Available online on www.ijcpr.com
International Journal of Current Pharmaceutical Review and Research, 4(4), 128-138

ISSN: 0976-822X

Research Article

Hepatoprotective Activity of Guazoma Tomentosa Leaf Extracts Against CCl_4 Induced Liver Damage in Rats

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Available online: 1st November 2013

ABSTRACT

Enhanced lipid peroxidation may result in hepatitis. As *Guazuma tomentosa* is rich in flavonoids and tannins so leaves of *Guazuma tomentosa* is selected for screening of leaves for hepatoprotective activity. In the present study, ethanolic, dichloromethane (DCM) and aqueous extracts of *Guazuma tomentosa* leaves were subjected to phytochemical evaluation, assay for flavonoid and tannin content. Evaluation for its protective effect on CCl_4-induced liver damage in Albino wistar rats. As oxidative stress is directly related to hepatotoxicity so in-vitro antioxidant activity was determined using DPPH assay. In-vivo anti-oxidant activity was also determined by estimation of TBARS and GSH. Serum biochemical parameters viz. serum glutamine oxaloacetate transaminase (SGOT), serum glutamine pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP) and total protein (TP) were also estimated. The ethanolic and dichloromethane extracts were found to be potential source of hepatoprotective agent.

Keywords: *Guazuma tomentosa*, Hepatoprotective, lipid peroxidation, Flavonoid content, Phenolic content.

INTRODUCTION

Liver play important role in regulation of physiological processes such as metabolism, secretion and storage. Moreover, detoxification of a variety of drugs and xenobiotic occurs in liver. The bile secreted by the liver has significant role in digestion. Liver diseases are

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classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). The main cause of liver diseases are toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune/disorder[1]. To consider this leaves of *Guazuma tomentosa* is selected for screening of different extract of leaves for hepatoprotective activity. *Guazuma tomentosa* is a plant native to tropical America, Ecuador and Colombia. Traditionally whole plant is used for its multipurpose benefits, e.g. as astringent, in cold, in cough, in diarrhoea, as diuretic, in dysentery, in venereal diseases, etc. Despite of its ethno pharmacological uses, presently it is proven to have many therapeutic valuable uses because of the presence of many phyto-constituents e.g. colistin, colatannins, catechins, caffeine, kaempferol, procyanidin B-2, procyanidin B-5, procyanidin C-1, tartaric acid, theobromine, xanthan gum, etc. The proven pharmacological activities involve, anti-diabetic, anti-hypertensive, anti-microbial, anti-oxidant, anti-ulcer, neurological, anti-secretory, cytotoxic, uterine stimulating activity and as a hair growth promoter. As per literature the plant is rich in purine, flavonoids, and tannins. The capability of flavonoids to interact with protein phosphorylation and antioxidant, iron chelating, and free radical scavenging activity may account for the hepatoprotective activity of *Guazuma tomentosa*[2,3,4].

MATERIALS AND METHODS

Plant materials: The leaves of *Guazuma tomentosa* were collected in the month of September from the Veermata Jijabai Bhosale Udyan Byculla, Mumbai and get authenticated at Chinmaya College of Sciences, BHEL, Haridwar. The herbarium of plant is kept in School of Pharmaceutical science, Lovely Professional University. The leaves were washed with tap water followed by drying under shade (temperature 30-40°C). Dried leaves were grinded to coarse powder and packed in suitable container.

Animals: The activity was carried out on Albino wistar rat of either sex which was approved from IAEC (Registration no. 954/ac/06/CPCSEA/11/20) and ethical norms were followed during all experimental procedure.

Preparation of Extracts: Aqueous extract of leaves was prepared using reflux. Alcoholic and dichloromethane extract of leaves were prepared separately using soxlet apparatus.

Preliminary phytochemical screening: Ethanolic, DCM and aqueous extracts were subjected to phytochemical screening to detect presence and absence of secondary metabolite[5].

Estimation of total phenolic contents: Total phenolic content of extracts of *Guazuma tomentosa* was determined by Folin Ciocalteu method. 1 ml of sample solution was mixed with 1ml of Folin Ciocalteu reagent. After 3 min of incubation at room temperature, 1ml of saturated Na_2CO_3 (35% aqueous solution) was added to the mixture, followed by the addition of 7 ml of distilled water. The reaction mixture was kept in dark for 90 min and its absorbance was taken at 725 nm. The total phenolic content of the samples was expressed as gallic acid equivalents (GAE), which reflected the phenolic content as the amount of gallic acid (µg) in 1 mg of sample[6].

