INVESTIGATION OF THE PHYTOCHEMICAL COMPOSITION AND HEPATOPROTECTIVE EFFECT OF LEUCANTHEMOPSIS TRIFURCATUM GROWING IN LIBYA

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ABSTRACT

Leucanthemopsis trifurcatum is common to Mediterranean countries and widely used in traditional medicine especially for north African countries. Due to the scarcity of researches about the pharmacological activities of *L. trifurcatum*, we intended to screen the *L. trifurcatum* ethanolic extract (LEE) for hepatoprotective effects against paracetamol induced chronic toxicity. LEE is subjected to preliminary phytochemical and pharmacological screening. Hepatotoxicity is induced in rats by chronic oral administration of paracetamol (PCM) for 30 days. LEE (300 and 500 mg/kg), and silymarin (25 mg/kg) were administered orally, for 30 days, along with PCM to explore their hepatoprotective activities. Phytochemically, LEE revealed the presence of alkaloids, carbohydrates, and flavonoids as well as nine separated spots on TLC paper using toluene : ethyl acetate: formic acid (5:4:1) solvent system. The treatment with LEE (300 and 500 mg/kg, p.o.) caused hepatoprotective activity in a dose dependent manner through significant reduction of liver enzymes (AST, ALT, and ALP) compared to paracetamol toxicity. 500 mg/kg of LEE exerted a profound hepatoprotective action comparable to silymarine. This activity may be attributed to the synergistic action between the phytochemical constituents of LEE.

KEYWORDS: *Leucanthemopsis trifurcatum*, antinflammatory, hepatoprotective, analgesic, anticonvulsant, phytoconstituents.

INTRODUCTION

The *Leucanthemopsis* genus, golden flower in Greek, belongs to the Asteraceae family and includes about 300 species. This genus synthesizes and accumulates a variety of secondary metabolites such as pyrethroids, monoterpenoids, sesquiterpenoids, triterpenoids, flavonoids, coumarins, steroids, phenolics, purines, lipids, and aliphatic compounds[1-5].

Leucanthemopsis trifurcatum (Desf.) are annual or rarely biennial herbs with small yellow flowers and distributed in North African countries; Morocco, Algeria, Tunisia, and Libya[6,7]. The species, *Leucanthemopsis trifurcatum*, has been known in Algeria and Tunisia as "gueredfa" or "ouazouaza". The heads of plant flower are locally used as health diet such as "assida" or "chorba". This plant is used traditionally in Tunisia to treat constipation, hepatic disorders, intestinal transit problems and to ameliorate women pain after parturition. In Libyan traditional medicine, the aerial parts of *L. trifurcatum* are used as an anti-inflammatory and antispasmodic agent[1,8].

Many studies has demonstrated the potential medicinal effects including antibacterial, antifungal, anti-HSV1, and antioxidant effects for some Chrysanthemum species[3,4,9-11], However, a few number of studies has investigated the biological activities and phytochemically analysed *L. trifurcatum* extracts[1,3,8,5,12].

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To the best of our knowledge, the secondary metabolites of *L. trifurcatum* growing in Libya as well as its pharmacological activities have not been studied yet,. We carried out this study to analyze the phytoconstituents and investigate the hepatoprotective activity of Libyan *Leucanthemopsis trifurcatum.*

MATERIALS AND METHODS

Collection and authentication of plant material:

For research and experiment, we collect fresh aerial parts of plant *Leucanthemopsis trifurcatum* from the basin of the Mediterranean Sea, Libya in May 2014, which was authenticated by Dr. Huda Elgubbi, Department of Botany, College of Science, Misurata University, Misurata, Libya.

Extraction:

The air-dried aerials parts of plant of *L. trifurcatum* were prepared as a coarse powder and 500 g of this powdered material was macerated with distilled water and 95% w/v alcohol separately for 24 hrs and 72 hrs, respectively. Then, the extract were filtered through a muslin cloth and the resultant filtrates were concentrated under reduced pressure and vacuum dried. The yield of ethanolic extract was 13 % w/w. The extract were reconstituted in their extraction solvent to give the required concentration needed in this study. Freeze-dried extracts were collected in small glass bottles and kept at 30° C for further evaluation.

Preliminary Phytochemical Screening and thin Layer Chromatography (TLC):

Preliminary phytochemical screening for the detection of various phytoconstitunts such as alkaloids, carbohydrates, steroids, flavonoids, saponins, and

glycosids was carried out by using standard procedures described by Harborne *et al*[13]. Thin layer chromatography studies of the extracts of *L. trifurcatum* carried out in various solvents at 30^{O} C using pre-coated plates silica gel 60 F_{254}, 7X6 cm (Merck)as adsorbent and the $R_f$values were determined.

