

Original Article

PHYTOCHEMICALS INVESTIGATION AND HEPATO-PROTECTIVE STUDIES OF IRAQI *BRYONIA DIOICA* (FAMILY CUCURBITACEAE)

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ABSTRACT

Objective: many herbal remedies have been employed in various medical systems for treatment and management of different diseases. The plant *Bryonia dioica* has been used in different system of traditional medication for the treatment of diseases and ailments of human beings. The current study is therefore reviewed to provide requisite phytochemical and pharmacological detail about the plant.

Methods: preliminary qualitative phytochemical screening of various secondary metabolites by a specific chemical tests was carried out on the ethanolic extract of the leaves plant part, then, general procedure for extracting the plant leaves using 80% ethanol in soxhlet apparatus was done. The plant leaves extract was evaluated for its efficacy in rats by inducing hepatotoxicity with CCl₄. Single oral dose of 250mg/kg of different fractions extract was given to rats for 7 days. Serum activities of transaminases (ALT and AST) were used as the biochemical marker of hepatotoxicity. Histopathological changes in rat's liver section were also examined.

Results: The results of the study indicated that this plant part contained alkaloids, flavonoids, anthraquinoin, sterols and terpenoids also the pretreatment of rats with *Bryonia* extract before the hepatotoxins agent (CCl₄) offered a hepato- protective action.

Conclusion: It can be concluded that this study is a good step to show that *Bryonia* extract possess a potent hepato-protective activity against CCl₄ induced liver damage in rats.

Keywords: *Bryonia dioica*, Phytochemistry, Hepato-protective effect, Medicinal plants

INTRODUCTION

Professor H.L.Chakravarty mentioned in his book "Plant Wealth of Iraq" that there are more than three thousands species of plants in Iraq. He mentioned also that about 1500 species are of economical value. Those economic plants were classified as: plants needed for basic food; others of medicine and drug industry needs, which are called as "medicinal plants". In addition, there are a large number of plants that are considered as raw materials for numerous transformative industries [1]. Quite a large number of medicinal and poisonous plants occur in Iraq, which are mostly used for home remedies. Investigation and study of the active constituents of these plants might bring good revenue for the drug industries; analysis of some of wild drugs gave very satisfactory results. Of these wildly grown and widely distributed plant species, Iraqi *Bryonia dioica* (Family Cucurbitaceae) which was chosen for this study. *Bryonia dioica* Jacq. a climbing perennial herb with tuberous roots which occurs in temperate Europe, North Africa, and western Asia [2], belongs to the genus *Bryonia* in which some species may contain cytotoxic cucurbitacines [3]. *Bryonia dioica* is used for both internal and external uses [4] It is taken orally in small quantities for the treatment of various inflammatory conditions, bronchial complaints, asthma, intestinal ulcers, hypertension and arthritis. Externally, it is applied as a rubefacient to muscular and joint pains and pleurisy. In Iraq it has been reported that the plant is used in folk medicine to treat bronchitis and as anti-diabetic agent [5], also traditional healers use the leaves and the seeds of this plant for treatment of fevers. This plant is generally and popularly considered toxic to humans. This study was undertaken to determine the scientific basis for the traditional uses of *Bryonia dioica* as hepato-protective plant. In addition, the phytochemical composition was studied.

Plant material

The whole plant of *Bryonia dioica* of the Family Cucurbitaceae was collected from Najaf, a city in Iraq about 160 km (roughly 100 miles) south of Baghdad. The plant was authenticated by the National

Herbarium at Abu-Graib, the plant leaves were dried in the shade for several days at room temperature and then grinded as powder and weighed.

The experimental work is divided into

- The experimental preliminary phytochemical screening of various secondary metabolites like alkaloids, flavonoids, steroids, tannins, saponins, anthraquinoin, terpenoids and cardiac glycosides) in the leaves *Bryonia* plant.
- Extraction of different active constituents.
- Investigation of the some pharmacological activity of the plant

Preliminary qualitative phytochemical analysis

Chemical tests were carried out using the ethanolic extracts from plants and or the powdered specimens, using standard procedures to identify the active constituents. [6-8]

Test for alkaloids

Alcoholic extract (10 ml) was stirred with 5 ml of 1% HCL on a steam bath. Mayer's (1.35gm mercuric chloride in 60ml water + 5gm potassium iodide in 10ml water)and Wagner's reagents (1.27g of iodine and 2g of potassium iodide in 100ml of water) were added, white and reddish brown color precipitate respectively, were taken as evidence for the presence of alkaloids.

