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HEPATOPROTECTIVE AND GASTRO PROTECTIVE STUDIES OF *Terminalia arjuna* LEAVES EXTRACT AND PHYTOCHEMICAL PROFILE

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Abstract. The methanol extract of *T. arjuna* leaves was evaluated as hepatoprotective and gastroprotective against Swiss albino rats. In hepatoprotective study the liver damage induced by paracetamol while the gastric lesion induced by absolute ethanol in the gastro protective study. The extract was given orally (250,500 and 1000 mg/kg) in different experimental models in both studies. The extract at dose (500mg/kg) showed significant reduction in ALT serum level by 19.9% while at dose (1000mg/kg) it reduced significantly serum ALT, AST and ALP levels by –26.15,–25.46 and –23.69 % respectively as compared with paracetamol treated group. In the gastro protective study the extract produced significant reduction in the number and severity of mucosal lesion by (–52.1 &–67.3 %, –66.7 &–71.2 % and –68.8 &–77.6%) respectively.

Key words: *T. arjuna*, hepatoprotective, gastroprotective, mucosal lesion.

Introduction

Liver diseases considered one of worldwide problem with major threats to public health up till now there is no effective drug available that stimulates liver function, offer protection to the liver from damage or help to regenerate hepatic cells.

The medicinal plants and their derivatives are still used all over the world in one form or another for the treatment of liver diseases.

The main constituents in liver protective plants are mainly glycosides, flavonoids, triterpenes and phenolic compounds [ADEWUSI and AFOLAYAN 2010].

Gastric ulcer is an illness that affects a considerable number of people worldwide.

Peptic ulcers are caused when natural balances between aggressive factors of acid and pepsin and defensive mechanisms of mucus, bicarbonate, mucosal turnover and blood supply (mucosal barrier) are disturbed [MAHMOOD *et al*., 2010].

Genus Terminalia belongs to family Combretaceae which is mostly found in tropical and subtropical regions.

In Egypt several Terminalia species have been introduced since 1890 and grown as shade and ornamental trees in public gardens and streets [YOUSIF, 1996].

In Indian system of medicine the bark is used as astringent, cooling, aphrodisiac, cardiotonic, tonic, anti ulcer, spermatorrhoea, leucorrhoea, diabetes, cough, tumour, excessive perspiration, asthma, inflammation and skin disorders [PAARAKH, 2010].

Recently, it has been reported that *T. arjuna* exhibit hypocholesterolemic [RAM *et al.*, 1997], hypolipidemic [SHAILA *et al.*, 1998], antioxidant, antimutagenic [KAUR, 2001], antibacterial [SAMY *et al.*, 1998], antiviral [CHENG *et al.*, 2002], and anticancer activities [NAGPAL *et al.*, 2000]. The bark is responsible for nearly all medicinal uses of *T. arjuna* [GUPTA *et al.*, 2001, CHENG *et al.*, 2002] and there is a little bit of work done on leaves so, it will be of great interest to investigate gastro and hepatoprotective activity of *T. arjuna* leaves.

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Material and methods

Plant material

The leaves of *T. arjuna* (Combretaceae) were collected from El–Zohria Botanical Garden, Giza, Egypt.

In March 2007, and were kindly authenticated by, Dr. Salwa El–Kawatchy, Department of Plant Systematic, National Research Centre (NRC) Giza, Egypt and by Mrs. Tereez Labib Consultant of Plant Taxonomy at Ministry of Agriculture and Director of Orman Botanical garden.

A voucher specimen was deposited in the herbarium of El–Zohria Garden, Giza, Egypt.

Preparation of the extract:

The air–dried powdered leaves of *T. arjuna* (1.6 kg.) were extracted by maceration with 70 % MeOH (3 × 3 L) at room temperature, and the extract was filtered and evaporated to give residue (140 g). The crude extract (100 g) was suspended in H_2O (250 mL) and partitioned with hexanes (6 × 500 mL), DCM (6 × 500 mL), EtOAc (6 × 500 mL), and n–BuOH (6 × 500 mL). The extracts were evaporated to yield 19, 16, 8.6, and 14 g, respectively.

