



Hepatoprotective, Therapeutic and *in vivo* anti-oxidant activities of *Tagetes lucida* leaves alcoholic extract against paracetamol-induced hepatotoxicity rats

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Abstract: Objective: Current study was carried out to evaluate the potential therapeutic, hepatoprotective and antioxidant effect of *Tagetes lucida* leaves alcoholic 70% extract using paracetamol-induced liver injury Wistar albino rats.

Methods: Over seven days therapeutic, hepatoprotective and antioxidant effect of *T. lucida* extract at dose 500 mg/kg was tested on Wistar albino rats after or before paracetamol force fed, compared with recommended dose of silymarin standard drug. *T. lucida* therapeutic or hepatoprotective effect evaluated by recommended standard drug; silymarin at recommended dose (25 mg/kg/day).

Results: Force feeding with single dose of paracetamol (800 mg/kg) after or before extract caused liver disorders represented as an elevation on AST and ALT activities and lipid peroxidation. On the other hand, paracetamol decreased all antioxidant defense system; reduced L. glutathione (GSH), glutathione reductase (GR), glutathione -S- transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) of rats compared to normal levels. Administration *T. lucida* extract after or before paracetamol significantly reduced liver enzymes activities; AST and ALT as well as lipid peroxidation with respect of normal status. As well as, Antioxidants defense non-enzymatic (GSH) or enzymatic; GR, GST, GPx, CAT and SOD activities were significantly improved compared with paracetamol controls. *T. lucida* extract caused protective effect like that of silymarin effect. *T. lucida* extract improved liver histology and reduced interstitial hemorrhage and hyaline degeneration of hepatocytes in damaged liver tissue by paracetamol.

Conclusion: *T. lucida* leaves alcoholic extract has a hepatoprotective, therapeutic and antioxidants characters against paracetamol-induced hepatotoxicity with a good safety margin. The possible mechanisms of therapeutic and hepatoprotective properties of *T. lucida* leaves extract are; inhibition of lipid peroxidation, amelioration oxidative stress and increase of enzymatic defense system. This *T. lucida* extract action is evidently originating from relatively high contents of polyphenolic and flavonoids.

Key words: Hepatoprotective, Therapeutic, Antioxidant, *Tagetes lucida* leaves alcoholic extract, Paracetamol, liver functions, histopathology.

1. Introduction

Liver is the biggest solid organ, the largest gland and the main metabolic organ in human body. Liver is responsible for many biological processes like protein synthesis, production of digestion biochemical, detoxification of toxic compound and metabolism of ats. Many medicinal agents cause liver injury, when taken in overdoses and sometimes even in therapeutic doses. Paracetamol (acetaminophen) is the most causes of the liver injury worldwide ¹. Most paracetamol is glucuronidated, passed into bile and excreted, but some is metabolized by the cytochrome P450 pathway into N-acetyl-P- benzoquinone imine (NAPQI), a strong oxidizer that is toxic to liver cells ². The amount of NAPQI produced from a therapeutic dose is small enough to be conjugated to glutathione (GSH) and passed out of the body as bile in urine. A greater dose causes a significant increase in liver cell death due to the oxidative processes mediated by NAPQI increasing mitochondrial glutathione disulfide (GSSG) levels ^{3,4}.

Tagetes lucida Cav., (*Asteraceae*) is a perennial plant native to Mexico and Central America. *T. lucida* common names include sweets cented marigold, Mexican marigold, Mexican mint marigold, Mexican tarragon, Spanish tarragon, cloud plant and winter tarragon ⁵. *T. lucida* is a half-hardy sub-shrub that grows 46-76 cm tall. It is cultured as an oriental flower and its pungent aromatic foliage, which is used as a flavoring. It bears clusters of small yellow flower heads on the ends of the stems. The flower heads are about 1.3 cm across and have 3-5 golden-yellow ray florets. It has been recommended for treating numerous diseases, some of which may associate with altered liver function, such as dysentery, fever, tumors, and gastrointestinal disorders ⁶. Of special relevance, *T. lucida* extracts show significant free radical scavenging compared with tocopherol ⁷. *T. lucida* have been proven as antidepressant agent ^{8,9,10}, platelet anti-aggregant ¹¹, nematocidal agent ¹² and antimicrobial agent ^{13,14}. Essential oils of *T. lucida* possess anti-inflammatory activities by inhibiting both nitric oxide and prostaglandin E2 production ¹⁵.

Although the major essential oil throughout the plant was methyl chavicol ⁵, the major essential oil in leaves was estragole ¹⁶. *T. lucida* also contains flavonoids and coumarins ⁵, of which the major flavonoids in leaves are patuletin, quercetin and quercetagenin ¹³.

This study was carried out to investigate the antioxidant, therapeutic and hepatoprotective effects of an alcoholic extract of *T. lucida* leaves following paracetamol-induced cell damage in Wester Albino rats.

2- Materials and methods

2.1. Chemicals

Paracetamol was provided by EIPICO Company (Egyptian Int Pharmaceutical Industries Company, 10th of Ramadan City, El-Sharkia, Egypt). Silymarin was obtained from CID Company (Egyptian Chemical Industries Development Company, El Haram, Giza, Egypt). All used Kits obtained from BIODIGONESTIC Company (29 El-Tahrer St.- Dokki- Giza- Egypt). Folin-Ciocalteu reagent and Authentic samples was purchased from Sigma-Aldrich Company (St. Louis, MO, USA). All chemicals were analytical grade.

2.2. Plant material and preparation of extract

Plant material used in this study was leaves of *Tagetes lucida* L. The seeds of *T. lucida* L. were imported from Canada Company of Johnny's Selected Seeds-Superior Seeds & Gardening, www.johnnyseeds.com/, (product ID: 2273). Seeds were cultivated at the SEKM Company Farm at Bilbase, El-Sharkya Governorate, Egypt during winter season of 2010. The leaves were collected in May and were air dried. Leaves were coarsely powdered and subjected to 70% ethanol solution and kept at the dark for three weeks. The mixture was filtered and the filtrate was evaporated under reduced pressure with evaporator to obtain crud extract. Resides extract was lyophilized and remained powder was kept in -20°C until using.

