Preventive effects of Turmeric (*Curcuma longa* Linn.) Powder on hepatic steatosis in the rats fed with high fat diet

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Abstract: The aim of the present study was to evaluate the preventive effects of Turmeric (Curcuma Longa Linn.) powder on rat high fat diet-induced hepatic steatosis. For this purpose, male Wistar rats were treated in 4 experimental groups including: 1-healthy control group given standard diet, 2- high fat diet group for induction of hepatic steatosis, 3- high fat diet plus Clofibrate (320 mg/kg) as positive control, and 4- high fat diet plus Turmeric powder (5%) for protection of liver steatosis, at a period of 6 weeks. At the end of experiment, the groups were compared considering serum lipid profile, serum biomarkers of liver tissue injury and liver histopathological changes. The lipid peroxidation product and the activities of antioxidant enzymes were measured as indicators of antioxidation in liver tissue. After 6 weeks, high fat diet caused deleterious metabolic effects, including hypertriglyceridemia, hypercholesterolemia and liver dysfunction. Rats fed high fat diet alone showed increased activities of hepatocellular enzymes in plasma, significant decline in antioxidants, and elevated lipid peroxidation indices in liver. Turmeric treatment significantly reduced elevated markers of liver tissue injury and lipid peroxidation product (MDA), and brought back the liver antioxidants and the over accumulation lipids in serum towards normal. Histopathology of the liver confirmed the changes induced by high fat diet and the hepatoprotective effect of Turmeric powder. The results obtained showed turmeric powder exerts protective effects against hepatic steatosis in rats fed with high fat diet possibly through its antioxidant actions.

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***Key words*:** High fat fed diet, Turmeric (*Curcuma longa* Linn.), Hepatic steatosis.

Abbreviations: MDA, Malondialdehyde; SOD, Superoxide dismutase; CAT, Catalase; GPX, Glutathione peroxidase; GR, Glutathione reductase; Alb, Albumin; TP, Total protein; AST, Aspartate aminotransaminase; ALT, Alanine aminotransaminase; LDH, Lactate dehydrogenase; GSSG, Oxide glutathione; GSH, Reduced glutathione; TB, Total bilirubin.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver injury in many countries around the world. It has a broad pathologic spectrum which ranges from simple fatty infiltration of the liver or steatosis, to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis and to liver failure (Assy et al., 2000). Nonalcoholic fatty liver disease (NAFLD) is now recognized as the most common type of liver disease and might lead to important public health problems (Clark et al., 2002).

Triglycerides and cholesterol are of important biological lipids of body that excess get them through the diet is resulted in hypertriglyceridemia (Hokanson, 2002; Kametani et al., 2002) and hypercholestrolemia (Walldius et al., 2004). NAFLD is diagnosed by accumulation of triglycerides in the hepatocytes in consequence of the esterification of free fatty acids and glycerol. Increase in free fatty acids in the liver is driven from three separate sources includes lipolysis (hydrolysis of glycerol and fatty acid from triglycerides) in adipose tissue, high fat diet and de novo lipogenesis (Postic and Girard, 2008). In contrast, fatty acids may use through β-oxidation, de novo esterification to triglycerides and store as fat droplets or excretion in the form of VLDL. Thus, accumulation of fat in the liver can occurs in results of increase the synthesis of fat, reduce in fat excretion or reduce in them oxidation. Donnelly et al. (2005), showed that 60% of liver triglyceride content is driven from influx of fatty acids from adipose tissue, 26% from de novo lipogenesis, and 15% from the diet. Nonalcoholic fatty liver is associated with some histopathologic changes, which is different from steatosis to cirrhosis (Dixon et al., 2001; Angulo and Lindor, 2002; Clark et al., 2002; Farrell, 2003). It was formerly believed that steatosis is a simple phenomenon and has no complications. However, nowadays it is known that fatty liver is vulnerable to factors such as oxidative stress and can lead to Steatohepatitis, which is

