glutamic-pyruvic transaminase), ALP (alkaline phosphatase), LDH (lactate dehydrogenase) and CRP (C-reactive protein) and various antioxidant parameters were measured from serum sample and liver homogenate, respectively. MC significantly ($p<0.05$) reversed I/R induced changes in liver damage marker -SGOT, SGPT and ALP, tissue damage marker LDH and inflammatory marker CRP. Changes in level of antioxidant parameters were also reversed by MC.MC possess protective effect against ischemic reperfusion injury that can be attributed to its antioxidant activity.

Keywords: Liver; Allopurinol; Oxidative stress; Inflammation; Liver markers

Introduction

The largest gland in the body, the liver, weighs around 1-2.3 kg. The cardinal physiological roles of liver include carbohydrate, fat and protein metabolism, breakdown of erythrocytes, defense against microbes, detoxification of drugs and noxious substances, inactivation of hormones, synthesis of vitamin A from carotene and secretion of bile [1].

Several diseases associated with liver are cholestatic liver diseases, primary biliary cirrhosis, primary sclerosing cholangitis, acute fulminant liver failure, alcoholic liver disease, autoimmune chronic hepatitis, biliary atresia (paediatric), chronic viral hepatitis, cryptogenic cirrhosis, hepatic malignancy, and metabolic disease [2]. Medical treatment of liver diseases and liver damage is always of prime importance. When drug therapy can no longer support life, a liver transplant is the ultimate option for end stage liver disease. Chronic viral hepatitis, alcoholic cirrhosis, and chronic cholestatic diseases are the most common liver diseases that require transplantation.

Liver Ischemia-Reperfusion (I/R) injury is a significant cause of morbidity and mortality in two principal settings. Firstly, it occurs in major liver resections [3] and transplantation [4,5] where anoxic or ischemic liver injury takes place. Secondly, it happens as a consequence of systemic hypoxia or with conditions that cause low blood flow to the liver resulting in insufficient perfusion. The latter occurs in hemorrhagic, cardiogenic, or septic shock with subsequent fluid resuscitation [6], in cardiovascular surgery with extracorporeal circulation [7], in laparoscopic surgery [8,9] and in abdominal compartment syndromes [10]. In the field of liver transplantation, I/R injury is closely related to the development of primary graft non-function (occurs in 5% of grafts) and primary graft dysfunction (occurs in 10-30% of grafts) [11]. Both conditions are associated with high rates of morbidity and mortality. I/R injury increase the incidence of subsequent graft rejection [12].

Liver injury induced by I/R is caused, by Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). There is evidence that during hepatic I/R there is generation and release of ROS and RNS with concomitant consumption of endogenous antioxidants and apoptotic or necrotic cell death [13-17]. Although the exact sources of ROS generation in liver I/R are still under investigation, the nicotinamide adenine dinucleotide phosphate oxidase (NADP), the xanthine/ xanthine oxido reductase system, and the mitochondria have been suggested to play key roles [18]. Administration of free radical scavenging agents that enhance the endogenous antioxidant system could reduce the post-ischemic tissue injury and so it can be useful in clinical settings against hepatic I/R damage [19].

The antioxidants with beneficial effect in hepatic ischemic reperfusion injury are: α-tocopherol [16], α-tocopherol/ascorbate [20], GSH [21], SOD derivatives [22], lipoic acid [23,24] and carvacrol [25]. Preconditioning is also another approach used in the patient prior to liver transplantation and resection. Ischemic preconditioning

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involves ischemia and reperfusion for a short period of time before exposure to prolonged ischemia and reperfusion.

Momordica charantia as an antioxidant is a strong free radical scavenger and also has hepatoprotective property [26]. Therefore, this study was carried out to evaluate the effect of *Momordica charantia* in hepatic ischemic reperfusion injury.

Materials and Methods

Collection of plant material

Fresh unripe fruits of *Momordica charantia*L. (Cucurbitaceae) were collected in the morning from the local market of Anand, Gujarat, India and were then authenticated by Dr. A. S. Reddy, B. R. Doshi School of Biosciences, Sardar Patel University, Bakrol, Anand, Gujarat, India. (Herbarium specimen no: APCH34).