Test for flavonoids

- Lead acetate test:** Lead acetate 10% (1 ml) solution was added to 5ml of alcoholic extract, The formation of a yellowish- white precipitate was taken as a positive test for flavonoids.
- NaOH test:** The extract (5 ml) was treated with aqueous NaOH and HCl, and looking for the formation of a yellow orange color.

Tests for steroids

- (i) **Liebermann-Burchard test:** Extract (3ml) was treated with chloroform, acetic anhydride and drops of sulphuric acid was added. The formation of dark pink or red color indicates the presence of steroids.
- (ii) **H₂SO₄ test:** The development of a greenish color was considered as indication for the presence of steroids, when the organic extract (2 ml) was treated with sulphuric and acetic acids.

Test for tannins

Plant material (10mg) in 10ml distilled water was filtered, then the filtrate (3ml) + 3ml of FeCl₃ solution (5%w/v) were mixed. The formation of a dark green or blue black precipitate was considered an indication for the presence of tannins.

Tests for anthraquinones

Borntrager's test: 3ml of alcoholic extract was shaken with 3 ml of benzene, filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the development of a pink, red or violet color in the ammonical (lower) phase indicates the presence of free anthraquinones.

Test for terpenoids

Alcoholic extract (2ml) was dissolved in chloroform (2ml) and evaporated to dryness. Concentrated sulphuric acid (2ml) was then added and heated for about 2 min. A grayish color was considered an indication for the presence of terpenoids.

Test for cardiac glycoside

Keller-kiliani test: Alcoholic extracts (2ml) +1ml glacial acetic acid+ FeCl₃+con.H₂SO₄. Formation of green-blue color indicates the presence of cardiac glycoside.

Preparation of extract

Shade-dried coarsely powdered leaves parts were defatted with hexane for 24 hours then allowed to dry at room temperature. The defatted plant materials was extracted with 80%ethanol in soxhlet apparatus until complete exhaustion. The alcoholic extract was evaporated under reduced pressure at a temperature not exceeding 40 C to give a dark-brown residue designated as a crude extract.

Investigation of the some pharmacological activity of the plant (Hepatoprotective studies)

1. Experimental animals

Eighteen – Albino male rats weighting 150-200 gm were used in this study. Animals were kept in the animal house, under standardized condition (12 hr light dark cycle at room temperature). The animals were fed standard chow and given water and libitum.

2. Experimental design

The animals were divided in to three groups (each group has 6 animals) and treated as follows:

Group (1): Six rats received normal saline for 7 day orally and secreted at along 7, saved as control

Group (2): Six rats received single oral dose of CCl₄ (1mg/kg) diluted by corn oil in ratio of 1:1 v/v for the induction of liver damage and animals were sacrificed after 24 hr of CCl₄ administration.

Group (3): Six rats received oral dose of the leaves extract of *Bryonia dioica* plant in amount equivalent to 250mg/kg by gavages tube for 7 days, before CCl₄ (1mg/Kg diluted by corn oil in a ratio of 1:1 v/v), then the rats were sacrificed after 24 hr, after CCl₄ administration.

3. Biochemical estimation

Serum was prepared from the collected blood and subjected to biochemical estimation of ALT and AST.

4. Histopathology

Portion of liver tissue in each group was fixed in 10% formalin (Formalin diluted to 10% with normal saline) and proceeded for histopathology. After paraffin embedding and block making, serial section of 5μ thickness were made, stained with Haematoxylin and Eosin and examined under microscope.

5. Statistical analysis

The significance of difference between the mean values was calculated using unpaired student's t-test. P-value less than 0.05 were considered significant for all data showed in our results.