Experimental Animals:

Thirty male and female Wistar rats (90-110 days) weighing 200-300 g. The animals were divided into groups and kept in plastic cages (47 * 34 * 18 cm) under a 12 h light/12 h dark cycle at room temperature (22 °C), with free access to standard ration and water. Animal care and the experimental protocol followed the principles and guidelines suggested by Faculty of Pharmacy-Misurata University and were approved by the local ethical committee.

Hepatoprotective Activity:

For evaluation the hepatoprotective activity of LEE against paracetamol-induced liver injury in experimental rats, 30 rats were used and randomly assigned to 5 groups (6 animals per group) and treated orally for 30 consecutive days. Group 1 served as normal control and received tween 80 (1%) (10 ml/kg body weight); groups 2 served as hepatotoxic control and received Paracetamol (PCM) (500 mg/kg); group 3 served as standard drug treatment group and received silymarin (25 mg/kg) + 500 mg/kg PCM; groups 4 received LEE (300 mg/kg) + 500 mg/kg PCM; groups 5 received LEE (500 mg/kg) + 500 mg/kg PCM. PCM was administered one hour before the administration of either silymarin or the LEE At the end of the treatment period all animals were eusthetized with ether and blood was collected into plain dry tubes and centrifuged at 2,000 rpm for 10 min.

Biochemical analysis:

The serum samples obtained were transferred into Eppendorff tubes and were analyzed by INTEGRA 400 plus for estimation of liver enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline Phosphatase (ALP), using commercially available kits (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical Analysis:

The results obtained were presented as mean ± standard error (SE). The significance of differences between means were analyzed statistically with one way analysis of variance (ANOVA; 95% confidence interval) and LSD post hoc tests using PSPP program (Linux operating system). Values of $p<0.05$ were taken to imply statistical significance.

RESULTS

Preliminary Phytochemical Screening and thin Layer Chromatography (TLC):

The preliminary phytochemical screening for the *L. trifurcatum* extracts showed the presence of Alkaloids, carbohydrates, and flavonoids. The tests also revealed that the absence of glycosids, saponins,steroids in LEE (table 1).

(Table 1) Qualitative phytochemical screening of CEE.

Constituents	CEE
Alkaloids	+ve
Carbohydrates	+ve
Glycosids	-ve
Flavonoids	+ve
Saponins	-ve
Steroids & Terpenes	-ve

Keys: +ve (Present), -ve (Absent).

Thin layer chromatography of the extracts of *L. trifurcatum* was carried out using toluene: ethyl acetate: formic acid (5:4:1) as mobile phase respectively and the *Rf* values were recorded (table 2). The peaks were visualised at a wavelength of 366 nm in UV chamber.

(Table 2) R_f values of TLC solvent system for CEE.

Solvent system	No. of spots	R_f values	Visualizing agent
Toluene: ethyl acetate: formic acid (5:4:1)	09	0.13, 0.17, 0.48, 0.55, 0.615, 0.73, 0.82, 0.88, 0.92.	UV-366 nm

Hepatoprotective Effect:

Chronic oral PCM (500 mg/kg) induced significant elevation in hepatic serum markers, AST, ALT, and ALP in rats. Meanwhile, oral administration of LEE (300 and 500 mg/kg) together with PCM reduced significantly, in a dose dependant manner, the increase in these hepatic markers in comparison to PCM treated group. Treatment with 500 mg/kg of LEE showed non significant differences with SLM (25 mg/kg) in serum AST , ALT, and ALP levels. These results are shown in (table 3).

(Table 3) Effect of LEE (300 and 500 mg/kg), and Silymarin (SLM) (25 mg/kg) on serum hepatic markers in PCM-induced chronic hepatotoxicity in rats:

Group	Liver Function Test		
	ALT (U/L)	ALP (U/L)	AST (U/L)
Control	51.20 ± 6.76^{a}	82.00 ± 16.81^{a}	85.00 ± 11.18^{a}
LEE 300 mg/kg + PCM (500 mg/kg)	93.00 ± 4.58^{b}	219.80 ± 9.36^{b}	652.60 ± 175.11^{b}
LEE 500 mg/kg + PCM (500 mg/kg)	76.80 ± 5.40^{c}	150.00 ± 20.26^{c}	510.20 ± 60.57^{c}
SLM (25 mg/kg) + PCM (500 mg/kg)	65.40 ± 9.76^{c}	120.60 ± 36.15^{c}	415.40 ± 49.22^{c}
PCM (500 mg/kg)	155.60 ± 16.33^{b}	229.40 ± 24.29^{d}	933.60 ± 191.87^{d}

Different letters indicate significant difference between means result ($p \geq 0.05$).