Extraction procedure

The fruits of *Momordica charantia* were air-dried and fine powdered, then extracted by repeated percolation which lasted about 6-8 h with hexane under reflux in Soxhlet extraction apparatus [27]. At the end of extraction process, the solvent was then evaporated and the remaining mass was measured. Extracted mass was stored at room temperature and labeled as **MC**. Extraction yield was 12.5%w/w. Pure powder of Allopurinol was obtained from Zydus Cadila Healthcare Ltd., Ahmedabad, Gujarat, India. Drug solutions were prepared freshly every day by suspending the drug in 0.5% Carboxymethyl Cellulose (CMC) suspension. All the chemicals used were of analytical grade and were obtained from Astron chemicals, Ahmedabad and SD fine chemicals, Mumbai. All the biochemical tests were performed using the standard reagent kits purchased from Coral Clinical Systems, Goa, India.

Experimental procedure

Wistar rats of either sex 200-250g obtained from Animal house of Anand Pharmacy College, Anand, were used as experimental animals for the study. The animals were housed in a group of 3 rats per cage under well-controlled conditions of temperature (22 ± 2°C), humidity (55 ± 5%) and 12hrs/12hrs light-dark cycle. Animals had free access to conventional laboratory diet (Pranav Agro Industries, Delhi, India) and R.O. water *ad libitum*. The protocol-APC/2012-IAEC/1211 of the experiment was approved by Institutional Animal Ethics Committee (IAEC) as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Animals were randomly allocated in six groups, with n=6 animals in each group, as follows: Group I Normal Control, Group II Model Control, Group III Sham operated, Group IV Vehicle control, Group V Standard Control, Group VI *Momordica charantia* 200 mg/kg (MC 200). Surgery was performed in Group II-VI. In Group III Sham surgery was performed in the absence of clamping of the vessels. Group IV received 0.5% CMC suspension (1ml/kg, i.p.) for 4 days, then surgery was performed on the fifth day. Group V and VI received Allopurinol 50mg/kg and *Momordica charantia* 200mg/kg respectively for 4 days then surgery was performed on fifth day. For induction of I/R animals were fasted for 12 h before experiments. Anesthesia was induced with phenobarbitone (10mg/kg; i.p) and body temperature was maintained throughout surgery at 37±0.5°C with a lamp. The left branches of portal vein and hepaticartery were clamped by vascular clamp to induce complete ischemia of the median and left hepatic lobes. Sham surgery did not involve clamping of the vessels. After 45 min of ischemia, reperfusion was allowed for 3 h by removing the clamp. Blood samples were collected after anesthesia just before sacrificing and liver was rapidly removed for further analysis.Serum samples were separated by centrifugation (Plastocrafts Rota 4R-V/FA) at 3000 rpm for 15 min and stored at –20°C until the analysis was carried out.

Table 1: Effect of Momordica charantia on Liver injury maker and CRP levels.

Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	LDH (U/L)	CRP (mg/dl)
I	50.34±0.03	38.02±0.22	42.38±0.88	254.54±0.11	0.35±0.21
II	353.08±0.07#	343.18±0.08#	257.34±0.09#	764.07±0.05#	8±0.33#
III	64.40±0.1	52.96±0.31	70.53±0.25	288.72±0.12	0.6±0.89
IV	333.09±0.03	331.06±0.09	244.8±0.05	750.58±0.55	7.2±0.34
V	97.87±0.12**	82.54±0.01**	78.18±0.22**	327.84±0.41**	4.4±0.78*
VI	121.05±0.16**	105.82±0.13**	94.40±0.03**	353.03±0.37**	2.15±0.91**

* significantly different from model at p <0.05
** significantly different from model at p <0.01
# significantly different from normal at p <0.01

SGOT, SGPT, ALP, LDH and CRP were estimated by spectrophotometry method by using standard kit.Antioxidant parameters- Malondialdehyde (MDA), total Glutathione (GSH), Superoxide dismutase (SOD) and Catalase (CAT) were measured in liver homogenate.

