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Effect of Ginger and curcum powder on fatty liver in Male Albino Rats intoxicated with oxytetracycline

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Abstract

Ginger and curcuma a widely used fruit in many countries for its nutritional benefits. The main target of this study was to investigate the effect of two different concentrations (2.5% & 5%) of Ginger and curcum powder on fatty liver (FL) of male albino rats. The experiment was carried out using forty-two male albino rats. These rats were fed on basal diet for one week before then divided into six main groups (seven rats of each). Group 1: Negative control group fed on basal diet. Group 2: Positive control group will be fed on basal diet + Oxytetracycline injection (120 mg / kg / body weight per day), that after 28 days. 'Rats will be injected intraperitonealy with Oxytetracycline for three consecutive days to induce fatty liver induction . Group 3 and Group 4: As the same of group 2 with 2.5 % and 5 % Ginger powder respectively. Group 5 and Group 6 : As the same of group 2 with 2.5 % and 5 % Curcum powder respectively. At the end of the experimental period rats were fasted overnight and sacrificed; blood samples were collected from the aorta to determine lipids profiles. Besides, biological parameters were recorded. From the obtained results

we concluded that feeding rats which were suffering from FL with ginger and curcum powder at 2.5% & 5% improved the body weight. Our results could be summarized that diet fortified with 2.5% and 5% ginger and curcum powder helped to improve blood lipid levels, liver and kidney function compared with the positive control group (injected with oxytetracycline) which were considered as a major risk factor for fatty liver disease.

Introduction

Liver is not only an important digestive organ, but also closely connected to inflammation, which is the innate defense system of the body for removing harmful stimulus ***(Lam et al., 2016).*** In fact, Nonalcoholic fatty liver disease (NAFLD) has become a major public health hazard worldwide. The prevalence rates range from 24% to 42% in Western countries and from 5% to 40% in Asian countries, also from 24% to 35% in African countries showed by ***Sasidharan et al., (2014).***

According to, ***Helal et al., (2012)*** showed that the prevalence of NAFLD has apparently increased in proportion to the increasing incidence of obesity in both adults and children.

NAFLD comprises a wide spectrum of hepatic damage, from simple steatosis alone, to inflammatory changes found in nonalcoholic steatohepatitis (NASH) and advanced fibrosis and cirrhosis of the liver. In Egypt according to Ministry of Health & Population – International Information Center for Health published that above 19.30 % men and women were reported during the year 2012 had non - alcoholic fatty liver disease.

Moreover, the prevalence of NAFLD was 38.5% in obese Egyptian children and adolescents, and the prevalence of NAFLD in general was 62.19% for males and 53.96% for females ***(Halaby et al., 2015).***

Ginger belongs to family Zingiberaceae, it's widely used as a food additive and coloring agent. It is one of the most commonly used spices around the world. It has long been used to treat gastrointestinal disorders and its constituents have shown anti-inflammatory, chemopreventive, antidiabetic, antihepatotoxic and antioxidant properties **(*(Young et al., 2005; Ahmad et al., 2006* and *Nwozo et al., 2014).***

Curcum commonly known as Turmeric is one of the most common medicinal plants; Turmeric is an herbaceous plant grown and harvested annually for its rhizomes, which contain high amounts of bioactive compounds ***(Hung & Duyen, 2016).*** Curcum is a rhizomatous perennial herb that belongs to family Zingiberaceae. It is a tropical plant and is the source of the spice turmeric, which is derived from the dried, ground rhizome. It is extensively grown and used as dietary pigment and spices. Curcum longa possesses antioxidant, antitumor, antimicrobial, anti-inflammatory, wound healing, lipid-reducing, chemopreventive, immunomodulatory, and gastroprotective activities and all these are documented by ***Maizura et al., (2011) and Nwozo et al., (2014).***

Materials and Methods

Materials:

Egyptian cultivar of ginger (zingiber officinale) root and curcum (curcuma longa L.) rhizome powder were purchased from Field Crops Research Institute, Ministry of Agriculture Giza, Egypt. Soybean oil and starch were purchased from the local market. Casein, cellulose, vitamins & minerals, dextrin, L-cysteine, choline chloride, and Cesplatin were obtained from the Cairo Company for Chemical Trading, Cairo, Egypt. Forty-two male albino rats (Sprague Dawley strain) were obtained from the laboratory animal colony. Helwan, Cairo - Egypt. Weighting were approximately (120 ± 15g) . Kits used to determine High density lipoprotein cholesterol (HDL-C), Low density lipoprotein cholesterol (LDL-C), Very low density lipoprotein cholesterol (VLDL-C). urea nitrogen,uric acid, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkalaine phosphatase (ALP) produced by Egyptian American Company for laboratory service and supplied by Alkan Company.