DISCUSSION

Natural products and their derivatives have been known for many years as a source of therapeutic agents and of structural diversity[14]. A bout 40% of all medicines is either natural products or their sem-

isynthetic derivatives. Clinical, pharmacological, and chemical studies of these traditional medicines, which were derived predominantly from plants, were the basis of most early medicines such as aspirin, digitoxin, morphine, quinine, and pilocarpine[15]. The phytochemical screening of *L. trifurcatum* has shown the presence of various bioactive constituents[2,3] which are responsible for most pharmacological activities of plants[16]. The results of the present study also supplement the folkloric usage of the *L. trifurcatum* which contain numerous known and unknown bioactive compounds with bio-activity. Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents. The chromatographic profile may serve as a characteristic finger print for qualitative evaluation of *L. trifurcatum.*

In the present study, the ethanol extracts of aerial parts of *L. trifurcatum* was observed to exhibit hepatoprotective effect as demonstrated by a significant decrease in serum marker liver enzymes AST, ALT, and ALP level in rat induced with paracetamol hepatotoxicity. Analgesic drug paracetamol is commonly used model for screening hepatoprotective drugs. It has been shown that high doses of paracetamol produce centrilobular liver necrosis in in experimental animals and human[17,18]. For screening of hepatoprotective agents, paracetamol-induced hepatotoxicity has been used as a reliable method. Paracetamol is primarily metabolized by sulfation and glucuronidation, but as the dose is increased, these pathways become saturated and a greater proportion of the drug is available for oxidation by the microsomal cytochrome P-450 system. Moreover, induced liver toxicity and cell death is due to formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450 to a highly reactive metaboliteN-acetyl-p-benzoquinoneimine.
(NAPQI)[19,20]. NAPQI produces oxidative stress and cause glycogen and glutathione depletion by irreversible conjugation with sulfhydral groups of glutathione, which leads to increased lipid peroxidation by abstracting hydrogen from a polyunsaturated fatty acid and ultimately, liver damage due to higher doses of paracetamol[21,22] The glutathione protects hepatocytes by combining with the reactive metabolite of paracetamol, thus preventing covalent binding to liver proteins[33]. Reactive metabolites can exert initial cell stress through a wide range of mechanisms including depletion of glutathione (GSH) or binding to enzymes, lipids, nucleic acids and other cell structures[24-26].

In the estimation of liver damage by paracetamol, the determination of these enzyme levels, especially ALT is largely used. It is generally acknowledged that administration of paracetamol caused a significant elevation of enzymes level such as AST, ALT and ALP, it has been attributed to damage structural integrity of liver, because they are cytoplasmic in location and released into circulation after cellular damages indicating development of hepatotoxicity. [27-39]. However, administration of the crude ethanol extract of *L. trifurcatum* whole plant (LEE) at various 300 and 500 mg/kg doses mediated a reduction in the levels of AST, ALT and ALP towards the normal value. This indicates a stabilization of plasma membrane as well as repair of hepatic tissue damage caused by paracetamol. This effect is in consonance with the common view that serum level of transaminases returns to normal following healing of hepatic parenchyma and regeneration of hepatocytes[30].

Silymarin is a well-known hepatoprotective compound isolated from Silybum marianum is reported to possess a protective effect on plasma membrane of hepatocytes and have multiple inhibitory multiple mechanisms of actions against different hepatotoxic agents. The antioxidant property and cell regenerating functions as effect of increased protein synthesis were considered as most important actions of silymarin[31]. Our study shows that ethanolic extracts of aerial parts of *L. trifurcatum* at higher dose (500 mg/kg) is comparable with standard drug Silymarin. Hepatoproective activites of LEE may be due to the antioxidant potential of flavonoid compounds as has been demonstrated from *Chrysanthemum trifurcatum*[1] and some other closely related plant species such as *Chrysanthemum balsamita*[32,33], *Chrysanthemum fontanesii*[34], *Chrysanthemum indicum*[34,35]. Moreover, a number of scientific studies of other plants indicated that contain flavonoids, triterpenoids and steroids have protective effects on liver due to its antioxidant properties[36-42].

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