associated with necrosis, inflammation, fibrosis and cirrhosis (James and Day, 1999; Orrenius et al., 2007). In the pathogenesis of nonalcoholic steatohepatitis is assumed that the accumulation of triglycerides in the liver or steatosis will yield to increases the susceptibility of liver to the damages caused by inflammatory cytokines and lymphokines, mitochondrial dysfunction and oxidative stress (Day, 2006; Day and James, 1998). Barbuio et al. (2007), showed that oxidative stress is effective in alteration of steatosis to steatohepatitis. However, although liver steatosis may lead to complete hepatic failure, but appropriate and ideal treatment is not established (Angulo and Lindor, 2002). Biological materials with plant origin forms modern branch pharmacotherapy of disease. Although various pharmacologic agents exist to treat various diseases, but most patients cannot tolerate the side effects of chemical drugs from one hand and plants have very few side effects on patients from other hands. Obviously, it is necessary that several studies must be done on the new drugs in several stages before their entrance to the field of medicine. Turmeric, the powdered rhizome of the plant Curcuma longa L. has been extensively used as colouring agent, a spice and in the treatment of infammatory conditions and other diseases (Govindrajan, 1980; Ammon and Wahl, 1991). Curcumin (diferuloylmethane), the major pigment and phenolic compound in turmeric has also been shown to possess both anti-infammatory (Srimal and Dhawan, 1973) and anti-oxidant properties (Sharma, 1976; Sreejayan and Rao, 1994). Dietary administration of turmeric or curcumin or ethanolic turmeric extract (ETE) has been shown to inhibit tumor induction by diverse carcinogens in various organs of mice (Nagabhushan and Bhide, 1987; Azuine and Bhide, 1992; Huang et al., 1994; Deshpande et al., 1997) and rats (Rao et al., 1995). Curcumin has been shown to reduce the hyperlipidaemia (Babu and Srinivasan, 1997), delay the development of cataract (Suryanarayana et al, 2005), ameliorate renal lesions (Babu and Srinivasan, 1998) and reduce cross-linking of collagen (Sajithlal et al., 1998) in a streptozotocin-treated diabetic animal model. Curcumin has also been shown to lower blood glucose levels in type 2 diabetic KK-Ay mice (Nishiyama et al., 2005) and streptozotocin-treated rats (Mahesh et al., 2005). Turmeric/alcoholic extract of turmeric/curcumin have not shown any toxic effects (even at high doses) in the acute and/or subchronic toxicity studies in rats, dogs, guinea pigs and monkeys (Wahlstrom and Blennow, 1978; Bhavani shankar et al., 1980; Sambaiah et al., 1982).

However, among the various protective mechanisms, the antioxidant activity of turmeric is considered responsible for its pharmacological effects. By consideration of antioxidant and hypolipidemic activity of turmeric, this matter it will probably be able to protect the liver from steatosis.

To our knowledge, no other biochemical investigations have so far been carried out concerning the effect of turmeric powder on the liver steatosis in high fat diet fed-rats are available in the literature. On the other hand, animal models of liver steatosis and dyslipidemia are valuable for studying the pathogenesis and treatment of steatohepatitis as well as its relationship to metabolic syndrome. Therefore, present study examined the hypothesis that turmeric supplementation prevents liver steatosis in a high fat diet model. The results of this study demonstrate that turmeric supplementation prevents liver steatosis and decreases oxidative stress in hepatocytes exposed to high levels of lipid.

2. Materials and methods

This study carried out during 2012 in the research center of Islamic Azad University. All procedures were conducted under supervision of Animal Rights Monitoring Committee of Islamic Azad University Research Center.

2.1. Animals

Forty male Wistar rats, weighted 180±20 gr and aged 10 weeks old were obtained from the animal breeding center of Islamic Azad University. The rats were divided into 4 equal groups of 10 animals including: 1- normal control, 2- normal rats fed high-fat diets, 3- normal rats fed high-fat diets plus Clofubrate (320 mg kg^{-1}/day) and 4- rats which are fed high-fat diets plus turmeric powder (5% turmeric diet). Management and husbandry conditions were identical in all groups with 12/12 h light/dark cycle at 21 ± 2°C. Food and water were provided ad libitum.