Statistical Analysis

Results represented are mean ± SEM. Statistical difference between the means of the various groups were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test with p value <0.05. Statistical analysis was done using GraphPad Prism software, version 5.03.

Results

Levels of SGOT, SGPT, ALP,LDH and CRP were found to be significantly (P<0.01) higher in model control animals as compared to normal control animals.Treatment with Allopurinol (50mg/kg) significantly (P<0.01) prevented the rise in SGOT, SGPT, ALP,LDH and CRP levels as compared tomodel controlgroup. Similarly, *Momordica charantia* (200mg/kg) significantly (P<0.01) prevented the rise in SGOT, SGPT, ALP, LDH and CRP levels as compared to

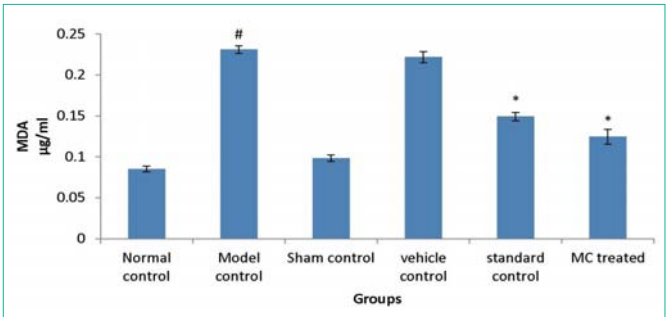

Figure 1: Effect of *Momordica charantia* extract on MDA level in I/R. values represented are mean±SEM. Statistical significance was set at p<0.05.
#significantly different from normal group at p<0.05
*significantly different from model group at p<0.05

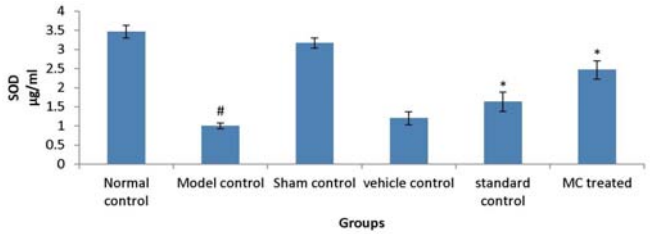

Figure 2: Effect of *Momordica charantia* extract on Catalase level in I/R. values represented are mean±SEM. Statistical significance was set at p<0.05. #significantly different from normal group at p<0.05 *significantly different from model group at p<0.05

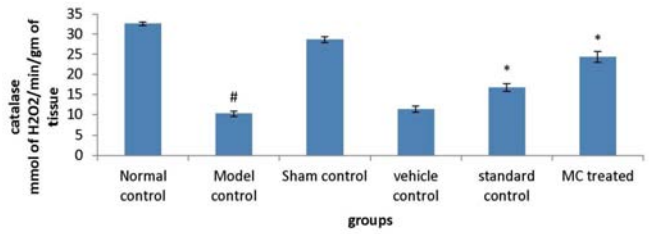

Figure 3: Effect of *Momordica charantia* extract on SOD level in I/R. values represented are mean±SEM. Statistical significance was set at p<0.05. #significantly different from normal group at p<0.05 *significantly different from model group at p<0.05

model control animals as shown in Table 1.

MDA level in liver homogenate of model control animals was significantly higher as compared to normal control animals. Treatment with Allopurinol (50mg/kg) and *Momordica charantia* (200mg/kg) significantly (P<0.05) prevented the rise in MDA levels as compared to model control group as shown in Figure 1.

Decreased levels of CAT, SOD and GSHas depicted by the model control group, were significantly (P<0.05) increased in treatment control group compared to the model group as shown in Figure 2, 3 and 4 respectively.