Estimation of total flavonoids: It was carried out by aluminium trichloride colorimetric method and 0.5 mL An aliquot (2 ml) of the extract was centrifuged for 5 min at 14000 rpm. 1 mL of aliquot was mixed with 2 ml aluminium trichloride in methanol (2%w/v). Blank was prepared from 1ml of standard solution and diluted to 25 ml with methanolic acetic acid (0.5%v/v). The absorbance of probe solution against standard solution was determined at 420 nm after 30 min. All the determinations were carried out in triplicate. Results were express as total (%) flavonoids contents (TFC) in extracts as quercetin equivalent was calculated by following formula**:**

$$TFC\ (\%) = \frac{\text{Absorbance x Dilution Factor x 100}}{\text{E1\% 1cm x Weight of sample (mL)}}$$

E1% 1cm = Specific absorption of the quercetin $AlCl_3$ complex (500)[7].

Evaluation of invitro antioxidant activity: The antioxidant activity of the plant extract was measured in terms of radical scavenging ability, according to the DPPH method. [43] Methanolic solution (1ml) of sample of various concentrations (10-100µg/ml) was placed in the cuvette and 4ml of 0.004% freshly prepared Methanolic DPPH solution was added and then kept in dark for 50 minutes. This mixture was then shaken vigorously and then absorbance is measured at 520 nm using a U.V. Spectrophotometer and the inhibitory percentage was calculated using the following formula[8].

$$\%\text{inhibitory effect} = \frac{\text{absorbance}_{(control)} - \text{absorbance}_{(sample)}}{\text{absorbance}_{(control)}} \boxed{\text{X 100}}$$

Treatment Protocol: Albino Wistar rats (120-150g) divided into six groups**;** group I (control), group II (CCl_4 treated), group III (CCl_4 + silimarin treated), group IV (CCl_4 + ethanolic extract treated), V (CCl_4 + DCM extract treated) group VI (CCl_4 + aqueous extract treated). Animals of group I were given distilled water via oral route in volume of 10 ml/kg body weight**.** Animals of groups II, III, IV, V, and VI administered 50% (v/v) CCl_4 in olive oil for 14 days via subcutaneous route**.** Simultaneously but at different hour of the day animals of

group III silimarin suspension (10ml/kg) and group IV, V and VI were fed with ethanolic (200 mg/kg) DCM (200 mg/kg) and aqueous extracts (200 mg/kg) extract respectively for 14 days via oral route. Animals were anaesthetized and blood collected by retro orbital puncture. Separation of serum by centrifugation at 3000 rpm for 10 min followed by estimation of biochemical parameters: SGOT, SGPT, Total protein and alkaline phosphatase[8,9].

Assessment of liver function

(SGOT, SGPT, ALP, Total Protein Content)

SGOT (serum glutamic oxaloacetic transaminase)

Procedure: Working reagent was prepared by mixing the reagent in 20 ml aqua and preserved. The reagent was used within a month. 50 µl of the test sample was added to 500 µl of the working reagent and aspirated immediately using Photometer 5010.

SGPT (serum glutamic pyruvic transaminase)

Procedure: Working reagent was prepared by mixing the reagent in 20 ml aqua and preserved. The reagent was used within a month. 50 µl of the test sample was added to 500 µl of the working reagent and aspirated immediately using Photometer 5010.

ALP (alkaline phosphatase)

Procedure: 20 µl of the test sample was added to 1000 µl of the working reagent and aspirated immediately using photometer 5010.

Total protein content

Procedure: 50 µl of serum was added to a mixture of 1000 µl Reagent I and 20 µl Reagent II. The mixture was incubated for 5 min at 37ºC. Total protein content was measured using Photometer 5100. Mixture of 1000 µl of Reagent III and 10 µl of Reagent II was incubated for 5 min at 37ºC and 50 µl of serum was added. Direct protein content was measured using Photometer 5010[8,9].

In vivo antioxidant activity: The in-vivo antioxidant activity of the ethanolic, DCM and aqueous extract were carried out in CCl_4 intoxicated rats. The liver samples collected were washed with chilled normal saline, weighed and 10% (w/v) liver homogenate was prepared in ice cold 0.15 M KCl solution. The suspension was centrifuged at 2000 rpm at 4°C for 10 min and the clear supernatant was used for the estimation of thiobarbituric acid reactive substance (TBARS) and reduced glutathione (GSH)[10].

