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BIOCHEMICAL STUDY ON THE PROTECTIVE EFFECT OF CURCUMIN ON THIOACETAMIDE INDUCED HEPATOTOXICITY IN RATS

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A B S T R A C T

The main objective of this study was to investigate the protective effect of curcumin in hepatotoxicity induced by thioacetamide (TAA) in rats. Sixty mal albino rats divided into six groups containing 10 rats each. Group I: (Control group) rats received no drugs. Group II: (TAA-induced liver toxicity group) rats injected TAA (50 mg/kg b.wt /twice/ week, i.p) for 3 weeks. Group III: (curcumin group) rats administered curcumin (100 mg/kg b.wt/daily, orally) to 6 weeks. Group IV: (TAA + curcumin pretreated group) rats administered curcumin for 3 weeks and then injected TAA for 3 weeks. Group V: (TAA +curcumin Post-treated Group) rats injected TAA for 3 weeks and then administered curcumin for 3 weeks. Group VI (TAA + Curcumin co-treated group) rats injected TAA and at the same time administered curcumin for 6 weeks (end of experiment). The obtained results revealed that, a significant increase in serum GGT, ALT, AST, ALP and LDH activities, total Bilirubin, TNF- α and liver L-MDA concentrations were observed in TAA injected rats. However, administration of curcumin in TAA induced liver toxicity in rats exhibited a significant decreased in all mentioned parameters and attenuated the increased MDA level in liver tissues. On the other hand, a significant decreased in serum total protein, albumin concentrations and in liver antioxidant enzymes (SOD, CAT) activities were observed in TAA induced hepatic toxicity in rats when compared with control normal group. Meanwhile, curcumin administrations resulted in significant increase in all mentioned parameters and enhanced the activity of antioxidant enzymes in liver tissues. It could be concluded that, inhibition of peroxidation, inflammation and oxidative stress and enhanced antioxidant status in rat liver tissues by curcumin suggest the potential efficacy of curcumin as an addition hepatoprotective, anti-inflammatory and anti-hepatotoxic agent in treatment of liver toxicity.

Keywords: Thioacetamide (TAA), curcumin, oxidative stress, liver toxicity

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1. INTRODUCTION

Liver plays a crucial role in the metabolic elimination of most drugs and other foreign compounds, thus making it an important target for toxicity. Hepatotoxic agents can react with the basic cellular components and consequently induce almost all types of liver lesions. Toxins and drugs are among the basic etiopathogenetic agents of acute liver failure in Western countries (Grattagliano et al., 2009).

It is not surprising that hepatoprotective action against liver toxic injury remains one of the major challenges for clinical therapy. Studies of effective protection require knowledge of the mechanisms leading to liver damage, which are, unfortunately, limited by the lack of satisfactory experimental models. Nevertheless,

chemical toxins (including acetaminophen, carbon tetrachloride, galactosamine and thioacetamide) are often used as the model substances causing experimental hepatocytes injury. (Dominical et al., 2009; Kucera et al., 2006)

Hepatic damage is a crucial factor to determine the severity of hepatic encephalopathy. Any drug or manipulation which ameliorates liver injury might improve hepatic encephalopathy in animals (Chang et al., 2010).

When liver injury occurs, intracellular components released from necrotic cells are able to activate immune cells and trigger the reactive oxygen species (ROS) mediated cell killing process, which leads to more necrosis and amplified inflammation (Jaeschke, 2011).

TAA Known as Thioacetamide acid, or acetothioamide, is a widely used sulfur-containing compound both in the laboratory and in various technical application and can also be present in the environment as organic sulfur compounds (Zaleska et al., 2007).

Thioacetamide is a highly specific hepatotoxic material causing liver injury and dysfunction, containing thiono-sulfur compound and is well known to induce hepatic damage by generation of ROS (Wang et al., 2012). Shortly after administration, the thiono-sulfur group of TAA undergoes an extensive metabolism by the mixed function oxidase system in the body to produce acetamide that does not have liver necrotizing properties and TAA-S-oxide by a microsomal monooxygenases requiring NADPH and cytochrome P450 (Baskaran et al., 2010). In a further step, TAA-S-oxide is transformed to TAA-S-dioxide, which is a highly reactive unstable compound that is thought to covalently binding to liver macromolecules and responsible for initiation of hepatic damage and centrilobular necrosis, (Chilakapati et al., 2005) and generation of ROS that leads to hepatocellular death via oxidative stress (Sarkar and Sil, 2007).