2.2. Experimental plan

In rats were fed with high-fat diets used of high-fat emulsion, which its formula is mentioned in table 1, to induce hepatic steatosis based on Zou et al., (2006) method. All treatment groups received high-fat emulsion at the dose of 10 ml kg-1 daily at morning 8 o'clock for 6 weeks. Simultaneously, control group received normal saline in same dosage. In groups 4 beside of high-fat emulsion, turmeric powder weighed and added to the preweighed standard laboratory diet and thoroughly mixed up to 5% concentration. Group 3 beside of high-fat emulsion received Clofubrate at the dose of 320 mg kg^{-1}/day through gavage as suspension in the 2 ml kg^{-1} methylcellulose 0.5% (Sheng, et al 2006). Control group received 2 ml kg^{-1} methylcellulose 5%.

2.3. Biochemical factors evaluation

At the end of the experiment, blood samples were collected from the retro-orbital plexus and the sera prepared through centrifuging at 2500 ×g for 15

minutes at 30°C. After 12 hours fasting, blood glucose and serum biomarkers of liver function including ALT, AST (Reitman and Frankel, 1957), LDH (Martinek, 1972), albumin, TP (Lowry et al., 1951) and total bilirubin (Malloy and Evelyn, 1937) were measured using commercially available kits.

Table 1: Composition of high-fat emulsion gavaged to rats

Constituents	Amount
Corn oil	400 g
Sacarose	150 g
Milk powder	80 g
Cholesterol	100 g
Sodium deoxy colat	10 g
Tween 80	36.4 g
Propilen glikol	31.1 g
Multi vitamin	2.5 g
Salt	10 g
Minerals	1.5 g
Normal saline	300 ml

2.4. Measurement of antioxidant activity

All experimental rats were euthanized by cervical dislocation. The rat's Liver were removed immediately and washed in normal saline and homogenate 10% prepared in 1.15% w/v of potassium chloride. The homogenate was centrifuged in 7000 ×g for 10 minutes at 4°C and supernatant were used for measurement of Oxidative stress by determination of malondialdehyde (MDA) as well as antioxidant enzymes (AOE) such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-PX) and glutathione reductase. MDA, SOD, CAT and GSH-PX, GR were measured by using commercially available kits according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Liver homogenate MDA levels were expressed as nmol MDA per mg protein and antioxidant activity was expressed as arbitrary units per mg protein.

Degree of lipid peroxidation in kidney tissue homogenates was determined in terms of thiobarbituric acid reactive substances (TBARSs) formation by following the protocol of Esterbauer and Cheesman (Esterbauer and Cheesman, 1990). SOD activity was measured by Nishikimi method (Nishikimi et al., 1972) and was modified by Kakkar method (Kakkar et al., 1984). Each unit of SOD activity was determined as required enzyme concentration for prohibition of creation color at 1 minute, under study conditions. CAT activity was measured by Claiborne method (Claiborne, 1985) and was based on hydrogen peroxide breakdown. GPX activity was measured by Rotruck method (Rotruck et al., 1973) and was expressed as micromole of GSSG /minute/milligram of protein, based on blew reaction:

$2H_2O+GSSG \longrightarrow H_2O_2+2GSH$

GR activity was measured by Mohandas method (Mohandas et al., 1984), based on blew reaction:

$NADPH+H^+ +GSSG \longrightarrow NADP^+ +2GSH$

2.5. Microscopic studies

A small piece of hepatic tissue from the anterior portion of the left lateral lobe was removed for histological analysis. The sample was fixed by immersing it in 10% neutral-buffered formalin. The sample was then embedded in paraffin, sliced into 5 µm sections, and stained with hematoxylin-eosin for blinded histological assessment (Lee and Luna, 1968). Hepatocytes were assayed from fatty changes aspect like a mentioned method by Wang et al 2009 and steatosis were degreed from 0 to 4 (0: without steatosis, 1: <25% steatosis, 2: approximately 26-50% steatosis, 3: approximately 51-75% steatosis, 4: >76% steatosis). The stained 5 µm sections were graded as follows: 0, absent; I, minimal; II, mild; III, modest; IV, severe. The histological changes were evaluated in nonconsecutive, randomly chosen ×200 histological fields using light microscope, NIKON ECLIPSE E200 (Shen et al., 2009).

2.6. Statistical analysis

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean ± SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. $P<0.05$ was considered statistically significant.