Estimation of TBARS: TBARS was carried out as an index of lipid peroxidation and measured in terms of malondialdehyde (MDA) content by following the method. MDA and other aldehydes have been identified as product of lipid peroxidation that reacts with TBA to

give pink coloured species that absorbs at 535 nm. The TBARS was determined using the method set by Lowry et al. The value of TBARS were presented as nmol MDA / mg protein[11,12].

Estimation of reduced glutathione (GSH): GSH was estimated using the Ellman reagent (5, 5-dithiobis-2-nitrobenzoic acid) according with Ellman's method and the attained values were expressed as µmoL/g of liver tissue[13].

STATISTICAL ANALYSIS

The result of Assessment of liver function and invivo antioxidant activity are reported as mean ± SEM.

RESULT

Preliminary phytochemical screening showed the presence of blends of chemical constituent as mention in table 1. The total phenolic and flavonoid content was also determined. The overall phenolic content was found to be 137.2 ± 3.25 mg, 112±2.87 and 15±0.86 for ethanolic, DCM and aqueous extract respectively in gallic acid equivalent/g extract and 70.3 ± 3.21, 56±1.94 and 13±0.72 mg for ethanolic, DCM and aqueous extract respectively in quercetin equivalent/g extract, respectively. The SGPT, SGOT, ALP and TP value for control, CCl4, silymarin, ethanolic extract, DCM extract, and aqueous extract is shown in table 2. Free radical scavenging activity is considered to be a good measure of the antioxidant activity of ethanolic, DCM and aqueous extract for new chemical entity and plant extracts. The radical scavenging activity of methanolic, aqueous and DCM extract of *G. tomentosa* leaves increased with increase in concentration and was found to be 84, 83 and 48 % respectively against 93% of ascorbic acid. The Calculated EC_{50} values are 0.190±0.005, 0.168±0.002 and 0.371±0.011for ethanolic, DCM and aqueous extracts. Prevention of oxidative stress to cell or tissue play a major role in prevention of hepatotoxicity. To consider this TBARS and GSH level were determined and reported in table 3.

DISCUSSION

The prepared ethanolic, DCM and aqueous extract was subjected to phytochemical screening because the secondary metabolite present in plant directly related to the biological action. As it is well known fact that phenolic specially flavonoids and tannins are good antioxidant and antioxidant play important role in prevention of hepatotoxicity due to oxidative stress[14,15]. As

Table 1 Phytochemical screening of different extract of *Guazuma Tomentosa*

Phytochemical	Dichloromethane extract	Ethanol extract	Aqueous extract
1.Alkaloids			
a. Wagner's test	+	+	-
b. Hager's test	+	+	-
c. Mayer's test	+	+	-
d.Dragendroff's test	+	+	-
2.Carbohydrates			
a. Molisch's test	-	+	+
b.Fehling's test	-	+	+
3.Glycosides			
a. Borntrager's test	-	-	-
b.Modified	-	-	-
Borntrager's test			
4.Saponin			
Froth test	-	-	-
5.Phytosterols			
a.Salkowski's test	+	+	-
b.Libermann	+	+	-
Burchard's test			
6.Fixed oil & Fats			
Stain test	-	-	-
7.Resins	+	+	-
8.Phenols			
Ferric chloride test	+	+	+
9.Flavonoids			
a.Alkaline reagent test	+	+	+
b.Lead acetate test	+	+	+
10.Diterpenes			
Copper acetate test	-	+	+
11.Tannins			
Gelatin test	+	+	+

Table 1 Phytochemical screening of different extract of *Guazuma Tomentosa*

Phytochemical	Dichloromethane extract	Ethanol extract	Aqueous extract
12.Protein & amino acid			
Ninhydrin test	_	+	+