2.3. Determination polyphenolics content on *T. lucida* extract

The amounts of total phenolic compounds in *T. lucida* extract was determined using the Folin Ciocalteu assay for total phenolics as described by Gorinstein et al., ¹⁷ with slight modification. The absorbance was read at 760 nm using a UV-vis spectrophotometer (Schimadzu UV/V is-240 IPC). Total phenolic concentrations were expressed as gallic acid equivalents (GAE).

2.4. HPLC analysis of phenolic compounds of *T. lucida* extract

HPLC analysis was carried out according to Kim *et al.*¹⁸ with slight modifications using an Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was Agilent Eclipse XDB C18 (150 x4.6 μm ; 5 μm) with a C18 guard column. The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 mL min⁻¹ for a total run time of 70 min and the gradient program was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. There was 10 min of post-run for reconditioning. The injection volume was 10 μL and peaks were monitored simultaneously at 280, 320 and 360 nm for the benzoic acid, cinnamic acid derivatives and flavonoids compound, respectively. All samples were filtered through a 0.45 μm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectrum and compared with those of the standards.

2.5. Acute toxicity assay

The oral acute toxicity test for *T. lucida* extract was carried out to evaluate any possible toxicity. Swiss albino mice weighted 25 to 30 g (n = 10) were administered with different doses of the extract by increasing or decreasing the dose according to the response of animal¹⁹. The dosing patron was 500, 1000, 2000, 3000, 4000 and 5000 mg/kg while the control group received only the normal saline. All groups were observed for any gross effect or mortality during 24 hours. After 24 hours, alive animals were kept for further 14 days and observed daily for behavioral and body weight changes. The obtained results revealed to *T. lucida* extract was safe up to dose 5000mg/ kg.

2.6. Experimental animals

2.6.1. Animals

This study was approved by Medical Research Ethics Committee, National Research Center, Egypt, under registration no. 15/192. Rats and pellets diet were obtained from central animal house of National Research Centre, Dokki, Giza, Egypt and experiment was carried completely in it. Healthy male Wistar rats of (150-170g) were housed in plastic cages under laboratory conditions (25 \pm 2 C, 60 \pm 5% humidity, 12-12 hour's light/dark cycle) at the animal facility of animal house. Rats were maintained at free food and water *ad libitum*.

2.6.2. The tested dose

The used dose of the extract was estimated by LD₅₀. The extract treated with one dose, which is 1/10 of LD₅₀ (500 mg/kg b. wt. /day). The standard was silymarin drug, which used as hepatoprotective drug, at recommended dose; 25 mg/ kg. Paracetamol was suspended in normal saline 0.9% and orally administrated in dose of 800 mg/kg b. wt.²⁰. All dose prepared in 1.0 ml solution. Rats force fed by these solutions by stomach tube.

2.6.3. Design of the experiment

After adaptation period (1 days) animals were divided into three main groups including negative control group (-ve), positive control groups (+ve) and treated group. The first main group was negative control -ve (6 rats), which were force fed with normal saline (1.0 ml/day for 7 days) and then blood samples and livers were collected. The second main group was the positive control group, was divided into four subgroups (each 6 rats) as follow;

- The first subgroup; rats were force fed with extract at dose 500 mg/kg b. wt. /7 days and then blood samples and livers were collected (extract +ve)
- The second subgroup; rats force fed with recommended dose of silymarin (25 mg/ kg b. wt. /7 days) and blood samples and livers were collected (silymarin +ve).
- The third subgroup; rats were force fed with paracetamol at dose 800 mg/kg b. wt. once at the first experiment period, and were force fed with saline for 7 days and then blood samples and livers were collected to evaluate the therapeutic properties of silymarin and extract (therapeutic control).

- d) The fourth subgroup; rats were force fed with saline for 7 days then were force fed with paracetamol once dose (800 mg/kg b.wt.) and after 48 hrs blood samples and livers were collected to evaluate the hepatoprotective properties of silymarin and extract (hepatoprotective control).

The third main group was treated group, which was divided into four subgroups (each 6 rats) as follow:

1. The first subgroup, rats were force fed with paracetamol at dose 800 mg/kg b. wt. once dose firstly and after 48 hrs rats were force fed with extract at dose 500 mg/kg b.wt. /7 days and then blood samples and livers were collected (extract as a therapeutic agent).
2. The second subgroup, rats were force fed with paracetamol (800 mg/kg b. wt.) once firstly and then rats were force fed with silymarin at dose 25 mg/kg b.wt./ 7 days then blood samples and livers were collected (silymarin as a therapeutic agent).
3. The third subgroup, rats were force fed with extract at dose 500 mg/kg b.wt. / 7 days and then rats were force fed with paracetamol at dose 800 mg/kg b. wt. once dose and then blood samples and livers were collected after 48 hours of paracetamol (extract as a hepatoprotective agent).
4. The fourth subgroup, rats were force fed with silymarin at dose 25 mg/kg b.wt./ 7 days and then were rats force fed with paracetamol at dose 800 mg/kg b. wt. once dose and then blood samples and livers were collected after 48 hrs of paracetamol (silymarin as a hepatoprotective agent).

2.6.4. Samples of blood and livers

At the end of the experimental period, animals were fasted for overnight. Following anesthesia, blood samples were obtained from retro- orbital plexus. The blood samples centrifuged (4000 g, 10 min by using Sigma labor zentrifugen), and serum was separated. Following sacrifice, each liver was separately washed in ice-cold 1.15% KCl solution, blotted and weighed. A piece of liver from each rat was separately homogenized in homogenizing buffer (ice-cold Tris-HCl buffer, 0.1M, pH 7.4), using ultrasonic homogenizer. The resulting homogenate in each case was centrifuged at 4000g for 15 minutes at 4 °C by using Sigma labor zentrifugen²¹ for antioxidant enzymes analysis and lipid peroxidation parameter. Another piece of liver was kept immediately in 10% formalin for pathological examination.