3. Results

3.1. Effect of Turmeric powder on the biochemical parameters of liver damage caused by feeding high-fat diet

In group 2, ALT, AST, ALP and TB increased and TP and Alb decreased significantly ($p<0.01$) in compared with control group. In group 3, high levels of ALT, AST, ALP and TB significantly decreased ($p<0.01$) to normal levels and levels of TP and Alb increased to their normal boundaries. In group 4, levels of ALT, AST, ALP and TB significantly decreased ($p<0.05$) and levels of TP and Alb significantly increased ($p<0.05$) but not reached to normal levels (Table 2).

Table 2: Effect of Turmeric powder on serum biochemical parameters in hepatic steatosis consequence of high-fat diet

Groups	Biochemical parameters					
	ALT U/L	AST U/L	ALP IU/L	TB Mg/dl	Alb g/dl	TP g/dl
Control	49.51±2.31 [bd]	64.72±1.55 [bd]	187.72±9.03 [bd]	0.83±0.04 [bd]	4.42±0.44 [bd]	7.95±0.54 [bd]
High-fat diet	66.75±3.21 [acd]	89.35±2.74 [acd]	275.56±11.25 [acd]	1.31±0.08 [acd]	3.16±0.25 [acd]	5.21±0.21 [acd]
High-fat diet+ Clofubrate	50.69±2.15 [b]	64.20±1.27 [b]	199.87±7.63 [b]	0.89±0.06 [b]	4.35±0.38 [b]	7.05±0.44 [b]
High-fat diet+Turmeric 5%	58.26±2.74 [ab]	74.15±2.42 [ab]	227.43±5.82 [ab]	1.08±0.07 [ab]	3.77±0.20 [ab]	5.88±0.39 [ab]
ANOVA	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000

Values are presented as mean ± SEM for 10 rats in each group.
a, significant difference with group 1; b, significant difference with group 2; c, significant difference with group 3; d, significant difference with group 4 ($p<0.05$).

3.2. Histopathological findings

In microscopic studies no abnormalities was found in the livers of control group rats (fig 1-A). But in group 2 rats' which fed with high-fat diet for 6 weeks, sever steatosis was found as micro and macrovesicular fatty changes accompanied hepatitis (fig 1-B). Clofubrate prevented from steatosis in group 3 rats (fig 1-C). In groups 4, Turmeric powder prevented from fatty changes in hepatocytes obviously (fig 1-D).

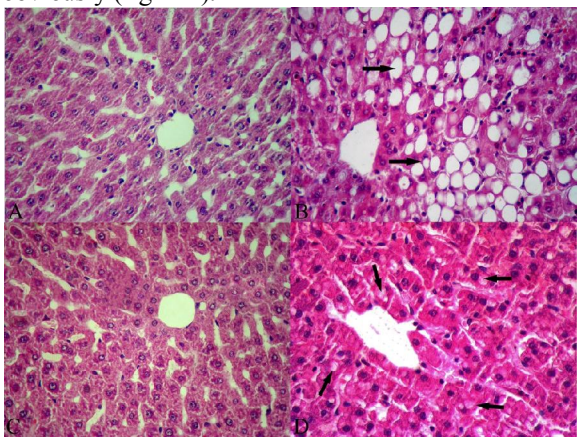

Fig 1: A, control group. **B**, high-fat diet group. **C**, high-fat diet+Clofubrate. **D**, high-fat diet+Turmeric 5%. Arrows show micro and macrovesicular lipid droplets. H&E 40×

Effect of Turmeric powder on the pathologic grading of hepatic steatosis in rats fed high-fat diet is listed in Table 3.

Table 3: Effect of Turmeric on the hepatic steatosis in rats fed high-fat diet

Groups	Hepatic steatosis grading					P
	0	1	2	3	4	
Control	10	0	0	0	0	
High-fat diet	0	0	1	2	7	a
High-fat diet+ Clofubrate	5	3	2	0	0	c
High-fat diet+Turmeric 5%	1	3	5	1	0	bd

Each group contains 10 rats. a: $p<0.01$; b: $p<0.05$ in compared with control group. c: $p<0.01$; d, $p<0.05$ in compared with high-fat fed diet group.

3.3. Effect of Turmeric powder on metabolism of fat due to high-fat diet

Clofubrate in groups 3 significantly ($p<0.001$) decreased, markedly increased serum levels of TG, total cholesterol, LDL and VLDL compared with group 2 and significantly ($p<0.01$) increased slightly decreased serum levels of HDL than group 2. In group 4, Turmeric powder significantly ($p<0.01$) decreased serum levels of total cholesterol, LDL and VLDL compared with group 2 and significantly ($p<0.05$) increased serum levels of HDL than group 2 (table 4).