Methods:

Egyptian cultivar of ginger and curcuma were purchased from Field Crops Research Institute, Ministry of Agriculture Giza, Egypt.

Experimental design:

Forty-two male Albino rats weighting (120 ± 15g) were fed on basal diet ***Gue et al. (2002).*** for one week prior to commencement of the experiment for adaptation, housed in well aerated cages under hygienic condition. Water was introduced *ad-libitum*. After this week,

rats were divided into 6 main groups (seven rats of each) fed on diets for six weeks as follows:

Group 1: Negative control group fed on basal diet. Group 2: Positive control group fed on basal diet + Oxytetracycline injection (120 mg/kg), that after 28 days. 'Rats will be injected intraperitonealy with Oxytetracycline for three consecutive days for fatty liver induction ***(Nicola et al., 1996)***'. Group 3: As the same of group 2 with 2.5 % Ginger powder. Group 4: As the same of group 2 with 5 % Ginger powder. Group 5: As the same of group 2 with 2.5 % Curcum powder. Group 6: As the same of group 2 with 5 % Curcum powder.

Blood Sampling:

At the end of the experiment period, the rats were fasted overnight and Body weight gain (BWG) and feed consumption were recorded weekly then anaesthetized and sacrificed and blood samples were collected from the aorta.

Biochemical analysis of serum:

Serum samples were used for the determination of total cholesterol (TC) ***(Allain et al., 1974),*** triglycerides (TG) ***(Fossati & Prencipe 1982),*** High density lipoprotein cholesterol (HDL-C) ***(Lopes, 1977),*** low density lipoprotein cholesterol (LDL-C) and Very low density lipoprotein cholesterol (VLDL-C), were calculated by using the method of ***Friedewald et al., (1972).*** Serum alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST) activity and alkaline phosphatase (ALP) were determined by colorimetric methods according to ***Rietman and Frankel (1957).*** Serum kidney function including uric acid, ***(Fossati et al., 1980),*** urea nitrogen, ***(Patton & Crouch 1977),*** serum Creatinine , ***Bartels et al., (1972).***

Statistical analysis:

Results are expressed as mean ± SD. Data were statistically analyzed for variance using one-way analysis of variance "ANOVA" according to ***Armitage & Berry, (1987).*** Computer software system SPSS (version 15) was used for these calculations.

Results and Discussion

Effect of feeding fatty liver rats on ginger and curcum powder at different ratios on body weight gain, feed intake and feed efficiency ratio

The mean values of body weight gain (BWG %); feed intake (g/day for each rat) and feed efficiency ratio (FER) of rats fed negative control group, positive control group; fatty liver group {oxytetracycline injection (120mg/kg body wt.) for three days respectively} fed on basal diet containing ginger and curcum powder at 2.5% & 5% in groups {which fed on basal diet containing ginger and curcum powder at 2.5% & 5% & injected with oxytetracycline after 28 days for three days respectively , were investigated and summarized in Tables (1).

On the other side, all treated groups showed significant $p<0.05$ in body weight, Other studies have revealed that High fat diet promote hyperlipidemia and hyperglycemia so it can be used to generate a valid rodent model for the analysis of the pathophysiology of dyslipidemia ***(Puccinelli et al., 2015).***

Effect of feeding on ginger and curcum powder on triglyceride and total cholesterol of fatty liver rats (table 2)

Results in Table (2) indicated that there were significant changes in the serum levels of Triglyceride (TG) and Total Cholesterol (TC) of negative control group when compared with fatty liver rats (positive control group) **.** Confirmed in the previous studies by ***(Khandouzi et al., 2015).*** suggested that intake of different forms of ginger (powder, warm or cold extract) in amount 2% of basal diet in rabbits resulted in significant decline in serum level of TG, TC and LDL-C, while it increased the level of blood glucose and HDL-C. Reduction of lipid peroxidation by ginger has been attributed to it`s antioxidant activity, because ginger have many phenolic compounds, which have inhibitory effects on lipid peroxidation and preserve the antioxidant compounds.