2.6.5. Biochemical analysis

Liver function of rats were determined in sera samples spectrophotometrically using a UV-vis spectrophotometer (Schimadzu UV/Vis-240IPC). Liver enzyme biomarkers; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated according to Reitman and Frankel,²².

Non-enzymatic antioxidant; GSH concentration was measured spectrophotometry at 405nm³ and the unit of concentration was mg/g tissue using Ellman's reagent (5,5'-dithiobis 2-nitrobenzoic acid; DTNB), which was reduced by thiol groups to form 1 mol 2-nitro 5-mercaptobenzoic acid/mol thiol and with maximal absorption at 412 nm. Antioxidant enzymes of liver were determined in liver homogenate spectrophotometrically. Glutathione reductase (GR) activity was measured according to Goldberg and Spooner,²³ and the amount of the enzyme reducing 1µmol GSSG per min per mg protein was regarded 1 activity unit. Glutathione -S- transferase (GST) activity was measured according to Habiget *al.*,⁴ and the amount of the enzyme that conjugate 1, chloro-2, 4- dinitrobenzene with reduced glutathione per min per mg protein was regarded 1 activity unit. Glutathione peroxidase (GPx) activity was assayed according to Paglia and Valentine,²⁴ and the amount of the enzyme converting 1µmol GSH per min per mg protein was taken as 1 activity unit. Catalase (CAT) activity was determined by following decomposition of H₂O₂ according to the methods Beers and Sizer²⁵. Superoxide dismutase (SOD) was measured according to Fridovich,²⁶ as the reduction suppression rate of nitrotetrazolium blue and for 1 unit of activity, the amount of protein was taken which provided 50% inhibition of nitrotetrazolium blue reduction under standard conditions.

Malondyaldehyde (MDA) the lipid peroxidation biomarker determined in sera and liver homogenate was assayed according to Ohkawa *et al.*,²⁷ and the unit of concentration was µmol/ g tissue.

2.7. Histopathological studies

Specimens of all animals were dissected immediately after death and fixed in 10% neutral-buffered formal saline for 72 hours at least. All the specimens were washed in tap water for half an hour and then

dehydrated in ascending grades of alcohol (70% - 80% - 90% and finally absolute alcohol), cleared in xylene, impregnated in soft paraffin wax at 55°C and embedded in hard paraffin. Serial sections of 6 µm thick were cut and stained with Hematoxylin and eosin ²⁸ for histopathological investigation. Images were captured and processed using Adobe Photoshop version 8.0

2.8. Statistical analysis

Data were analyzed by one-way ANOVA test for comparisons among means at $p \leq 0.05$.

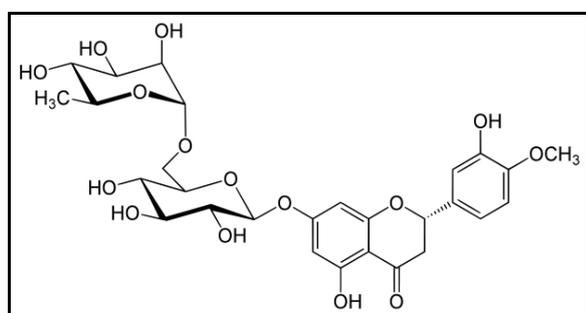
3. Results

3.1. Polyphenolic content and composition of *T. lucida* extract

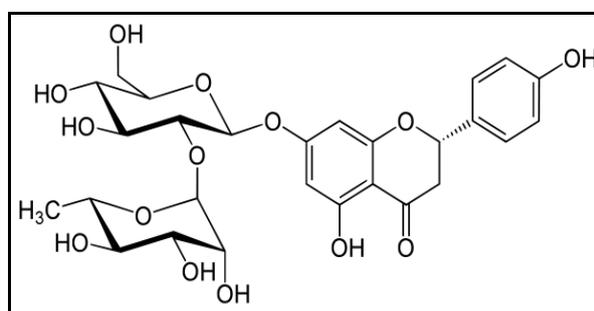
The *T. lucida* alcoholic 70% extract contains polyphenolics about 15.31±0.90 g as gallic acid/100 g extract. HPLC analysis showed sixteen compounds in the extract as presented in Table (1). Hesperidin was the most predominant phenolic compound in the extract (171.379 mg hesperidin/g extract), followed by naringin (96.730 mg naringin/ g extract) (Fig 1).

Table 1. The polyphenolics composition of *Tagetes lucida* alcoholic 70% extract (mg/g extract).

Compound	mg/g
Gallic acid	1.944
Protochatchuic acid	11.627
Syrngic acid	15.271
Vanillic acid	2.985
Coumarin	14.689
Naringin	96.730
Hesperidin	171.379
Chyrsin	12.273
Chlorogenic acid	7.185
Caffeic acid	3.842
Ferulic acid	39.623
Sinapic acid	25.091
Rosmarinic acid	32.589
Rutin	10.264
Qurecetin	13.735
Kaempferol	2.123



Hesperidin



Naringin

Figure. 1 the structure of the major components in *Tagetes lucida* leaves alcoholic extract.

3.2 Effect of *T. lucida* extract on relative weight of liver

Paracetamol administration caused significant reduction on relative weight of liver (g/100 g b. wt.) when force fed at the first or the end of experiment in comparison with \bar{v} e control (Data showed in Fig. 2). *T. lucida* extract either therapeutic agent or hepatoprotective one elevated the relative weight of liver rats compared with each paracetamol control. Relative weight of liver of treated with extract still close to that of \bar{v} e control ($P < 0.05$, LSD 0.58).