Table 2

Treatment	Dose (mg/kg)	SGPT (U/ml)	SGOT (U/ml)	ALP (U/ml)	TP (g/dl)
Control	10 ml/kg	66.7±0.20	77.72±2.15	32.75±1.19	3.95±0.32
CCl_4	10 ml/kg	128.5±1.60	171.20±3.45	62.0±0.85	2.85±0.09
Silymarin	10 mg/kg	65.6±0.35	75.60±2.35	32.20±1.30	4.2±0.35
Ethanolic extract	200 mg/kg	72.2±0.65	84.35±2.15	63.25±0.95	4.85±0.20
Dichloromethane extract	200 mg/kg	66.2±1.25	72.60±3.15	69.35±1.20	4.61±0.05
Aqueous extract	200 mg/kg	85.6±1.60	129.20±3.25	68.83±1.15	3.45±0.05

all three extract shows the presence of flavonoid and tannins so total flavonoid and tannins content were determined. Flavonoids prevent tissue injury initiated by free radicals in various ways. One of the simple way is the direct scavenging of free radicals. Flavonoids gets oxidized by radicals which may leads to more stable, less-reactive radical. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical. Due to high reactivity of the hydroxyl group of the flavonoids, radicals are made inactive, according to the following equation.

Flavanoid (OH) + R* → Flavanoid (o^-) + RH

Where $R^{\cdot}$ is a free radical and $O^{\cdot}$ is an oxygen free radical

Like flavonoids and other polyphenolics, tannin also prevent *oH formation[16]. Moreover in-vitro antioxidant activity using DPPH assay were also carried out to prove the hypothesis[17].

Carbon tetrachloride is used to induce hepatotoxicity in the experiment studies. Carbon tetrachloride bio transformed by cytochrome p450 system to produce the trichloromethyl free radicals which in turn covalently binds to cell membranes and organelles to elicit lipid

Table 3

Treatment	Dose	TBARS	GSH
Control	10 ml/kg	0.66 ± 0.036	67.62 ± 5.23
CCl_4	10 ml/kg	2.65 ± 0.0872	11.38 ± 4.73
Silimarin	10 mg/kg	0.58 ± 0.051	62.34 ± 11.28
Ethanolic extract	200 mg/kg	0.59 ± 0.027	61.54 ± 2.65
DCM extracr	200 mg/kg	0.69 ± 0.0332	62.75 ± 11.67
Aqueous extract	200 mg/kg	1.47 ± 0.0875	32.98 ± 5.34

peroxidation which may leads to liver cell necrosis[14.] This is evidenced by the elevation of TBARS and a decrease in the activity of free radical scavenging enzymes such as GSH in CCl4 intoxicated animals. In addition an elevation in the serum marker enzymes is also witnessed. The increase in the level of total protein indicates about pathological condition. An increase in transaminases was a clear indication of cellular leakage and a loss of the functional integrity of the cell membrane. The ethanolic and DCM extract significantly reduced the level of total serum protein indicating hepatoprotection[18]. Glutathione is a naturally occurring tripeptide and a non- enzymatic biological antioxidant that is abundant in many living creatures. It is widely known that deficiency in GSH within living organism can lead to tissue injury and disorder. The increase in the level of MDA in liver induced by CCl4 suggests enhanced lipid peroxidation. This leads to tissue damage and the failure of antioxidant defence mechanisms preventing the formation of excessive free radicals. Antioxidants, especially inhibitors of lipid per-oxidation can prevent such toxicity. Oxidative attack of essential cell components by reactive oxygen species has been directly associated with hepatitis. TBARS low-molecular-weight end products mainly malondialdehyde (MDA) that are formed during the decomposition of lipid peroxidation products. Increased levels of TBARS have been demonstrated in inflammation of liver. GSH play important role in defence processes against oxidative damage. It protects cells against free radicals, peroxides and other toxic compounds[19]. Indeed, glutathione depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects. It is well known fact that deficiency of GSH within living organisms may lead to tissue disorder and injury [20]. In our

study the liver GSH level in the CCl_4 treated group was significantly decreased compared with that of the control group. However, the same parameter for CCl_4 in the ethanolic and DCM extract treated groups were increased compared with the CCl_4 treated group. This means that the situation is sourced by antioxidant feature of *Guazuma tomentosa*l[21]. As the drug is reach in phenolic (flavonoids and tannins) which is prominent antioxidant. Antioxidant compound prevent oxidative stress by oxidising itself instead of cell membrane and macromolecules and may act as hepetoprotective[14]. On the basis of the above results it was concluded that ethanolic and dichloromethane (DCM) extracts were found to be potential source of hepatoprotective compound.

ACKNOWLEDGEMENT

The help and encouragement rendered by the management of Lovely Professional University, Phagwara, for conducting this research is acknowledged.

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