Previous studies have suggested that improvement of hyperlipidemia is achieved with curcumin decreased cholesterol and TG levels could be related to the inhibition of Diabetic Nephropathy (DN). Like type 2 diabetes mellitus, high levels of cholesterol, TG, and free fatty acid could stimulate ectopic lipid accumulation in the organs, except adipose tissue, and especially lipid accumulation of renal tissue could be a crucial factor in the development of chronic kidney disease ***(Kim et al., 2016).***

Effect of feeding on ginger and curcum powder on serum High density lipoprotein, low density lipoprotein and very low density lipoprotein (table 3)

Injected rats with oxytetracycline increased the mean values of LDL-C ; while VLDL-C , HDL-C decreased, as compared to the negative control group. Treating rats which were suffer from FL with the two levels of ginger or curcum led to significant improvement of

all parameters (HDL-C, LDL-C and VLDL-C) as compared to the positive control group. ***Helal, et al., (2012)*** In this study, oxytetracycline injection to rats was resulted in highly significant increased in serum cholesterol, triglycerides, LDL-cholesterol, while HDL-cholesterol was highly significant decreased. It was also induced acute pathological changes in the liver included narrowed blood sinusoidal lumina due to the enlarged, fat-laden hepatocytes together with necrosis. This finding correlates with the marked increase in serum cholesterol, triglycerides and LDL-cholesterol. The increased of these parameters in the blood is in correlation with the fatty degeneration of the liver. Biochemical mechanism for oxytetracycline toxicity based on mitochondrial damage **.**

The ameliorative effects of ginger on lipid profile in the present data are in agreement with ***(Bhandari et al., 2005)*** who revealed that, ethanolic extract of ginger produced significant decrease in serum total cholesterol and triglycerides levels and increased HDL-cholesterol level as, compared to diabetic rats, and the extract exhibit a significant lipid lowering activity and protect the tissues from lipid peroxidation.

Effect of feeding on ginger and curcum powder on serum uric acid, urea nitrogen and creatinine of fatty liver rats (table 4)

Results from table (4) indicated that there were significant decreased in the serum levels of uric acid of negative control group when compared with positive control group. There was significant difference between positive control group and groups of rats fed on 2.5% and 5% ginger and curcum powder. In fact, oral administration of 2.5% and 5% ginger and curcum powder was significant reduced the uric acid level . It can be observed that serum creatinine

increased in groups of rats with oxytetracycline injection which induced fatty liver.

Injection rats with oxytetracycline was a significant increased serum urea and creatinine when compared with control group. The mechanism of renal damage includes inhibiting protein synthesis and provoking a catabolic effect. Investigators have reported the propensity for renal side effects. In addition, an increase in kidney functions may occur due to the drug's anti-anabolic effect which, significantly impaired renal function, can lead to azotemia, hyperphosphatemia, and acidosis. Treating rats with oxytetracycline caused kidney dysfunction which appeared through high increase in serum urea and creatinine, while after treating rats with curcum, renal function markers returned back to normal values which may be due to the protective effect of curcumin (bioactive compound of curcum) against renal injury ***Eman et al .,(2011) and Helal, et al (2012).***

The present study revealed that, post-administration of ginger to fatty liver rats reduced and normalized the levels of serum creatinine and urea. On the other hand, the pre-treatment with this plant before the induction of fatty liver inhibited the higher increase of plasma creatinine and urea but they did not normalized them. Moreover, the study shows that, ethanol extract of ginger rendered significant protection against induced nephrotoxicity, which was evident from the lowered serum urea and creatinine levels in the mice that pre-treated with ginger extract. The presence of polyphenols and flavonoids in ginger extract might be responsible for the antioxidant nephroprotective activities and the reduction of serum urea and creatinine levels. Furthermore, the treatment of ginger extract could significantly prevent the depletion of antioxidant concentration and antioxidant enzymes activities in the kidneys.

Effect of feeding on ginger and curcum powder on Aspartate Amine Transferase, Alanine Amine Transferase and alkaline phosphatase

Results revealed that, control positive group with oxytetracycline injection showed a significantly higher of AST and ALT, as compared with those of negative control group. In fact, administration of rats with ginger and curcum powder at 2.5% and 5% for six weeks improved the mean values of Aspartate Amino Transferase (AST) , Alanine amino transferase (ALT) and Alkaline phosphatase (ALP) activity , as compared to the positive control group, and the best results of liver enzymes recorded for 5% ginger , curcum, it appears from our results that high concentration of ginger and curcum safe and improves liver functions.

Our results are in agreement with ***Helal, et al (2012)*** who reported that**,** Treated rats with oxytetracycline for three consecutive days caused fatty liver, necrosis and inflammation. These histological changes were associated with the high significant increase in activities of serum ALT, AST, gama glutamate transferase (GGT) and Lactate dehydrogenase (LDH). This significant increase may be due to rise in free radicals and decrease in the antioxidant enzyme levels. The significant decrease in serum transaminases AST, ALT and GGT activities in ginger treated group elucidates the hepatoprotective effect of ginger.