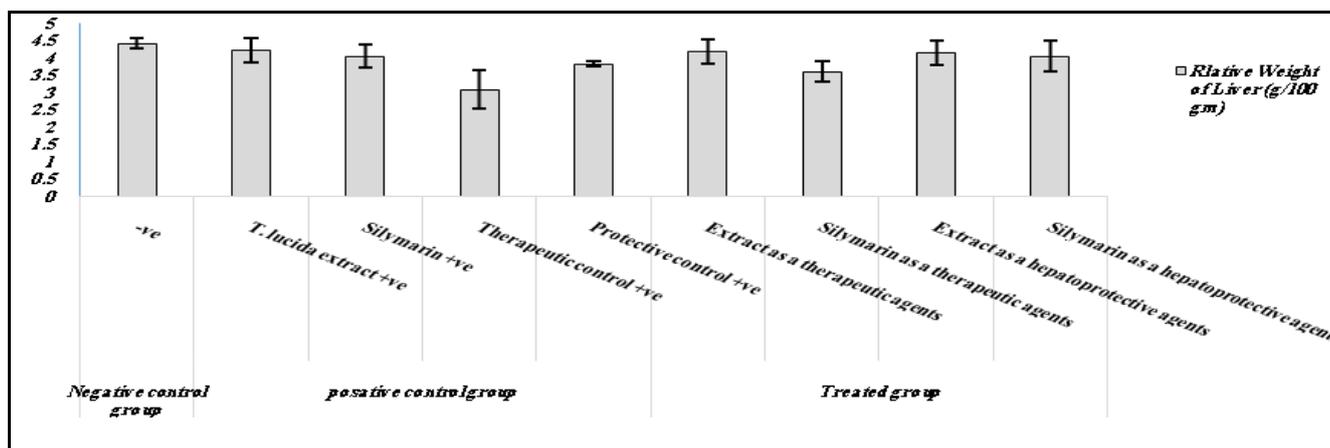


Figure 2. Influence of *Tagetes lucida* leaves extract on relative weight of liver of paracetamol-induced rats liver injury.

Data are presented as the means \pm S. D of three replicates. Data analyzed by ANOVA, $P \leq 0.05$, Therapeutic control was rats force fed with paracetamol at the first of experiment, while protective control was rates force fed at the end of experiment.

3.3. Effect of *T. lucida* extract in liver enzymes (AST and ALT) activities

Paracetamol force feeding either in the first or in the end caused significant elevation in AST and ALT activities compared with \bar{v} e control (Table. 2). *T. lucida* extract as a therapeutic agent or a hepatoprotective agent caused significant reduction on AST and ALT of both groups in comparison with each controls. Silymarin as a therapeutic agent or hepatoprotective agent showed the same effect. The \bar{v} e of silymarin or *T. lucida* extract did not change ALT significantly, with respect of \bar{v} e control. *T. lucida* extract \bar{v} e recorded significant decrease on AST, compared with \bar{v} e control. *T. lucida* extract as a hepatoprotective agent was more effective than that as a therapeutic agent. *T. lucida* extract was more effective on ALT than that on AST. The protective effect of *T. lucida* extract was outperformed than silymarin ($P < 0.05$).

Table 2. Liver enzymes activities of paracetamol-induced rat's liver injury treated with *Tagetes lucida* leaves alcoholic extract.

Group	Subgroups	AST (U/L)	ALT (U/L)
Negative control	-ve	85.0 ^c ±4.3	51.1 ^c ±3.5
Positive control group	<i>T. lucida</i> extract ^{+ve}	78.8 ^d ±4.0	49.9 ^c ±0.7
	Silymarin ^{+ve}	64.6 ^e ±5.4	53.3 ^c ±4.3
	Therapeutic control ^{+ve}	96.4 ^a ±5.32	59.6 ^b ±3.0
	hepatoprotective control ^{+ve}	97.0 ^a ±4.4	72.7 ^a ±2.9
Treated group	<i>T. lucida</i> as a therapeutic agents	92.7 ^{ab} ±3.4	53.5 ^c ±3.5
	Silymarin as a therapeutic agents	89.3 ^{bc} ±4.1	48.9 ^c ±3.2
	<i>T. lucida</i> as a hepatoprotective agents	89.9 ^{bc} ±6.9	52.0 ^c ±3.4
	Silymarin as a hepatoprotective agents	86.0 ^c ±4.7	70.2 ^a ±3.0
LSD _{0.05}		5.20	5.54

Data are presented as the means ±S.D of three replicates, Data analyzed by ANOVA, $P \leq 0.05$, Value with the same letter has no significant but value with different letter has significant at 0.05. therapeutic control was rats force fed with paracetamol at the first of experiment, while hepatoprotective control was rates force fed at the end of experiment. AST; aspartate aminotransferase, ALT; alanine aminotransferase.

3.4. Effect of *T. lucida* extract on liver antioxidants

Paracetamol administration caused remarkably decrease on antioxidant non-enzyme or enzymes system of paracetamol ^{+ve} controls as compared with -ve control. Therapeutic control recorded significant reduction on GSH concentration (57.66%), and specific activity of antioxidant enzymes; GR (58.82 %), GPx (58.57%), GST (57.90%), CAT (43.83%) and SOD (42.86%) in comparison with -ve control ($P < 0.05$). Hepatoprotective control showed the same observation and all of them were reduced significantly; GSH (40.88%), GR (41.18%), GPx (42.86%), GST (41.23%), CAT (24.90%) and SOD (42.86%), compared with -ve control (Table. 3) ($P < 0.05$).

Significant elevation was recorded in GSH concentration of rats treated with *T. lucida* extract or silymarin after paracetamol administration (156.90 and 131.03% respectively) compared with therapeutic control. The same trend was noticed in hepatoprotective effect of *T. lucida* extract and silymarin, which significantly increased GSH concentration (183.95 and 87.65% respectively) with respect of hepatoprotective control. *T. lucida* extract caused significant increment on GSH concentration of ^{+ve} rats about 57.66%, compared with -ve control ($P < 0.05$).

T. lucida extract either therapeutic agent or hepatoprotective one ameliorated antioxidant system in liver significantly compared with both paracetamol controls. GR was significantly elevated on rats force fed paracetamol before extract (157.14%) and those force fed paracetamol after extract (180.00%) as compared with each control. GR of *T. lucida* extract ^{+ve} control rats was significantly increased (58.82%) compared with -ve control ($P < 0.05$). *T. lucida* extract effect on GR was similar with that of silymarin, and the effect of *T. lucida* extract suppressed that of silymarin.