During the few past years, a large number of natural products and dietary component have been evaluated as potential chemo preventive agent (Sharma et al., 1994).

In natural products are a rich source of potentially therapeutic drugs but many natural products have to be structurally modified and optimized to become useful pharmacological agents. In the case of curcumin, the poor aqueous solubility and relatively low bioavailability have been major obstacles for its clinical development as a therapeutic drug (Anand et al., 2007).

The phenolic hydroxyl groups are important for curcumin's anti-oxidant activities, the C7 linker and its carbonyl functions are important for anti-inflammatory activity and the conjugated enones have been shown to act as Michael acceptors for curcumin's anti-cancer activity for a comprehensive review see (Mosley et al., 2007).

In particular, curcumin may slow the growth of gastrointestinal cancers including esophageal, mouth, intestinal, stomach and colon, probably due to its increased bioavailability in the gastrointestinal tract. Curcumin, a hydrophobic polyphenol, is the yellow pigment in the Indian spice turmeric derived from the rhizome of the herb Curcuma longa. Curcumin is also known as diferuloylmethane and chemically is a bi-α, β-unsaturated β-diketone. Differing in methoxy substitutions on the aromatic ring, turmeric contains three natural analogues, the so-called curcuminoids, with curcumin being the most abundant (77%) and the less common demethoxycurcumin (17%) and 3% bisdesmethoxycurcumin (Anand et al., 2008). Accordingly, the present study aimed to evaluate the hepatoprotective effects of curcumin as natural antioxidants agent on hepatotoxicity induced by Thioacetamide (TAA) in rats.

2. MATERIALS AND METHODS

2.1. Experimental animals:

Sixty Male albino rats, 6-8 weeks old, and average body weight 150 - 200 gm, were used in the experimental investigation of this study. Rats were obtained from "The Laboratory Animals Research Center", Faculty of Veterinary Medicine, Benha University, and housed in separate wire mesh cages, exposed to good ventilation, humidity and to a 12-hr light/dark cycle. Constant supplies of standard pellet diet, fresh and clean drinking water were supplied ad-libitum. The animals were left for 7 days for acclimatization prior to the beginning of the experiment, and kept at constant environmental and nutritional conditions throughout the period of the experiment.

2.2. Chemicals and antioxidants

Thioacetamide (TAA): Cadmium chloride has molecular weight 218.41. TAA Known as thioacetamide acid, or acetothioamide the molecular formula (CH3CSNH2). TAA compound that exists at room temperature as colorless to yellow crystals with a slight odor of mercaptans. It is soluble in water and ethanol, miscible with benzene and petroleum ether, and sparingly soluble in ether. It is hydrolyzed by acids or bases and reacts with salts of heavy metals. Thioacetamide was purchased from Sigma Aldrich Company Co. for Trading Chemicals, Medicines and Medical Appliances (IARC 1974, HSDB 2009).
Preparation and dosage of TAA: - TAA was freshly dissolved in distilled water, and administered to rats at a dose (50 mg/kg b.wt / injected intraperitoneal) twice week for 3 weeks for induction of liver toxicity (Ayden, et al., 2010)**.**

Dimethyl sulphoxide (DMSO) as solved to curcumin: it is purchased from Elgoumhouria Co for trading chemicals medicines and medical appliances, Egypt.

Curcumin (CUR): Melatonin was Physical properties: Curcumin is an orange yellow powder, with the molecular formula C21H20O6 , molecular weight 368.39 , melting point 175-180 ºC, and soluble in Dimethyl sulphoxide (DMSO) (Aggarwal, et al., 2003). Curcumin (purity ~99%) was manufactured by Fluke Co for chemicals and purchased from Elgoumhouria Co for trading chemicals medicines and medical appliances, Egypt.
Preparation and dosage of Curcumin: - Curcumin was freshly dissolved in 7% DMSO solution, and administered to rats at a dose level of (100 mg/kg b.wt /p.o) once daily. (Aggarwal , et al., 2003).