On the other hand, The antioxidant, anti-inflammatory and free radical scavenging property of ginger was ameliorated the effect of oxytetracycline on serum LDH activity through scavenge the free radicals, minimize lipid peroxidation, thereby preventing membrane damage and leakage of enzymes **.**

Table (1): Effect of feeding Ginger and Curcum powder) on body weight gain (BWG) feed intake (FI) and Feed efficiency ratio (FER)

paramaters / Groups	BWG (g)	FI (g/day)	(FER)
Control (- ve) fed on basal diet	163.60^{d} ±9.50	13.60^{c} ±0.16	23.80^{a}±5.17 12.44^{b} ±0.72
Control(+ve) fed on basal diet + Oxytetracycline injection (120 mg/kg)	128.00^{a} ±4.53	5.18^{a} ±0.08	
Control (+ ve) + 2.5 % Ginger powder	131.80^{ab} ±6.42	10.60^{c} ±0.15	12.01^{b} ±0.74
Control (+ ve) + 5 % Ginger powder	147.80^{c} ±5.26	6.18^{b} ±0.49	23.17^{a} ±0.89
Control (+ ve) + 2.5 % Curcum powder	150.40^{c} ±5.98	9.40^{c} ±0.16	16.00^{c} ±0.67
Control (+ ve) + 5 % Curcum powder	149.60^{c} ±5.13	9.08^{d} ±0.08	16.47^{c} ±0.57

The mean difference is significant at the 0.05 level.

Table (2): Effect of feeding fatty liver rats on diet containing Ginger and Curcum powder on triglyceride and total cholesterol

Paramaters / Groups	Triglyceride mg/dl	Cholesterol mg/dl
Control (- ve) fed on basal diet	106.40^{c} ±6.11	141.60^{c} ±8.62
Control (+ ve) fed on basal diet +Oxytetracycline injection (120 mg/kg)	190.00^{a} ±5.09	252.00^{a} ±6.44
Control (+ ve) + 2.5 % Ginger powder	131.40^{b} ±6.69	153.60^{b} ±2.97
Control (+ ve) + 5 % Ginger powder	112.00^{c} ±1.58	149.40^{b} ±4.04
Control ve) + 2.5 % Curcum powder	110.40^{c} ±3.85	152.80^{b} ±5.26
Control (+ ve) + 5 % Curcum powder	111.80^{c} ± 1.92	151.00^{b} ±3.94

Values are expressed as mean± SD, n7

Values at the same column with different letters are significant at $P < 0.05$

Table (3) : Effect of feeding fatty liver rats on diet containing Ginger and Curcum powder on HDL-C , LDL-C and VLDL-C

Paramaters / Groups	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl
Control (- ve) fed on basal diet	45.00 ^{a} ± 2.23	75.32 ^{c} ±10.12	21.28 ^{c} ±1.22
Control (+ ve) fed on basal diet + Oxytetracycline injection (120 mg/kg)	35.60 ^{c} ±1.51	178.40 ^{a} ±5.91	38.00 ^{a} ±1.02
Control (+ ve) + 2.5 % Ginger powder	40.60 ^{b}±1.82	86.72 ^{b} ±4.95	26.28 ^{b} ±1.34
Control (+ ve) + 5 % Ginger powder	42.40 ^{ab} ±1.82	84.60 ^{b} ±4.72	22.40 ^{c} ±0.32
Control (+ ve) + 2.5 % Curcum powder	44.00 ^{a} ±1.58	86.72 ^{b} ±7.26	22.08 ^{c} ±0.77
Control (+ ve) + 5 % Curcum powder	44.40 ^{a} ±1.34	84.24 ^{b} ±5.49	22.36 ^{c} ±0.39

Values are expressed as mean± SD , n7

Values at the same column with different letters are significant at $P < 0.05$

Table (4): Effect of feeding on diet containing Ginger and Curcum powder on serum uric acid, urea nitrogen and creatinine of fatty liver rats

Paramaters / Groups	Uric acid mg/dl	Urea nitrogen mg/dl	Creatinine mg/dl
Control (- ve) fed on basal diet	4.18 ^{c} ±0.84	38.40 ^{b} ±1.82	0.90 ^{b} ±0.28
Control (+ ve) fed on basal diet + Oxytetracycline injection (120 mg/kg)	5.46 ^{a} ±0.19	45.80 ^{a} ±1.92	1.20 ^{a} ±0.10
Control (+ ve) + 2.5 % Ginger powder	4.44 ^{b} ±0.23	36.80 ^{c} ±0.84	0.86 ^{b} ±0.06
Control (+ ve) 5 % Ginger powder	4.30 ^{b} ±0.16	37.20 ^{b} ±1.30	0.86 ^{b} ±0.05
Control (+ ve) + 2.5 % Curcum powder	4.56 ^{bc} ±0.18	40.60 ^{b} ±2.07	0.86 ^{b} ±0.03
Control (+ ve) + 5 % Curcum powder	4.40 ^{bc} ±0.27	39.20 ^{b} ±1.30	0.91 ^{c} ±0.01