T. lucida extract showed significant therapeutic characters corresponding as significant increment on GST of rats compared with therapeutic control. Protective characters of extract recorded as significant increment of GST of rats in comparison with protective control. *T. lucida* extract improved GST of ^{+ve} control rats also (57.90%) more than silymarin and -ve control. No significant differences were observed between the effect of *T. lucida* extract and silymarin on GST ($P < 0.05$).

Table 3. Glutathione concentration and antioxidant enzymes activities of paracetamol induced - liver injury Wister Albino rats treated with *Tagetes lucida* leaves alcoholic extract.

Group	Subgroups	GSH (mg/g liver tissue)	GR (umol /mg protein /min)	GST (umol/ mg protein/min)	GPx (umol /mg protein/min)	SOD (U/ mg protein)	CAT (U/ mg protein)
Negative control	̄ve	1.37 ^b ±0.11	1.7 ^b ±0.1	1.14 ^b ±0.09	0.70 ^b ±0.05	8.4 ^c ±0.6	16.95 ^b ±0.63
Positive control group	<i>T. lucida</i> extract ^{+ve}	2.16 ^a ±0.17	2.7 ^a ±0.2	1.80 ^a ±0.15	1.09 ^a ±0.09	16.2 ^b ±0.8	18.37 ^b ±1.18
	Silymarin ^{+ve}	1.38 ^b ±0.23	1.7 ^b ±0.1	1.15 ^b ±0.10	0.70 ^b ±0.09	14.4 ^c ±0.9	19.07 ^b ±1.06
	Therapeutic control ^{+ve}	0.58 ^c ±0.07	0.7 ^c ±0.1	0.48 ^c ±0.07	0.29 ^c ±0.04	4.8 ^e ±0.7	9.52 ^d ±0.58
	hepatoprotective control ^{+ve}	0.81 ^c ±0.07	1.0 ^c ±0.1	0.67 ^c ±0.06	0.40 ^c ±0.02	7.2 ^f ±0.4	12.73 ^e ±0.63
Treated group	<i>T. lucida</i> as a therapeutic agents	1.49 ^b ±0.10	1.8 ^b ±0.1	1.24 ^b ±0.09	0.75 ^b ±0.01	19.2 ^a ±0.7	18.21 ^b ±0.64
	Silymarin as a therapeutic agents	1.34 ^b ±0.24	1.7 ^b ±0.1	1.12 ^b ±0.15	0.67 ^b ±0.07	13.2 ^c ±0.7	16.87 ^b ±0.80
	<i>T. lucida</i> as a hepatoprotective agents	2.30 ^a ±0.13	2.8 ^a ±0.2	1.92 ^a ±0.11	1.16 ^a ±0.07	16.9 ^b ±0.8	20.88 ^a ±1.18
	Silymarin as a hepatoprotective agents	1.52 ^b ±0.25	1.9 ^b ±0.1	1.2 ^b ±0.14	0.76 ^b ±0.10	11.9 ^d ±0.8	17.80 ^b ±1.21
LSD _{0.05}		0.32	0.39	0.26	0.16	1.22	1.57

Data are presented as the means \pm S. D of three replicates. Data analyzed by ANOVA, $P \leq 0.05$, Value with the same letter has no significant but value with different letter has significant at 0.05. Therapeutic control was rats force fed with paracetamol at the first of experiment, while hepatoprotective control was rates force fed at the end of experiment. GSH; glutathione L- reduced, GR; glutathione reductase, GST; glutathione-S-transferase, GPx; glutathione peroxidase, CAT; catalase, SOD; superoxide dismutase.

The therapeutic and hepatoprotective properties of *T. lucida* extract was pronounced as a significant raise on GPx of rats administrated paracetamol before or after administration extract (158.62 and 190.00% respectively) compared with each control. As well as, GPx of extract ^{+ve} control rats did not affect significantly with respect of ̄ve control. Silymarin took the same trend of *T. lucida* extract and elevated GPx of all treated groups ($P < 0.05$).

T. lucida extract and silymarin as a therapeutic agents caused significant elevation on SOD of rats by about; 300.00 and 175.00% respectively with respect of therapeutic control; 4.80 ± 0.7 U/ mg protein. The same raise recorded on protective rats treated with *T. lucida* extract or silymarin (134.72 and 65.28% respectively), compared with hepatoprotective control; 7.20 ± 0.40 U/ mg protein. SOD of ^{+ve} controls rats were treated with *T. lucida* extract was elevated significantly by about 92.86% as compared to ̄ve control; 8.40 ± 0.60 U/ mg protein ($P < 0.05$).

T. lucida extract either therapeutic agent or protective agent recorded remarkably elevation on CAT levels of therapeutic rats (91.33%) or hepatoprotective rats (63.80%) as compared with each control. *T. lucida* extract had the same effect of silymarin on CAT. CAT of ^{+ve} control rats did not change significantly by *T. lucida* extract or silymarin, compared with ̄ve control ($P < 0.05$).

Generally, *T. lucida* extract restored liver non-enzymatic antioxidant; glutathione (GSH) and enzymatic; GST, GR, GPx, SOD and CAT of all rats treated by extract better than those of ̄ve control. The highest value of GSH, GR, GST, GPx, CAT and SOD recorded on rats force fed with extract firstly and then administrated paracetamol, compared with those of ̄ve control. The protective properties of *T. lucida* extract were pronounced than the therapeutic one. *T. lucida* extract as a hepatoprotective agent outperformed silymarin hepatoprotective effect on antioxidants liver system.

3.5. Effect of *T. lucida* extract on lipid peroxidation biomarker

Administration paracetamol raised serum and liver lipid peroxidation biomarker; malondialdehyde (MDA) significantly of therapeutic control (45.26 and 46.15% respectively) or hepatoprotective control (115.79

and 38.46% respectively) with respect of \bar{v} e control. *T. lucida* extract as a therapeutic agent did not change serum MDA significantly, compared with therapeutic control. Contrary, *T. lucida* extract and silymarin as a hepatoprotective agent significantly declined serum MDA (49.27 and 80.98% respectively), compared with protective control ($P < 0.05$, LSD; 1.31) data showed in Table 4.