Each extract was tested for the presence of the phytoconstituents according to following standard tests, Molisch's test for carbohydrates, Shinoda test for flavonoids, forth test for Saponins, Salkowski's for terpenes and sterols, $FeCl_3$, and Mayer's reagents for detecting of tannins and alkaloids, respectively [SOFOWRA, 1993; TREASE, and EVANS, 1989; HARBORNE, 1973, ROSS *et al.*, 2013].

Reagents

Drugs and Chemicals

The 1–Paracetamol (Paramol) (Misr Co. for pharmaceutical industries, Cairo, Egypt). 2–Silymarin (SEDICO, for pharmaceutical industries, Cairo, Egypt).

They were used in the experiments.

The doses employed were based upon the human dose after conversion to that of rat according to Paget and Barnes. [PAGET and BARNES 1964]. 3–absolute ethanol (AL–Gomhoryia Co. chemical industry)2–2 Biochemical kits: ALT (Alanine aminotransferase) (Biodiagnostic, Egypt) AST (Aspartate aminotransferase). (Biodiagnostic, Egypt) ALP (alkaline phosphatase). (Biodiagnostic, Egypt).

Animals

Adult rats of both sexes weighing 150–200gm were used in the experiments.

Animals were housed under standardized conditions of light and temperature and received standard rat chow and tap water ad libitum.

Animals were randomly assigned to different experimental groups each of six, kept in separate cage.

All animal procedures were performed after approval from the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals [NIH publication No. 85–23, revise 1985].

Hepatoprotective study

Thirty six rats were divided into six groups of six animals each as following:

Group 1: Normal control group received a daily oral dose of 1ml saline.

Group 2, 3, 4: Received a daily oral dose of methanol extracts of *T. arjuna* leaves (250, 500 and 1000 mg/kg) alone for successive 7 days before paracetamol injection (1000 mg/kg)

Group 5: Received single oral dose of paracetamol (1000 mg/kg) according to [SILVA *et al.*, 2005].

Group 6: Received a daily oral dose of Silymarin (25 mg/kg) alone also for successive 7 days before paracetamol injection (1000mg/kg) used as a reference drug.

At the end of the experimental period (24h after paracetamol injection), the blood was obtained from all groups of rats after being lightly anaesthetized with ether by puncturing rato–orbital plexus [SORG and BUCKNER 1964], the blood was allowed to flow into a clean dry centrifuge tube and left to stand 30 minutes before centrifugation to avoid hemolysis.

Then blood samples were centrifuged for 15 minutes at 2500, rpm the clear supernatant serum was separated and collected by Pasteur pipette into a dry clean tube for the following biochemical tests: Alanine

aminotransferase (ALT) accord to [BERGMEYER *et al.*, 1985].

Aspartate aminotransferase, (AST) accord to [KLAUKA *et al.*, 1993].

Serum Alkaline phosphatase (ALP) accord to [TIETZ and SHUCY 1986].

Gastroprotective study

Gastric lesions was induced in rats by ethanol (1 mL of 100 % orally).

Rats were divided into four groups each of six, one group received ethanol as control, and the remaining groups received 70 % methanol extract of *T. arjuna* leaves (250, 500 and 1000 mg/kg) one hour before ethanol was given.

Rats were killed 1/2 h after ethanol administration by cervical dislocation after being lightly anaesthetized with ether and stomach was excised, opened along the greater curvature, rinsed with saline, extended on a plastic board and examined for mucosal lesions.

The number and severity of mucosal lesions were noted and lesions were scaled as follows:

–Petechial lesions = 1
–lesions less than 1 mm = 2
–lesion between 1 and 2 mm = 3
–lesions between 2 and 4 mm = 4
–lesions more than 4 mm = 5.

A total lesion score for each animal is calculated as the total number of lesions multiplied by respective severity scores. Results were expressed as the severity of lesions/ rat [MÓZSIK *et al.*, 1982].