Values are expressed as mean± SD , n7

Values at the same column with different letters are significant at $P < 0.05$

Table (5): Effect of feeding fatty liver rats on Ginger and Curcum powder on AST , ALT and ALP

Paramaters / Groups	AST (u/l)	ALT (u/l)	ALP (u/l)
Control (- ve) fed on basal diet	44.60 ^{c} ±0.55	33.00 ^{c} ±2.83	831.20 ^{c} ±5.55
Control (+ ve) fed on basal diet + Oxytetracycline injection (120 mg/kg)	113.60 ^{a} ±7.56	66.40 ^{a} ±1.82	850.80 ^{a} ±3.84
Control (+ ve) + 2.5 % Ginger powder	52.40 ^{b} ±5.51	52.40 ^{b} ±5.51	847.80 ^{a} ±3.11
Control (+ ve) + 5 % Ginger powder	49.00 ^{bc} ±2.92	40.60 ^{d} ±2.70	845.20 ^{a} ±3.56
Control (+ ve) + 2.5 % Curcum powder	55.60 ^{b} ±2.30	45.80 ^{c} ±1.92	844.40 ^{b} ±3.29
Control (+ ve) + 5 % Curcum powder	50.60 ^{b} ±4.72	39.80 ^{d} ±1.64	839.40 ^{b} ±4.83

Values are expressed as mean± SD , n7

Values at the same column with different letters are significant at $P < 0.05$

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التأثير الوقائي للزنجبيل والكركم على علي الفئران المصابة بالكبد الدهنى بعد حقنها بالاوكسى تيترا سيكلين

عبدالرحمن محمد عطيه ، أمنيه جلال رفعت ، منى سامى حلبى ، الاء أسامه أبوريه ، ساره عاطف على محمود ٢٠١٩

قسم التغذيه وعلوم الاطعمه – كليه الاقتصاد المنزلى- جامعه حلوان

المستخلص العربى

تهدف هذه الدراسه الى معرفه تاثير الكركم والجنزبيل بنسبه (٢,٥ % و ٥%) على الكبد الدهنى لعدد اثنى واربعون من ذكور الجرذان البيضاء ، والتى وضعت على نظام غذائى مثالى لمده اسبوع واحد قبل تقسيمها الى مجموعات . وبعد هذه الفتره قسمت الى سته مجموعات رئيسيه متضمنه سبعه فئران فى كل مجموعه وذلك على النحو التالى :

المجموعه (١) المجموعه الضابطه السالبه تغذت على النظام الغذائي الاساسى . المجموعه (٢) المجموعه الضابطه الموجبه تغذت على الغذاء الاساسى مع حقن الفئران بالاوكسى تيترا سيكلين (١٢٠ جم / كجم تحت الغشاء البريتونى) بعد اليوم الثامن والعشرون تم حقن الفئران ثلاثه ايام متتاليه لاحداث الكبد الدهنى . المجموعه (٣) كما فى المجموعه (٢) + مسحوق الجنزبيل بنسبه ٢,٥ % . المجموعه (٤) كما فى المجموعه (٢) + مسحوق الجنزبيل بنسبه ٥ % . المجموعه (٥) كما فى المجموعه (٢) + مسحوق الكركم بنسبه ٢,٥ % . المجموعه (٦) كما فى المجموعه (٢) + مسحوق الكركم بنسبه ٥ % .

فى نهايه فتره التجربه ، تم جمع عينات الدم من الشريان الاورطى لتقدير نسب الدهون فى الدم ، وتقدير وظائف الكلى والكبد ، وبالتالى سجلت القياسات البيوكيميائيه والبيولوجيه .
وقد اوضحت هذه الدراسه النتائج التاليه : ان تغذيه الفئران المصابه بالكبد الدهنى على مسحوق الكركم والجنزبيل بنسب ٢,٥ % و ٥ % ادت الى تحسن ملحوظ فى وزن الجسم وفى الغذاء المتناول . هذا الى جانب تحسين مستويات الدهون فى الدم وكذلك تقليل المخاطر على وظائف الكلى والكبد مقارنه بالمجموعه الضابطه الموجبه (الحقن بالاوكسى تيترا سيكلين) والتى اعتبرت عامل خطرا كبيرا لمرض الكبد الدهنى .