Table 4. MDA in serum and liver homogenate of paracetamol-induced rat's liver injury treated with *Tagetes lucida* leaves alcoholic extract.

Group	Subgroups	MDA	
		Serum (nmol/mL)	Liver (nmol/g)
Negative control group	\bar{v} e	9.5 ^{de} ±0.7	2.6 ^b ±0.6
Positive control group	<i>T. lucida</i> (+ve)	8.5 ^e ±0.7	2.2 ^{bc} ±0.7
	Silymarin (+ve)	8.7 ^e ±0.9	2.8 ^b ±0.4
	Therapeutic control (+ve)	13.8 ^b ±0.7	3.8 ^a ±0.6
	Hepatoprotective control (+ve)	20.5 ^a ±0.9	3.6 ^a ±0.7
Treated group	<i>T. lucida</i> as a therapeutic agents	13.4 ^b ±0.9	2.0 ^{bc} ±0.6
	Silymarin as a therapeutic agents	11.4 ^c ±0.6	2.4 ^{bc} ±0.1
	<i>T. lucida</i> as a protective agents	3.9 ^f ±0.3	1.8 ^{bc} ±0.2
	Silymarin as a protective agents	10.4 ^{cd} ±0.8	1.6 ^c ±0.2
LSD _{0.05}		1.31	0.90

Data are presented as the means \pm S.D of three replicates. Data analyzed by ANOVA, $P \leq 0.05$, Value with the same letter has no significant but value with different letter has significant at 0.05. Therapeutic control was rats force fed with paracetamol at the first of experiment, while hepatoprotective control was rats force fed at the end of experiment. MDA; malondyaldehyde.

T. lucida extractor silymarin as a therapeutic agent caused significant reduction on liver MDA (47.37 and 36.84% respectively) of rats, compared with the therapeutic control. Both of *T. lucida* extract and silymarinas a protective agent significantly decreased liver MDA (50.00 and 55.56% respectively), compared with protectivecontrol. There is no significant difference noticed among all groups treated by *T. lucida* extract or silymarin ($P < 0.05$). MDA of serum and liver of +ve controls of extract or silymarin rats did not significantly change compared with \bar{v} e control. *T. lucida* extract showed therapeutic and hepatoprotective properties against lipid peroxidation on serum and liver more than silymarin drug.

3.6. Effect of *T. lucida* extract on Histopathological examination of liver

Histopathological investigation revealed that paracetamol caused marked damage to liver tissue in the form of dilatation and congestion of central veins with thickening of their walls, large areas of interstitial hemorrhage and hyaline degeneration of many hepatocytes (Fig. 3). These results were reduced greatly by the use of *T. lucida* extract as a therapeutic agent giving better results than silymarin, where dilatation and congestion of blood vessels was reduced and the hepatocytes retained their normal shape and structure except for a few cells that showed acidophilic cytoplasm. By using silymarin as a therapeutic agent, mild degree of degeneration in hepatocytes, pyknotic nuclei, inflammatory cells and mild dilatation of blood vessels were still observed. Best results were achieved by using *T. lucida* extract as a hepatoprotective agent being also better than those obtained by using silymarin as a hepatoprotective agent. By using *T. lucida* extract the liver tissue almost regain its normal architecture, while by using silymarin a few hepatocytes were still suffering from acidophilic cytoplasm or slight degeneration.