2.3. Experimental design

After acclimatization Rats were randomly divided into six main groups, each group contains 10 rats placed in individual cages and classified as follow:
Group I: (Control group) rats received no drugs served as control for all experimental groups.
Group II: (TAA-induced liver toxicity group) rats injected with TAA (50 mg/kg b.wt /twice/ week, ip) for 3 weeks for induction of liver toxicity.
Group III: (curcumin treated group) rats administered curcumin at a dose of (100 mg/kg b.wt/daily, orally) all over the experimental periods (6 weeks).
Group IV: (TAA + curcumin pretreated group) rats administered curcumin at a dose of (100 mg/kg b.wt/daily, orally) for 3 weeks before TAA injection and then administered with TAA (50 mg/kg b.wt /twice/ week, ip) for 3 weeks.
Group V: (TAA +curcumin Post-treated group) rats injected with TAA (50 mg/kg b.wt /twice/ week, ip) for 3 weeks and administered with curcumin (100 mg/kg b.wt/daily, orally), for 3 successive weeks, from beginning of the 4th week until the end of experiment (6 weeks).

Group VI: (TAA + Curcumin co-treated group) rats injected TAA (50 mg/kg b.wt /twice/ week, ip) and at the same time co-administered with curcumin(100 mg/kg b.wt/daily, orally) for 3 weeks, followed by curcumin treatment alone 3 weeks later (end of experiment, 6 weeks).

2.4. Sampling:

At the end of the experimental period, rats were fasted overnight, blood samples were taken from retro-arbitral plexus. The blood samples were collected in dry, clean test tubes and allowed to clot for 30 min and serum was separated by centrifugation at 3000 rpm for 15 min at 4 ºc.

The serum was separated by automatic pipette and received in dry sterile tubes, processed, (ALT), (AST), (ALP), (LDH) , (GGT) and (TNF-α). Then kept in a deep freezer at -20°C until used for subsequent biochemical analysis All serum samples were analyzed for the following parameters: Albumin, Total Protein, Total Bilirubin, were determined according to the methods described by((Reitman and Frankel(1957); Kind and King (1954); Mallay and Evelyn (1937); Dito, W.R., (1979); Lowry (1951); Kaplan et al., (1984); Allain et al.,(1974); Buccolo, et al.,(1973); Tietz et al.,(1995)) respectively.

Then liver samples were collected for determination of L-Malondialdehyde (L-MDA), Catalase (CAT) and Superoxide Dismutase (SOD). Concentrations were determined according to the methods described by (Mesbah, et al., (2004); Xu, et al.,(1997); Paoletti and Macali,(1990)) respectively.

2.5. Statistical Analysis

The results were The results were expressed as mean + Standard Error (SE) of 6 rats per group to evaluate variations in data, a one-way analysis of variance (one-way ANOVA) was performed followed by a Student's t-test using the Bonferroni correction for multiple comparisons; when the analysis indicated the presence of a significant difference, the means were compared with the Duncan test. Significance was accepted at $p<0.05$.
All calculations were performed using the (SPSS 14.0) statistical software.

3. RESULTS

The obtained data in table (1) revealed a significant increase in ALT, AST, ALP, GGT, LDH, Total Bilirubin and TNF-α in TAA induced hepatotoxicity group, accompanied with significant decrease in Albumin and Total protein levels, when compared with control normal group While in pretreated ,postterated and co-treated groups with Curcumin there were significant decreases in ALT, AST, ALP, GGT, LDH and Total Bilirubin, accompanied with significant increases in Albumin and Total protein levels, in comparison with TAA treated group.

The obtained data in table (2) revealed a significant increase in L-MDA level and significant decreases in SOD, CAT activities level in liver tissue homogenate in TAA induced hepatoxicty group, when compared with control normal group, pretreated, posttreated and co-treated groups with curcumin there were significant decrease in L-MDA level and significant increases in SOD, CAT activities level in liver tissue homogenate, when compared with TAA treated group.

Table (1) Effect of Curcumin administration on serum hepatic function tests and pro-inflammatory cytokine (TNF-α) in normal and Thioacetamide - induced hepatotoxicity in rats.