Table 4: Effect of Cr Turmeric powder ocin on lipid levels in rats fed high-fat diet

Groups	TG mg/l	Total cholesterol mg/l	LDL mg/l	VLDL mg/l	HDL mg/l
Control	88.68±4.21	83.65±3.58	13.69±0.83	19.45±1.16	50.51±3.26
High-fat diet	233.61±6.90	218.14±7.81	122.72±4.75	49.52±2.21	45.90±2.34
High-fat diet+ Clofubrate	95.87±3.42 [c]	110.28±4.29 [c]	25.54±1.09 [c]	31.32±1.15 [c]	53.42±4.38 [b]
High-fat diet+Turmeric 5%	173.62±5.31 [b]	134.15±4.56 [b]	49.62±3.71 [b]	33.07±2.50 [b]	51.46±3.41 [a]
ANOVA	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000

Values are presented as mean ± SEM for 10 rats in each group.
a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ in compared with high-fat fed diet group.

Effect of Turmeric powder on anti-oxidative activity of liver in damage induced by high fat diet

In group 2, Hepatic levels of antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase compared with group 1 (normal control), significantly ($p<0.01$) reduced and the levels of malondialdehyde significantly ($p<0.01$) increased. Clofubrate in groups 3 significantly ($p<0.01$) increased, markedly decreased levels of SOD, CAT, GPX and GR compared with group 2 and significantly ($p<0.01$) decreased slightly increased levels of malondialdehyde than group 2. Data are showed in table 5.

Table 5: Effect of Turmeric powder on anti-oxidative activity of rat livers in steatosis induced by high fat diet

Groups	Biochemical parameters				
	MDA nmol/g protein	SOD U/mg protein	CAT U/mg protein	GPX U/mg protein	GR U/mg protein
Control	3.54±0.16 [bd]	13.64±0.54 [bd]	64.66±2.13 [bd]	22.84±1.65 [bd]	123.37±5.65 [bd]
High-fat diet	5.18±0.21 [acd]	9.13±0.32 [acd]	41.74±1.15 [acd]	17.49±0.83 [acd]	88.85±3.52 [acd]
High-fat diet+ Clofubrate	3.59±0.18 [b]	12.53±0.52 [b]	60.84±1.74 [b]	21.95±1.54 [b]	116.13±3.42 [b]
High-fat diet+Turmeric 5%	4.86±0.28 [ab]	10.74±0.47 [ab]	52.14±1.85 [ab]	19.12±1.14 [ab]	106.57±3.12 [ab]
ANOVA	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000

Values are presented as mean ± SEM for 10 rats in each group.
a, significant difference with group 1; b, significant difference with group 2; c, significant difference with group 3; d, significant difference with group 4 ($p<0.05$).

Discussion

The increased activities of marker enzymes, AST, ALT and ALP are suggestive of liver injury (Chidambarama and Venkatraman, 2010). Because these serum liver biomarkers disorders have been documented in hepatic steatosis (Angulo, 2002, Wang et al., 2009; Chidambarama and Venkatraman, 2010), their levels were studied. Increased plasma activities of AST, ALT and ALP were found in high fat diet fed rats, indicating damage to liver cells. These results were consistent with the findings reported by Chidambarama et al. (2010).Treatment with turmeric powder notably prevented the elevation of these enzymes to an extent that was comparable to the Clofibrate.

The biochemical findings were matched with histopathological verification. Rats fed with high-fat emulsion for 6 weeks developed a higher degree of steatosis. Howevere, histopathological assessment of liver tissues from high fat emulsion induced rat hepatic steatosis, displayed the antihepatosteatosis effects of turmeric powder. Administration of turmeric powder resulted in prevention of hepatic fatty deposition in hepatocytes. Histopathological changes in agreement with biochemical findings were concordant with those of previously reported (Wang et al., 2009).