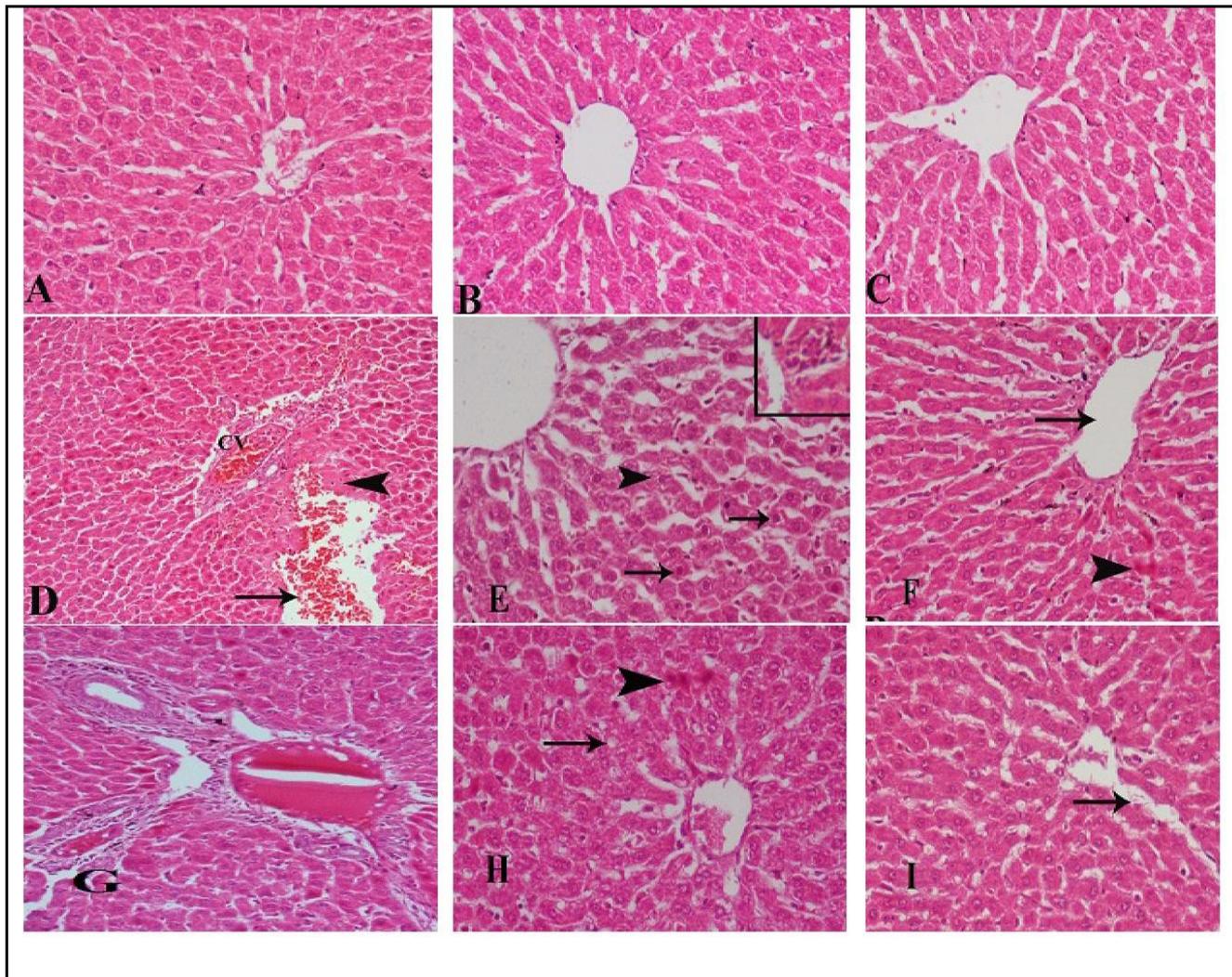


Fig. 3. A photomicrograph of sections of liver tissue.

- a. Negative control rat (ve) showed the normal structure of the liver tissue.
- b. Silymarin ⁺ve control rats received silymarin only showed a quite normal architecture of liver tissue.
- c. Extract ⁺ve control rats received *T. lucida* extract only showed slight dilatation of blood sinusoids.
- d. a therapeutic control rats, force fed by paracetamol firstly and after 48 hours received saline for 7 days showed dilatation with congestion of central vein (CV), areas of interstitial hemorrhage (arrow) and hyaline degeneration of some cell.
- e. Rats force fed by paracetamol firstly and after 48 hours received silymarin as a therapeutic agent showed only a few cells with pyknotic nuclei (arrow), mild degeneration (arrow head). The upper right corner of the figure showed some inflammatory cells beside central vein.
- f. Rats force fed by paracetamol firstly and after 48 hours received *T. lucida* extract as a therapeutic agent shows amelioration of liver tissue except for a few acidophilic cells (arrow head) and slight dilatation of blood vessels (arrow).
- g. Hepatoprotective control rats received saline for 7 days and then force fed by paracetamol at the end showed dilatation and congestion of blood vessels with fibrosis around.
- h. Rat received silymarin firstly for 7 days as a hepatoprotective agent and then force fed by paracetamol, showed only few cellular changes in the form of acidiphication of cytoplasm (arrow head) or slight degeneration (arrow).
- i. Rat received *T. lucida* extract firstly for 7 days as a hepatoprotective agent then force fed by paracetamol showed normalization of liver.

4. Discussion

Paracetamol with a high dose often causes acute hepatocellular necrosis with high morbidity and mortality²⁹. Paracetamol is not toxic but it's convert via the cytochrome P₄₅₀ to a highly toxic metabolite, N-acetyl-p-benzoquinone-imine (NAPQ1)²⁹. NAPQ1 depletes glutathione (GSH) by conjugation and excreting it in the urine. Accumulation NAPQ1 causes mitochondrial dysfunction developing to acute hepatic necrosis. GSH depletion enhances the expression of tumor necrosis alpha (TNF α). TNF α primes phagocytic NADPH+ H⁺ oxidase to the increase production of oxygen free radicals and leads to liver damage. AST and ALT enzymes release into blood circulation as a result of necrosis or membrane damage, which means cellular leakage and loss of functional integrity of cell membrane in liver³⁰.

In this report, the paracetamol-induced hepatotoxicity rats model was used to reflect disorders of the liver function. Over single dose of paracetamol (800 mg/kg) showed to elevate on the serum ALT, AST and serum and liver lipid peroxidation with respect of -ve control. The same dose reduced liver antioxidant; non-enzymatic (GSH) and enzymatic (SOD, CAT, GR, GPx and GST), compared to -ve control. Paracetamol force feeding decreased relative weight of liver in comparison with -ve control.

Force feeding with *T. lucida* extract (500 mg/kg for 7 days) was effectively protect all obvious parameters against paracetamol-induced hepatic toxicity. The treatment with *T. lucida* extract either as a therapeutic agent or as a hepatoprotective agent prevented the necrotic and the others histopathological changes induced by paracetamol treatment. *T. lucida* extract restored AST, ALT and serum and liver MDA equal or lower than those of -ve control. The same dose causes an elevation on antioxidant enzymes of liver: SOD, CAT, GR, GPx and GST, and non-enzymatic antioxidant GSH, which means recovering in the paracetamol-induced hepatic toxicity of rats. The protective properties of *T. lucida* extract were more pronounced than therapeutic one. *T. lucida* extract effect as a protective or as a therapeutic agent were similar with those of silymarin or outperformed that. These results agreed with those reported by Ibrahim *et al.*,³¹ on *Origanum syriacum*, Parmar *et al.*,³² on *Phyllanthus niruri*, Maytenus *emarginata*, *Eclipta alba*, *Aloe Vera*, *Solanum indicum* and *Aegle marmelos*, Soni *et al.*,³³ on *prunus domestica*, Danladi *et al.*,³⁴ on ethanolic extract of *Telfairia occidentalis* leaves, Aluko *et al.*,³⁵ on *Ocimum americanum* L. leaves, Grespan *et al.*,³⁶ on *Thymus vulgaris* essential oil and Sivakumar *et al.*,³⁷ on *Solanum xanthocarpum*, Rehman *et al.*,³⁸ on *Alhagim aurorum* Boiss, .

T. lucida extract significantly decreased the AST and ALT towards normal level, indicating that *T. lucida* extract recovered the structural integrity of the hepatocellular membrane and liver cell damage caused by paracetamol. The effect of *T. lucida* extract was nearly with the effect of silymarin. The serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes.