Discussion

Protection of liver function from the effects of I/R injury is a serious problem in transplant and resection liver surgery. Although ischemia can damage cells directly, liver cells have defense mechanisms to protect against such insults if the ischemic time is relatively brief [28]. However, if the liver cells survive even after the ischemic insult, reintroduction of blood flow (reperfusion) often leads to cellular damage [29]. Reperfusion of previously ischemic hepatic tissue initiates the complex cellular events that eventually results in necrosis and apoptosis of liver cells [30].

Currently available therapy for management of hepatic ischemic reperfusion injury is antioxidant and preconditioning [18]. But it has certain drawbacks like antioxidants are only scavenger of free radical and they do not possess any specific effect on inflammatory mediators. In case of preconditioning some studies suggest that it is not successful in all patient and other strategies are required for older patient. Generation of ROS in the reperfusion phaseplays key role in the pathogenesis of I/R [31].

Momordica charantia, a well-known herbal drug, is strong free

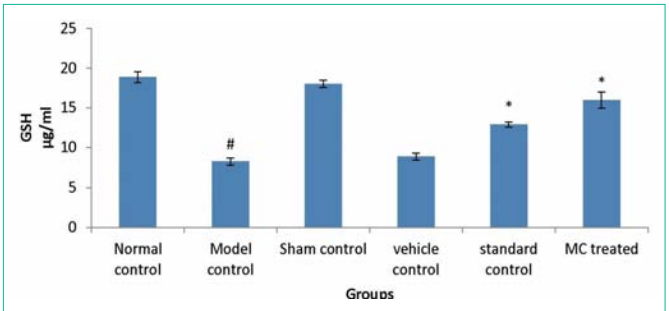

Figure 4: Effect of *Momordica charantia* extract on GSH level in I/R. values represented are mean±SEM. Statistical significance was set at p<0.05. #significantly different from normal group at p<0.05 *significantly different from model group at p<0.05

radical scavenger and also possess hepatoprotective property [27]. Therefore the present study was carried out to access protective role of *Momordica charantia* in I/R injury in rats.

It is a very well established fact that the extent of hepatic damage can be assessed by quantifying the activities of serum transaminase: SGPT, SGOT and alkaline phosphatase. ROS generation has been proposed to be a contributory factor to cellular mechanisms of inflammation and I/R injury [32]. ROS damage the liver cell by various mechanisms. When liver cells are damaged, the levels of SGOT, SGPT, ALP and LDH are increased after I/R injury [33]. Present result support these facts as level of SGOT, SGPT, ALP and LDH were increased in model control group with comparison to normal control animals. Treatment with MC (200mg/kg) prevented these change in SGOT, SGPT,ALP and LDH level as compared to model control animals.

C - reactive protein (CRP) is an acute phase protein produced by the liver in response to inflammation, infection and tissue injury. I/Rcause activation of kupffer cell. Activated kupffer cell (KCs) are thought to mediate hepatocytes injury via the production of TNF-alpha, IL-6 and ROS [34]. IL-6 induces CRP production in the liver by activating Janus kinases [35]. The estimation of CRP revealed significant elevation in CRP level in model control group as compared to normal control group while treatment with MC (200mg/kg) reversed these change in CRP level as compared to model control animals.

Hepatic ischemic reperfusion injury results in increased lipid peroxidation by the induction of free radical production. Malondialdehyde, an end product of lipid peroxidation, is extensively used as a biomarker of lipid peroxidation. Model control animals showed higher Malondialdehyde (MDA) levels compared to the normal control animals as a result of lipid peroxidation. Animals treated with MC (200mg/kg) tend to produce a decline in the MDA levels as compared to model control animals.

In present study, the results suggested that SOD, CAT and GSH level were markedly decreased in I/R injury compared to normal control and sham control which might be due to excess availability of O_2^- and H_2O_2 in the biological system, which in turns generate OH^- resulting in the propagation of lipid peroxidation, which increases the MDA content and which might be responsible for the cellular damage in ischemic rat liver. These changes were significantly prevented by *Momordica charantia*. *Momordica charantia*possess protective effect

against ischemic reperfusion injury that can be attributed to its antioxidant activity.

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