Our results show that high fat diet caused significant decreases in SOD, CAT, GPx and GR activities. The derangement in enzymatic antioxidant potential indicates that high fat diet fed rats is unable to cope up with excess free-radical formation which leads to tissue damage. A body of evidence indicates that accumulation of fat in the liver increases the susceptibility to other insults such as oxidative stress that results in the progression of steatosis to steatohepatitis, fibrosis and cirrhosis (Koteish and Diehl, 2002).

Considering the recently recognized association between oxidative stress and inflammation (Chidambarama and Venkatraman, 2010), the present experiment confirms that high fat diet could result in oxidative liver injury. Induction of oxidative stress is evident from the increased peroxidation marker (MDA) and inadequate antioxidant enzymes status in liver of rats fed high fat diet. We estimated antioxidant activities of turmeric by determination of hepatic MDA content and antioxidant enzymes activity. High fat diet fed caused an increase in liver MDA content but a decrease in liver antioxidant enzymes activity compared with normal control group. Turmeric powder supplementation significantly improved the antioxidant defense mechanisms in high fat diet fed rats.

These results suggest that the imbalance between oxidative stress generation and antioxidants formation could occur after high fat diet fed, and turmeric could prevent this pathological process, indicating its therapeutic and preventive effect on hepatosteatosis induced by high fat ingestion. Antioxidant activity of turmeric is concordant with those of other investigators (Sharma, 1976; Sreejayan and Rao, 1994). The results of biochemical tests together with histological observations suggest that turmeric treatment lowers steatosis and prevents peroxidative damage and the effects are comparable with that of Clofibrate.

To analyze the possible role of turmeric in lipid metabolism which is the key factor in fatty liver formation, serum TG, TC, VLDL-C, HDL-C and LDL-C were investigated. After 6 weeks of treatment, the serum levels of TG, TC, VLDL-C, and LDL-C was markedly increased in the high fat diet fed group compared to those in the control group. This finding was parallel to the previous study (Zou et al., 2006). Treatment of high fat diet fed rats with turmeric showed considerable restoration of lipid levels to that of control. The increased serum levels of TG, TC, VLDL-C and LDL-C were significantly suppressed, whereas the decreased serum HDL-C level was obviously elevated by turmeric treatment in high fat diet fed rat.

Results of the histological changes in high fat diet rats, widespread deposition of lipid droplets inside the parenchymal cells, are consistent with the result of the biochemical analysis. This result suggests that turmeric powder can prevent hepatosteatosis via downregulation of accumulation

of lipid in serum and liver. Liver plays a key role in lipid metabolism. Hepatic steatosis refers to the excessive accumulation of lipids within hepatocytes due to imbalance between lipid formation and lipid degradation (Burt et al., 1998). Hypercholesterolaemia, hypertriglyceridaemia, low level of HDL-C and high level of LDL-C are the most common impairments in lipid homeostasis in patients with steatosis (Angulo and Lindor, 2002). Previous study has showed turmeric has hypolipidemic effects (Babu and Srinivasan, 1997). In this study, turmeric powder significantly improved both the biochemical and histological evidence of hepatic lipid accumulation. These results indicate that turmeric attenuates the disorder of lipid metabolism in liver resulted from high fat diet fed.

This study reveals that turmeric, as a glycosylated carotenoid contained in the stigmas of *Crocus sativus* Linne and in the fruits of *Gardenia jasminoides* Ellis, prevents high fat fed induced accumulation of lipid in rat liver. The preventive effect of turmeric powder is mediated through downregulation the levels of TG, TC, VLDL-C and LDL-C and elevation HDL-C synthesis. These changes are associated with decreasing in serum biomarkers of hepatic injury as well as attenuation of oxidative stress formation by turmeric treatment. These results demonstrate that turmeric powder has preventive effects against high fat diet induced rat fatty liver. It is noteworthy that this experiment has been performed on animal, so further studies are needed to examine whether similar findings would be obtained in humans.