Parameters	Normal Group	TAA Group	Curcumin Group	Pretreated Group	Post-treated Co-treated	Group Group
Albumin (gm/dl)	4.6±0.05^{b}	3.8±0.08^{e}	4.85±0.06^{a}	4.57±0.03^{b,c}	4.2±0.05^{d}	4.45±0.04^{c}
T.Protein (gm/dl)	7.77±0.08^{a}	6.63±0.09^{c}	7.25±0.09^{b}	7.13±0.08^{b}	7.1±0.06^{b}	7.82±0.06^{a}
T.Bilirubin (mg/dl)	0.4±0.04^{d}	1.13±0.05^{a}	0.6±0.03^{c}	0.68±0.03^{b}	0.78±0.05^{b}	0.75±0.06^{b}
GGT(IU/l)	5.83±0.48^{d}	17.5±0.76^{a}	8.67±0.49^{c}	11.3±0.49^{b}	12.7±0.49^{b}	9.67±0.42^{c}
AST (IU/l)	52.33±0.76^{e}	527±1.38^{a}	55.00±1.32^{e}	120±1.02^{c}	135 ±1.46^{b}	77.83±1.49^{d}
ALT (IU/l)	39.7±0.67^{c}	395±1.34^{a}	39.67±0.67^{c}	61.83±1.08^{b}	60.67±1.02^{b}	60.5±1.06^{b}
ALP (IU/l)	197.17±0.79^{e}	413.00±2.81^{a}	200.83±1.49^{d,e}	207.7±1.80^{c}	213.67±1.17^{b}	204±1.98^{c,d}
LDH (IU/l)	228.83±0.87^{e}	782.33±2.54^{a}	232±0.86^{e}	298.67±0.84^{b}	291.67±0.88^{c}	253.5±1.15^{d}
TNF-α (pg/ml)	14.17±0.6^{e}	67.5±0.85^{a}	13.17±0.6^{e}	32.5±0.76^{c}	35.66±1.26^{b}	25.67±0.95^{d}

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at (*P≤0.05*).

Table (2) Effect of Curcumin administration on liver tissue antioxidant enzymes (SOD and CAT) activities and MDA concentration in normal and Thioacetamide – induced hepatotoxicity in rats.

Parameters	Normal Group	TAA Group	CUR Group	Pretreated Group	Postterated Group	Co-treated Group
SOD (U/g. tissue)	52.50±1.06c	39.33±0.42d	75.67±0.56a	61.50±0.76b	51±0.58c	60±0.52b
CAT (K/g.tissue)	57.33±0.67c	41.67±0.76e	62.83±2.56a	59.83±0.60b	51.33±0.49d	52.83±0.48d
MDA (nmol/g.tissue)	73.8±0.70e	108±0.86a	69.67±0.56f	76.33±d	89.83±0.60b	75.5±0.43c

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at (*P≤0.05*).

4. DISCUSSION

In the present study, administration of TAA caused elevation of serum AST, ALT, ALP, Bilirubin, LDH and GGT activities compared with control group and pretreated, Postterated and Co-treated Groups with Curcumin. This is in agreement with previous data (Abul et al., 2010), which could be taken as an index of liver damage. In our study, the rise in AST, ALT, and GGT levels of activities induced by TAA administration was significantly reduced by administration of CUR. When compared with TAA, suggesting that Curcumin. Protective activity might be due its effect against cellular leakage and loss of functional integrity of the cell membrane in hepatocyte. The increase in the activities of AST, ALT, and GGT in serum of rats treated with

TAA might be due to the increased permeability of plasma membrane or cellular necrosis leading to leakage of the enzymes to the blood stream (Atef, et al ., 2011) and this showed the stress condition of the TAA treated animals.

Animals exposed to TAA showed necrotic changes resulting in the release of hepatic enzymes (AST, ALT, ALP, GGT and bilirubin) that mark liver injury Baskaran, et al., (2010), Jain and Singhai, (2011) interpreted the elevated levels of AST and ALT as a result of the hepatocytes damage or alterations in the membrane permeability indicating the severity of hepatocellular damage induced by TAA, which is in accordance with previous reports of (Sehrawat et al., 2006).

In contrast, an increase in ALP activity and bilirubin level reflects the pathological alteration in biliary flow. Increase in serum total bilirubin concentration after TAA administration might be attributed to the failure of normal uptake, conjugation and excretion by the damaged hepatic parenchyma (Fan, et al., 2009).