Results and discussion

Hepatoprotective study

Both concentrations 500 mg/kg and 1000 mg/kg of the70% methanol extracts of *T. arjuna* leaves showed significant reduction in elevated serum ALT levels by –19.9, –26.15 % while The extract at concentration 250 mg/kg didn't achieve significant improvement in AST, ALT or ALP enzyme levels.

Only dose 1000 mg/kg showed significant reduction in serum AST and ALP levels by –25.46 and –23.69 % respectively as compared with paracetamol treated group which exhibited significant serum elevation of ALT, AST and ALP by 43.14, 32 and 38.48% respectively indicating enzymes leakage due to disturbed transport function of hepatocytes as a result of paracetamol hepatotoxicity leading to and increased serum levels.

Both doses 500 and 1000 mg/kg gave statistically significant improvements in AST, ALT and ALP when compared to paracetamol group.

The over dosage of paracetamol is known to be hepatotoxic in experimental animals, at normal doses, about 80% of it is eliminated mainly as sulphate and glucoronide conjugates before oxidation and only 5% is oxidized by hepatic cytochrom P450 (CYP2E1) to a highly reactive and toxic electrophile N–acetyl–p–benzoquineimine (NAPQI), glucoronidation and sulfation routes become at over dosage of paracetamol saturated and as a consequence, paracetamol is increasingly metabolized into NAPQI [DAR *et al.*, 2012].

The elevated Serum ALP levels are related to function of hepatic cell and indicating presence of billary pressure.

So it could be concluded that *T. arjuna* extract at concentration 1000mg/kg improve secretory mechanism of hepatocytes.

$$\text{No. of lesions change\%} = \frac{\textit{No. of lesion post drug administration} - \textit{No. of lesion after ethanol administration}}{\textit{No. of lesion after ethanol adminstration}} \times 100$$

$$\text{Severity of lesion change\%} = \frac{\textit{severity of lesion post drug administration} - \textit{severity of lesions after ethanol adminstration}}{\textit{severity of lesions after ethanol adminstration}} \times 100$$

In hepatic cells ALT, AST found in higher concentration in cytoplasm and ALT particularly in mitochondria.

So *T. arjuna* at concentration 500, 1000 mg / kg significantly reduce the elevated levels of AST, ALT and so consequently stabilize plasma membrane as well as repair hepatic tissue damage caused by paracetamol.

This is probably because of free radical scavenging activity of flavonoids

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and polyphenols present in *Terminalia arjuna* [DOORIKA and ANANTHI 2012].

Phytochemical screening of leaves of *T. arjuna* revealed the presence of Flavonoids which have been previously reported to have hepatoprotective activity.

Table 1.

Effect of oral administration 70 % methanol extracts of *T. arjuna* W. and A.(250 mg, 500 mg and 1000 mg/kg) on ALT, AST and ALP serum level in paracetamol induced hepatotoxicity in rats, (n=6).

Group	ALT(IU/L) X ± S.E	% of change	AST(IU/L) X ± S.E	% of change	ALP(IU/L) X ± S.E	% of change
Saline 1mL	42.23±2.1	———	63.74±1.5	———	47.17 ± 2.4	———
Paracetamol 1000mg/kg	60.45±3.5●	43.14	84.14±2.8●	32.00	65.32±3.1●	38.48
70% MeOH extract (250mg /kg)	56.31±2.3	33.34	80.2±5.1	25.82	62.71±4.2	32.94
70% MeOH extract (500mg /kg)	50.64±2.2*	19.91	72.99±3.5	14.51	56.85±2.4	20.52
70% MeOH extract (1000mg /kg)	44.64±1.8*	5.71	62.72±1.9*	–1.60	49.84±3.2*	5.66
Silymarin 25mg/kg	43.91±1.4*	3.98	62.89±3.2*	–1.33	50.73±2.3*	7.55

Values represent the mean ± S.E. of six animals for each group.
● = P <0.05: Statistically significant from saline control group.
* = P <0.05: Statistically significant from paracetamol group by using (Student´s t test)
Percent of change was calculated as regard saline control group.