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References

1. Assy N, Kaita K, Mymin D, Levy C, Rosser B, Minuk G. Fatty infiltration of liver in hyperlipidemic patients. Dig Dis Sci 2000;45:1929-1934.
2. Clark JM, Brancati FL, Diehl AM. Nonalcoholic fatty liver disease. Gastroenterology 2002;122:1649-1657.
3. Hokanson JE. Hypertriglyceridemia and risk of coronary heart disease, Curr Cardiol Rep 2002;4:488-493.
4. Kametani T, Koshida H, Nagaoka T, Miyakoshi H. Hypertriglyceridemia is an independent risk factor for development of impaired fasting glucose and diabetes mellitus: a 9-year longitudinal study in Japanese. Intern Med 2002;41:516-521.
5. Walldius G, Aastveit A, Jungner I. Hypercholesterolemia and hypertriglyceridemia-greatest cardiac risk in subjects with high apoB/apoA-I levels. Int Congr Ser 2004;1262:203-206.
6. Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. J Clin Invest 2008;118:829-838.
7. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest 2005;115:1343-1351.
8. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology 2001;121:91-100.
9. Angulo P, Lindor KD. Non-alcoholic fatty liver disease. J Gastroenterol Hepatol 2002;17:S186–190.
10. Farrell GC. Non-alcoholic steatohepatitis: what is it, and why is it important in the Asia-Pacific region. J Gastroenterol Hepatol 2003;18:124-138.
11. James O, Day C. Non-alcoholic steatohepatitis: another disease of affluence. Lancet 1999;353:1634-1636.
12. Orrenius S, Gogvadze V, Zhivotovsky B. Mitochondrial oxidative stress: implications for cell death. Annu Rev Pharmacol Toxicol 2007;47:143-183.
13. Day CP. From fat to inflammation. Gastroenterology 2006;130:207-210.
14. Day CP, James OF. Steatohepatitis: a tale of two 'hits'? Gastroenterology 1998;114:842-845.
15. Barbuio R, Milanski M, Bertolo MB, Saad MJ, Vellosa LA. Infliximab reverses steatosis and improves insulin signal transduction in liver of rats fed a high-fat diet. J Endocrinol 2007;194:539-550.
16. Angulo P. Non-alcoholic fatty liver disease. N Engl J Med 2002;18(346):1221–1231.
17. Govindrajan VS. Turmeric chemistry, technology and quality. CRC Crit. Rev Food Sci 1980;12:199-301.
18. Ammon HPT, Wahl MA. Pharmacology of Curcuma longa. Planta Med 1991;57:1-7.
19. Srimal RC, Dhawan BN. Pharmacology of diferuloylmethane (curcumin), a non-steroidal anti-infammatory agent. J Pharm Pharmacol 1973;25:447-452.
20. Sharma OP. Antioxidant activity of curcumin and related compounds. Biochem. Pharmacol 1976;25:1811-1812.
21. Sreejayan N, Rao MN. Curcuminiods as potent inhibitors of lipid peroxidation. J Pharm Pharmacol 1994;46:1013-1016.
22. Nagabhushan M, Bhide SV. Antimutagenic and anticarcinogenic action of turmeric (Curcuma longa). J Nutr Growth Cancer 1987;4:82-89.
23. Azuine MA, Bhide SV. Chemopreventive effect of turmeric against stomach and skin tumors induced by chemical carcinogens in Swiss mice. Nutr Cancer 1992;17:77-83.
24. Huang MT, Lou YR, Ma W, Newmark HL, Reuhl KR, Conney AH. Inhibitory effects of dietary curcumin on forestomach, duodenal and colon carcinogenesis in mice. Cancer Res 1994;54:5841-5847.
25. Deshpande SS, Ingle AD, Maru GB. Inhibitory effects of curcumin-free aqueous turmeric extract on benzo(a)pyrene-induced forestomach papillomas in mice. Cancer Lett 1997;118:79-85.