In this study, administration of Curcumin. combined with TAA decreased the levels of TNF-α compared with TAA alone, this result is in agreement with previous work by (Missima et al., 2009) who mentioned that TAA administration to Rats submitted to chronic stress stimulated pro inflammatory cytokines including TNF-α. In our study, administration of extracts of Curcumin to animals caused a decrease in the levels of TNF-α compared with TAA group, this result is in agreement with previous work by (Khayyal et al., 2003) who mentioned that daily administration of aqueous extract of Curcumin for two months to asthma patients decreased pro-inflammatory cytokines production suggesting the anti-inflammatory effect of Curcumin.

The obtained data demonstrated in table (2) revealed that, administration of TAA to normal rats exhibited a significant reduction in liver SOD, CAT activities after induction of Hepatoxicty when compared with control normal group. Studies in TAA models of liver toxicity indicate a higher free radical activity in the liver, as shown by the increase in mitochondrial superoxide radical and H2O2 and the induction of the microsomal cytochrome P-450 (Lettéron, et al., 1996). Higher pro-oxidant liver status in rats with TAA is likely to involve a high consumption of cellular and circulant antioxidants. This could be partly related to the decrease in liver activities of CAT. The antioxidant enzymes (CAT and SOD) assays showed that TAA treatment caused the depletion of these enzymes; therefore, it could be said that TAA caused the cellular damage by inhibiting the activity of the antioxidant enzymes (Sarkar and Sil, 2007).

The obtained data demonstrated in table (2) revealed that, administration of TAA to normal rats exhibited a significant increase in liver L-MDA concentration, when compared with control normal and other protected groups. These results came in agreement with those recorded by (Ahmad, et al., 2002; Sanz, et al., 2002) who reported that TAA administration to normal rats led to a significant increase in MDA level hepatic homogenates, when compared with normal control rats. Also, (Sarkar and Sil, 2007) recorded that TAA administration increased liver MDA level which indicates the extent of TAA-induced lipid peroxidation to 160% with respect to the normal cells MDA is the main product of lipid per oxidation and its concentration usually reflects the total level of lipid per oxidation (Tsai, et al., 2010); Ansil, et al.,

(2011) observed that TAA treatment caused a significant increase in hepatic MDA level, when compared with normal control group. TAA has been found to stimulate lipid per oxidation by generation of ROS (Bruck, et al., 1999). Curcumin contains -sitosterol, a component reported as a hepatoprotective agent and ellagic acid, a strong antioxidant and chemoprotictive agent (Das, et al., 2007). The identified class of components in single or in combination with other components present in the extract might be responsible for the anti hepatotoxic activity in both the treatment groups. The obtained data demonstrated in table (2) revealed that Curcumin treatment to hepatoxicty rats significantly attenuated the decreased MDA level. The obtained results are nearly similar with those of (Kamalakkannan, et al., 2005) oral administration of curcumin decreased the levels of plasma (MDA) and hydro peroxides and improved the levels of non-enzymatic antioxidant. In conclusion, the result of serum biochemical parameters, level of hepatic lipid peroxides, CAT and SOD support the dose dependent hepatoprotective and antioxidant activity of Curcumin. So this can be employed as main ingredient in medicine for disorders due to oxidative stress. However, further pharmacological evidences at molecular level are required to establish the mechanism of action of the drug.

CONCLUSION

It could be concluded that, Curcumin administration provided an effective protection against hepatic toxicity and oxidative damage in liver induced by TAA in rats, since these natural antioxidant agent were able to ameliorate serum biomarkers of hepatic function, enzymatic antioxidant defense system, prevent the lipid peroxidation and oxidative stress in hepatic tissues.

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دراسة كيميائية حيوية للتأثير الوقائي للكركم على التسمم الكبدى المحدث بالثيواسيتاميد في الفئران.