We reported previously the isolation of flavonoids apigenin, luteolin, Vitexin Isovitexin luteolin 3`glucrornic from the *T. arjuna* leaves [ROSS *et al.*, 2013].

So the hepatoprotective effect of *T. arjuna* leaves.

May be due to it's flavonoids content. The results were recorded in table 1 and showed in figure 1.

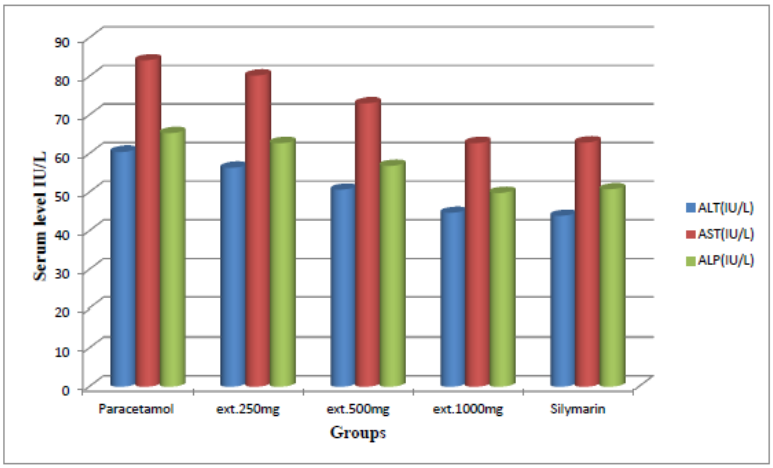

Figure 1. Effect of oral administration 70 % methanol extracts of *T. arjuna* W. and A. (250 mg, 500 mg and 1000 mg/kg) on ALT, AST and ALP serum level in paracetamol induced hepatotoxicity in rats.

Gastro protective study

All tested concentrations (250,500 and 1000mg/kg) of the70% methanol extracts of *T. arjuna* leaves showed significant reduction by –52.1 & –67.3 %, –66.7 &– 71.2 % and –68.8 &– 77.6 % in number and severity of gastric lesions induced by absolute ethanol in control group respectively.

Ethanol is commonly used for induction of ulcer in experimental rats; it leads to intense gastric mucosal damage resulting in increased vascular permeability, edema formation and produces necrotic lesions in gastric

mucosa by its direct toxic effect, reducing secretion of bicarbonates and production of mucus.

It is well known that many flavonoids display anti–secretory and cytoprotective properties in different experimental models of gastric ulcer.

Flavonoids possess anti–oxidant properties in addition to strengthening mucosal defense system through stimulation of gastric mucus secretion it also can scavenge for reactive oxygen species (super–oxide anions) and free radicals produced by ethanol which are potentially implicated in ulcerogenicity [MAHMOOD, 2010].

Tannins could also prevent ulcer development via their abilities to complex proteins where it precipitate micro proteins at ulcer sites, protecting under laying mucosa from irritation they also showed strong radical scavenging activities [HASLAM, 1996].

Phytochemical screening of leaves of *T. arjuna* revealed presence tannins which we reported previously its isolation (Gallic acid, Ellagic acid and methyl gallate) from *T. arjuna* leaves [ROSS *et al.*, 2013].

Table 2.

The effect of oral administration of 70% methanol extracts *T. arjuna* W. and A. (250, 500 and 1000 mg/kg) on gastric mucosal injury induced by 100 % ethanol (1 ml) in rats (n=6).