26. Rao CV, Rivenson A, Simi B, Reddy BS. Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occuring plant phenolic compound. Cancer Res 1995;55:259-266.
27. Babu P. Srinivasan K. Hypolipidemic action of curcumin, the active principle of turmeric (Curcuma longa) in streptozotocin induced diabetic rats. Mol Cell Biochem 1997;166:169-175.
28. Suryanarayana P, Saraswat M, Mrudula T, Krishna P, Krishnaswamy K, Reddy G. Curcumin and turmeric delay streptozotocin-induced diabetic cataract in rats. Invest Ophthalmol Vis Sci 2005;46:2092-2099.
29. Babu P. Srinivasan K. Amelioration of renal lesions associated with diabetes by dietery curcumin in streptozotocin induced diabetic rats. Mol Cell Biochem 1998;181:87-96.
30. Sajithlal GB, Chithra P, Gowri C. Effect of curcumin on the advanced glycation and cross-linking of collagen in diabetic rats. Biochem Pharmacol 1998;56:1607-1614.
31. Nishiyama T, Mae T, Kishida H, Tsukagawa M, Mimaki Y, Kuroda M. Curcuminoids and sesquiterpenoids in turmeric (Curcuma longa L) suppress and increase in blood glucose level in type 2 diabetic KK-Ay mice. J Agric Food Chem 2005;53:959-963.
32. Mahesh T, Balasubashini M, Menon V. Effect of photo-irradiated curcumin treatment against oxidative stress in streptozotocin-induced diabetic rats. J Med Food 2005;8:251-255.
33. Wahlstrom B, Blennow G. A study on the fate of curcumin in the rat. Acta Pharmacol Toxicol 1978;43:86-92.
34. Bhavani Shankar TN, Shanta NV, Ramesh HP, Indira Murthy AS, Sreenivasmurthy V. Toxicity studies on turmeric (Curcuma longa): Acute toxicity studies in rats, guinea pigs and monkeys. Ind J Exp Biol 1980;18:73-75.
35. Sambaiah K, Ratankumar S, Kamanna VS, Satyanarayana MN, Rao MVL. Infuence of turmeric and curcumin on growth, blood constituents and serum enzymes in rats. J Food Sci Technol 1982;19:187-190.
36. Zou Y, Li J, Lu C, Wang J, Ge J, Huang Y, Zhang L, Wang Y. High-fat emulsion-induced rat model of nonalcoholic steatohepatitis. Life Sciences 2006;79:1100-1107.
37. Sheng L, Qian Z, Zheng S, Xi L. Mechanism of hypolipidemic effect of crocin in rats: Crocin inhibits pancreatic lipase. Eur J Pharmacol 2006;543(1-3):116-122.
38. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am J Clin Pathol 1957;28:56-63.
39. Martinek RG. A rapid ultraviolet spectrophotometric lactic dehydrogenase assay. Clin Chem Acta 1972;40(1):91-99.
40. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-275.
41. Malloy HT, Evelyn KA. The determination of bilirubin level with the photoelectric colorimeter. J Biol Chem 1937;119(2):481-484.
42. Esterbauer H, Cheesman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol 1990;186:407-421.
43. Nishikimi M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. Biochem Biophys Res Commun 1972;46:849-854.
44. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984;21(2):130-132.
45. Claiborne A. Catalase activity. In: Boca Raton FL, editor. CRC Handbook of methods for oxygen radical research. Florida: CRC Press, Boca Raton 1985; 283-284.
46. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science 1973;179:588-590.
47. Mohandas J, Marshall JJ, Duggin GG, Horvath JSL, Tiller DG. Low activities of glutathione-related enzymes as factors in the genesis of urinary bladder cancer. Cancer Res 1984;44:5086-5091.
48. Lee G, Luna HT. Manual of histologic staining methods of the armed forces institute of pathology. Third Edition. The Blakiston Division Mc Graw. Hill Book Company 1988;32-107.
49. Shen X, Tang Y, Yang R, Yu L, Fang T, Duan JA. The protective effect of Zizyphus jujube fruit on carbon tetrachloride-induced hepatic injury in mice by anti-oxidative activities. J Ethnopharmacol 2009;122(3):555-560
50. Chidambarama J, Venkatraman AC. Cissus quadrangularis stem alleviates insulin resistance, oxidative injury and fatty liver disease in rats fed high fat plus fructose diet. Food Chemical Toxicol 2010;48(8-9):2021-2029.
51. Wang JQ, Li J, Zou YH, Cheng WM, Lu C, Zhang L, Ge JF, Huang C, Jin Y, Lv XW, Hu CM, Liu LP. Preventive effects of total flavonoids of Litsea coreana leve on hepatic steatosis in rats fed with high fat diet. J Ethnopharmacol 2009;121(1):54-60.
52. Koteish A, Diehl AM. Animal models of steatohepatitis. Semin Liver Dis 2002;21:89-104.
53. Burt AD, Mutton A, Day CP. Diagnosis and interpretation of steatosis and steatohepatitis. Seminars in Diagnostic Pathology 1998;15:246-258.

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