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الملخص العربي

تهدف هذه الدراسة إلى استخدام الكركم المذاب فى (DMSO 7%) مرة واحدة يومياً بجرعة مقدارها(100 مجم /كجم من وزن الفأر) لدراسة تأثيرها الوقائي والعلاجي لخلايا الكبد ضد التسمم الكبدى المحدث تجريبياً بحقن الفئران مرتين اسبوعياً فى الغشاء البريتونى للبطن بمادة الثيواسيتاميد بجرعة مقداراها 50 مجم /كجم من وزن الفأر . ولقد أجريت التجارب على عدد ستين فأراً قسمت إلى مجموعات كل مجموعة مكونة من عشرة فئران على النحو التالي المجموعة الأولى (المجموعة الضابطة). المجموعة الثانية (مجموعة الثيواسيتاميد المحدث بها التسمم الكبدى) تم الحقن بالثيواسيتاميد لمدة ثلاثة أسابيع.المجموعة الثالثة (مجموعه الكركم): تم فيها تجريع الفئران بالكركم لمدة ثلاثة أسابيع. المجموعة الرابعة (مجموعه الوقاية):- تم فيها تجريع الكركم لمدة ثلاثة أسابيع ثم حقن الفئران بمادة الثيواسيتاميد لمدة ثلاثة أسابيع أخرى المجموعة الخامسة (مجموعة العلاج) : تم حقن الفئران بمادة الثيواسيتاميد لمدة ثلاثة أسابيع ثم تم تجريع الكركم لمدة ثلاثة أسابيع أخرى المجموعة السادسة (مجموعة مختلطة) تم الحقن بماده الثيواسيتاميد وبعد ساعتين تم تجريع الكركم لمدة ثلاثة أسابيع ثم تم تجريع الكركم بمفرده لمدة ثلاثة أسابيع أخرى وبعد 6 أسابيع (نهاية التجربه) تم أخذ عينات الدم مرة واحدة لأجراء التحاليل البيوكيميائية الاتية انزيمات الكبد(الانين أمينو ترانسفيراز - أسبارتيت أمينو ترانسفيراز- الفوسفاتيز القلوى) - الألبيومين - البروتين الكلى - الصفراء الكلية - لاكتيت دى هيدروجينيز- جاما جلوتاميل ترانسفيريز(TNF) وانسجه الكبد لعمل التحاليل الآتية : مالون داى ألدهيد- قياس نشاط الأنزيمات المضادة للأكسدة مثل (الكتاليز – سوبراكسيد ديسميوتيز) وقد أظهرت النتائج مايلى:- ارتفاع ملحوظ فى إنزيمات الكبد والجاماجلوتاميل ترانسفيراز والبليروبين ولاكتيت داى هيدروجينيز و (TNF) فى مجموعه الماده السامه المحقونه بالثيواسيامايد مقارنه بالمجموعه الضابطه بينما فى المجموعات التى تم تجريعها بالكركم لوحظ انخفاض هذه القيم إلى المعدلات الطبيعية مقارنة بمجموعة الماده السامه كما أن هناك إنخفاض ملحوظ فى الألبيومين والبروتين الكلى فى المجموعة المحقونة بالثيواسيامايد مقارنة بالمجموعة الضابطة بينما فى المجموعات التى قد جرعت بالكركم لوحظ أن هذه القيم عادت قريباً من قيم المجموعة الضابطة . كما أوضحت النتائج انخفاضا ملحوظاً فى قيم إنزيمات مضادات الأكسدة سوبر أوكسيداز ديسميوتيز وكاتاليز وزيادة فى مستوى مالونيل الدهيداز فى أنسجة فئران مجموعة الثيو اسيتاميد مقارنة بنتائج المجموعة الضابطة بينما فى المجموعات التى تم تجريعها بالكركم لوحظ أن نتائج قيم إنزيمات مضادات الأكسدة سوبر أوكسيداز ديسميوتيز و كاتاليز قد ارتفعت إلى القيم الطبيعية وكذلك حدث انخفاض مستوى مالونيل الدهيداز ما يعطى حماية للكبد. ومن ثم فإن تلك النتائج أوضحت تأثيرالكركم كوقاية أو علاج لأمراض الكبد المختلفة ولذلك توصى الدراسة بضرورة استغلال تلك المزايا الهائلة للكركم كمادة طبيعية وقائية مضادة للتسمم الكبدي وإدخالها كمادة فعالة في صناعة العقاقير الطبية المستخدمة فى وقاية وعلاج أمراض الكبد الناتجة عن التسمم الكبدى.

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