Group	Number of lesions/ Rat X±S.E	% Change	Severity of lesions/ Rat X ±S.E	%Change
Ethanol 100%	9.6 ±0.8	———	19.6 ±1.5	———
70% MeOH extract (250mg /kg)	4.6 ± 0.3*	–52.1 %	6.4 ±0.5*	–67.3 %
70% MeOH extract (500mg /kg)	3.2 ±0.2*	–66.7%	5.6 ±0.4*	–71.2 %
70% MeOH extract (1000mg/kg)	3 ± 0.1*	–68.8 %	4.4 ±0.3*	–77.6 %

Data represent the mean value ± SE of six rat per group. Statistical comparison of difference between ethanol control group and treated groups. Values represent the mean ± S.E. of six animals for each group. P< 0.05: Statistically significant from Control. (Dunnett's test)

Oxidative stress plays an important role in the pathogenesis of various diseases including gastric ulcer.

The antioxidants play a significant role in protection of gastric mucosa against various necrotic agents it could be concluded that the gastroprotective effect exerted by *T. arjuna* could be attributed to its antioxidant property which counteract oxidative damage caused by absolute ethanol toxicity.

And it's the ability to strength mucosal barrier, which is first line of defense against endogenous and exogenous ulcerogenic agents.

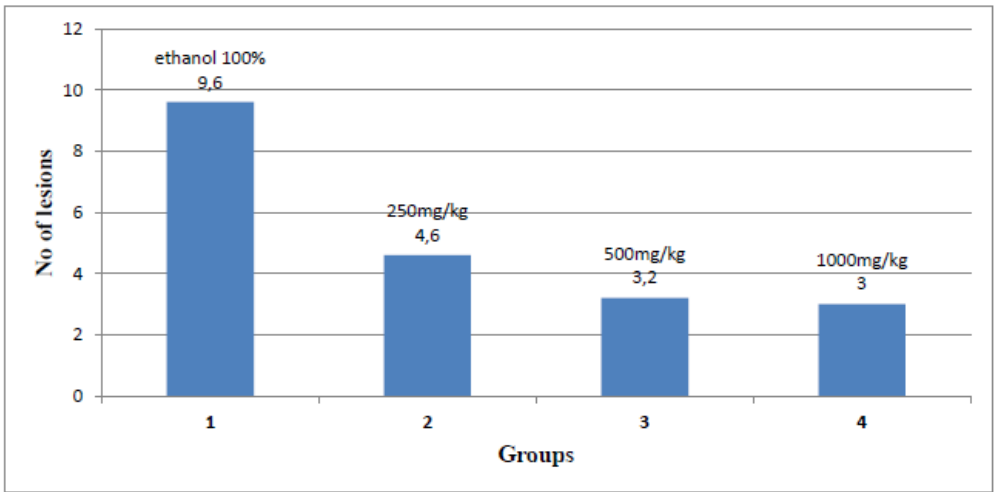

Figure 2. The effect of oral administration of 70 % methanol extracts *T. arjuna* W. and A. (250, 500 and 1000 mg/kg) on No. of lesions in gastric mucosal induced by 100 % ethanol

The results were recorded in table 2 and showed in figure 2. The phytochemical analysis revealed presence of flavonoids, tannins, sterols

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/or triterpenes, carbohydrate/or glycosides and absence of alkaloids table 3.

Table 3.

Results of phytochemical screening of the extracts of *T. arjuna* W. and A. leaves.

Constituents	Dichloromethane	Ethyl acetate	70% MeOH
Carbohydrate /or glycosides	–	–	+
Coumarins	–	–	–
Flavonoids	–	+	+
Alkaloids	–	–	–
Sterols/or triterpenes	+	–	+
Saponins	±	–	–
Anthraquinone	–	–	–
Tannins	–	–	+

(+): present, (–): absent, (±): traces

Conclusions

T. arjuna powdered leaves was extracted by maceration with 70% MeOH, the phytochemical screening revealed the presence flavonoids and tannins as major constituents.

The 70% MeOH extract showed very promising gastro protective activity in all tested concentrations also *T. arjuna* 70 % methanolic extract showed a dose dependant hepatoprotective effect and at dose 1000 mg/kg managed to render the liver enzyme levels back to normal and its effect at that dose is similar to Silymarin.

The Hepato and Gastro protective activities of 70% MeOH extract of *T. arjuna* leaves t is due to their flavonoids and tannins contents.

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