RESULTS

Preliminary qualitative phytochemical analysis

The results of phytochemical screening are given in (table-1). The results of preliminary phytochemical screening of plant extracts showed the presence of alkaloids, flavonoids, steroids, anthraquinoin and terpenoids in the leaves parts of Iraqi *Bryonia* and the absence of, tannins, saponins and cardiac glycosides in this plant parts.

Many researchers reported that the concentration of secondary metabolites are varying from plant to plant belong to the same genus and even in the different parts of the same plant[9], this is due to many factors like environmental heterogeneity, since the effect of environmental heterogeneity is highly scale-dependent. It may create high niche diversity and hence allow species to coexist at a large spatial scale [10], also the high complexity and heterogeneity of soil, like(soil structure, texture and depth, moisture retention characteristics, aeration) create a big variation in the chemical constituents even in the same country[11].

Table 1: Phytochemical screening of *Bryonia dioica* leaves extract

Alkaloids	Flavonoids	Steroids	Tannins	Saponins	Anthraquinoin	Terpenoids	Cardiac glycoside
+	+	+	-	-	+	+	-

+, - represent presence and absence of phytoconstituents respectively.

Table 2: Effects of leaves extract of *Bryonia dioica* on the activities of serum ALT and AST in rats treated with CCl₄.

Treatment	Serum ALT U/L	Serum AST U/L
Control N=6	11.31±1.21	46±3.7
CCl ₄ -treated N=6	66.4±7.63 ^a	68.6±1.67 ^a
Leaves extract + CCl ₄ N=6	14.6±1.34	52.4±3.28 ^b

- Each value represents Mean ± standard deviation.
- Values with non=identical superscripts (a, b) within each parameter are significantly different (P< 0.05)
- N= Number of animals.

Biochemical parameters

There is a significant elevation in the activities of both ALT and AST in CCl₄- treated rats compared to control group. Pre-treatment rats with leaves extract of *Bryonia dioica* (250mg/kg) showed a significant decline in the activities of ALT and AST compared with CCl₄ treated rats (Table -2, Figure- 1 and 2).

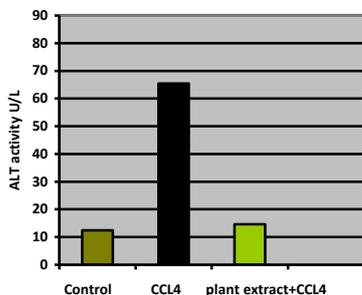


Fig. 1: Bar chart comparing the effects of leaves extract of *Bryonia dioica* pre-treated with CCl₄ on serum ALT activity

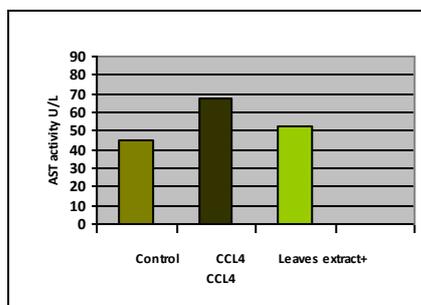


Fig. 2: Bar chart comparing the effects of leaves extract of *Bryonia dioica* pretreated with CCl₄ on serum AST activity.

Histological examination

Histological examination of rat's liver treated with CCl₄ showed that, there was centrilobular hemorrhage, with heavy inflammation and necrosis. In addition to steatosis and individual necrosis were observed compared with control (Figure 3 and 4). Pre-treatment of rats with leaves extract of *Bryonia dioica* before CCl₄, exhibit variable degrees of recovery with slight centrilobular congestion, marked reduction in inflammatory reaction. Furthermore, neither necrosis nor steatosis was observed in rat's liver section. (Figure 5)



Fig. 3: Section showing normal rat's liver. Histology of liver section of normal control animal (group 1) exhibited normal hepatic cells each with well defined cytoplasm, prominent nucleus and nucleolus and well brought out central vein. Magnification: 40X, staining: haematoxylline and eosin.