Lipid peroxidation has been implicated in the pathogens is of increased membrane rigidity, reduced erythrocyte survival and perturbation in lipid fluidity³⁹. MDA is the famous end products of lipid peroxidation process. Paracetamol administration enhanced lipid peroxidation leading to liver damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals, indicating by increasing MDA content. The significant decrease on MDA of rats treated with *T. lucida* extract indicated its ability to break the chain reaction of lipid peroxidation. Based on these results, we may suggest that the therapeutic and hepatoprotective potential of *T. lucida* extract is dependent on an antioxidant mechanism. These results agreed with those of Soni *et al.*,³³ on *prunus domestica*, Fakurazi *et al.*,⁴⁰ on *Moringa oleifera*, Datta *et al.*,⁴¹ on *Cyperus articulatus* Linn., Saleem *et al.*,⁴² on *Opuntia monacantha*, Ho *et al.*,⁴³ on *Vernonia amygdalina* water extract and El-Newary *et al.*,⁴⁴ on *Cordia dichotoma* fruits extract.

Antioxidant defense mechanisms of liver can neutralize free radicals. Antioxidant defense mechanisms of liver classified into enzymatic antioxidants (SOD, GR, GPx, GST and CAT) and non-enzymatic antioxidants (GSH)⁴⁵. The non-enzymatic antioxidant; GSH is one of the most abundant tripeptides present in the liver. The main functions of GSH are remove free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals, and upkeep membrane protein thiols which, is a substrate for GPx and GST. A significant decrease in GSH concentration which is an important factor in the paracetamol induced toxicity. Our study has shown that the level of GSH was restored following 800 mg/kg/day *T. lucida* extract administration to the nearly same concentration of normal control.

Superoxide dismutase (SOD) is the main antioxidant enzyme, which stabilizes oxidative reactions. SOD plays a role in the elimination of ROS derived of xenobiotics in liver tissues. Decreasing SOD activity is a sensitive index in hepatocellular damage⁴⁶. The observed increase of SOD activity in this study suggests that the *T. lucida* extract have an efficient protective mechanism in response to ROS and free radical-mediated tissue damage.

Catalase (CAT) is a common enzymatic antioxidant in all animal tissues. CAT decomposes H₂O₂ by dismutating it to H₂O and O₂ and protects the tissues from highly reactive hydroxyl radicals⁴⁷. Therefore, reduction in CAT activity causes a deleterious effect in hepatocellular. *T. lucida* extract increases the level of CAT as silymarin, the standard hepatoprotective drug.

Glutathione peroxidase (GPx) is a member of peroxidases enzyme whose function is to detoxify peroxides in cells. GPx play an important role in scavenging free radical and protecting cell from damage. GPx catalyzes the reduction of H₂O₂ to organic peroxides using glutathione²⁴. *T. lucida* extract increased GPx activity in all treated rats with 500 mg/kg/day extract higher than those treated with silymarin or that of normal control.

Glutathione reductase (GR) is the enzyme, which reduces the oxidized glutathione (GSSG) to reduced GSH⁴⁸. The level of GSH and the activities of GSH dependent enzymes were reduced significantly in rats administered with paracetamol. The depression in GR and GPx contents makes the cells more susceptible to toxic compounds. Administration of *T. lucida* extract caused an elevation of GR and GPx levels in liver homogenate, which make cell more protected.

Glutathione-S-transferase (GST) is multifunctional enzyme, which is responsible for cellular detoxification. GST protective cells by conjugating toxicant with glutathione, that way neutralizing their electrophilic sites, and making it more water soluble and excreted. Once formed, 4-hydroxynonenal (4-HNE) protein adduct is rapidly degraded by three major reactions: reduction by alcohol dehydrogenase, aldehyde dehydrogenase or formation of glutathione conjugate (GS-HNE) catalyzed by GST. The majority of 4-HNE is metabolized through forming GS-HNE⁴⁰. Thus, GST may play an important role in obstruction the formation of 4-HNE protein adduct with cellular macromolecules. *T. lucida* extract administration caused highly significant elevation on GST of normal, therapeutic and hepatoprotective rats treated with extract.

The main antioxidant activity and ability to scavenge free radical formation has been associated with phenolic content⁴⁹. Confirmation, phenolic compounds enhance production of GST and other antioxidant enzymes⁵⁰. Naringin inhibits some drug-metabolizing cytochrome P450 enzymes,⁵¹. *T. lucida* extract have high polyphenol content about 15.31±0.90%. HPLC analysis confirmed that hesperidin was the major component in extract (171.379 mg hesperidin/ g extract) followed by naringin (96.730 mg naringin / g extract).

Flavonoids are hepatoprotective, membrane-stabilizing and antioxidant properties. Flavonoids scavenge free radicals and prevent lipid peroxidation⁵². Flavonoids antioxidant and hepatoprotective activities attributed to epigallocatechin gallate⁵². Gallic acid report as antioxidant and hepatoprotective agent. Eid, et al.⁵³, reported that Coumarin-3-carboxylic acid and dicoumarol were shown to stimulate choleresis in rats. *T. lucida* contains coumarins in dry herb ranged from 1.2 to 1.95 mg/100 g dry herb⁵.

5. Conclusion

The present study scientifically confirms that potent antioxidant, therapeutic and hepatoprotective properties of *T. lucida* leaves alcoholic extract effect against paracetamol-induced liver injury Wistar Albino rats. Extract normalized liver functions and antioxidants defense system of liver concurrently with reduction on lipid peroxidation and amelioration in liver histopathology examination. The possible mechanisms of therapeutic and hepatoprotective properties of *T. lucida* leaves extract are; inhibition of lipid peroxidation, amelioration oxidative stress and increase of enzymatic defense system. Therefore, paracetamol-liver cell damage showed medicament of biological parameters, structural integrity of the hepatocellular membrane, healing of hepatic parenchyma and the regeneration of hepatocytes. This *T. lucida* extractation is evidently originating from relatively high contents of polyphenolic and flavonoids.

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