DISCUSSION

This study demonstrates the positive effect of *Bryonia dioica* as a hepato-protective agent since from ancient times, plants from different families have been used in herbal formulations for the treatment of various ailments especially that of liver. There are more than 600 commercial preparations available from the crude plant extracts, available as formulations for the treatment of liver ailments[12].

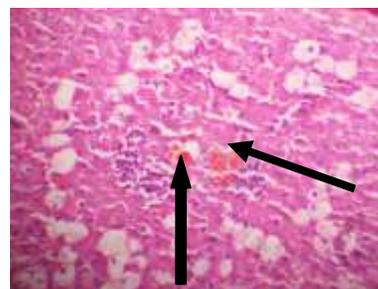


Fig. 4: Section showing morphological alteration of liver from CCl₄-treated rats. CCl₄-intoxicated group animal showed total loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes, vacuolization and congestion of sinusoids, kupffer cell hyperplasia, crowding of central vein and apoptosis. White arrow represents fatty changes (steatosis), black arrow represent hemorrhage. Magnification: 40X, staining: haematoxylin and eosin

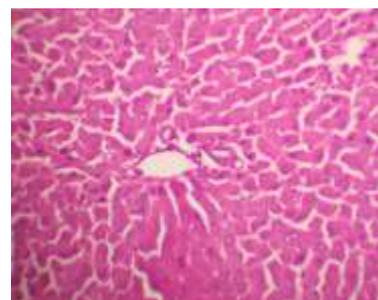


Fig. 5: Section showing the administration of leaves extract of *Bryonia dioica* improved CCl₄-induced hepatic damage. Magnification: 40X, staining haematoxylin and eosin.

Exploration of chemical constituents of the plants and pharmacological screening may provide us the basis for developing the leads for development of novel agents. In addition herbs have provided us some of the very important life saving drugs used in the armamentarium of modern medicine. The administration of CCl₄ to the animals resulted in marked increase in the levels of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, total bilirubin and liver weight as compared to normal (control) group. Histological sections of CCl₄ treated animals showed severe hepato-toxicity evidenced by prominent centrilobular necrosis with prominent and enlarged central vein and periportal inflammation with fatty deposition as compared to normal hepatic architecture of the control group. The CCl₄ has been used as a tool to induce hepatotoxicity in experimental animals[13]. The toxic chemical caused per oxidative degradation in the adipose tissue resulting in fatty infiltration of the hepatocyte. Administration of the plant extract showed protection against the toxic effects of CCl₄. Histopathological sections of plant extract treated animals suggest the recovery against the CCl₄ induced necrosis by returning to the normal hepatic architecture. The plant extract decreased the CCl₄ induced elevated enzyme levels, suggesting the protection of structural integrity of hepatocytes cell membrane or regeneration of the damaged liver cells, this is mainly related to different secondary metabolites

constituents like flavonoids, alkaloids, terpenoids, sterols and others, since flavonoids are reported to be potent therapeutic agents against microcrystin LR-induced hepatotoxicity[14], and are reported to show regeneration and hepato-protective effects in the experimental cirrhosis[15], also flavonoids inhibit lipid peroxidation *in vitro* at an early stage by acting as scavengers of superoxide anion and hydroxyl radicals. They terminate chain radical reaction by donating hydrogen atom to a peroxy radical thus, forming flavonoids radical, which, further reacts with free radicals thus terminating propagating chain[16]. Also anti-oxidant and anti-inflammatory effect of terpenoids and sterols may involve in the defense mechanism[17]. The results suggest that the traditional herbal formulation possess a potent hepato-protective activity against CCL₄ induced liver damage in rats, hence, justifying its use in traditional practice.

CONCLUSION

Phytochemical investigation of a new wild Iraqi plant used traditionally as hepato-protective drug named *Bryonia dioica* was done and the results revealed the presence of alkaloids, flavonoids, steroids, anthraquinoin and terpenoids in the leaves parts of Iraqi *Bryonia* and the absence of, tannins, saponins and cardiac glycosides in this plant parts, also this study demonstrates the positive effect of *Bryonia dioica* as hepato-protective agent and provides a scientific support for the claimed ethnomedical uses of plant extracts in the treatment of different disease.

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