International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 05, 2020
ISSN: 1475-7192

Isolation and Identification of Flavonoids from Arctium Lappa Stem and Study the Hepato Protective Effect on Acetaminophen Induced Liver Damage

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Abstract--- *The present study investigate the heap to protective and antioxidant effect of crude extract of Arctium lappa stem(E-ALS) and isolated total flavonoids(ITF) from the stem, With qualitative and quantitative determination of flavonoids by HPLC. Twenty four local adult male rabbits were used in this study which divided into four groups(6animal in each group),Control-1(C1) as control group, Control-2(C2):treated with 300mg/kg paracetamol administrated for one week, Group 1(G1): 300mg/kg paracetamol administrated for one week +Orally 250mg/kg of E-ALS administrated daily for 4 weeks, Group 2(G2): Orally 300mg/kg paracetamol administrated for one week +Orally 50mg/kg of ITF administrated daily for 4 weeks. The results identified five types of flavonoids for the first time in plant stem (Rutin, Myricetin, Quercetin, Apigenin, and Kaempferol) with quantified determination for the concentration of each type of flavonoids in E-ALS and also in ITF which identified the same five flavonoids. The hepatoprotective effect of E-ALS and ITF were observed by monitoring the antioxidant parameters and activity of liver enzymes. The results obtained from this study showed that the levels of glutathione-GSH, Glutathione peroxidase-GPX and Glutathione-S-Transferase-GST were significantly ($P \leq 0.05$) decreased in C2 treated with paracetamol as comparing with C1, with significant ($P \leq 0.05$) elevation of alanine aminotransferase-ALT activity and non-significant effect on Aspartate aminotransferase-AST, while the level of GSH and GPX were significantly ($P \leq 0.05$) elevated in G1 and G2 treated with E-ALS and ITF respectively as compared with C2, in which the levels of GSH and GPX are less than the levels in C1 and G1. Otherwise the levels of GST, ALT and AST significantly ($P \leq 0.05$) decreased in G1 and G2 as compared with C2. These findings show the hepatoprotective properties of E-ALS and IFT against liver injury induced by paracetamol and also the protective role of anti-oxidative defense system of flavonoids in the two extracts.*

Keywords--- *Arctium Lappa, Flavonoids, Acetaminophen, Glutathione, Glutathion-S-Transferase, Liver Enzymes.*

I. INTRODUCTION

The flavonoids are a category of natural substances belonging to the family of polyphenols, they are one of the important types of plant secondary metabolites, widely distributed in foods and Medicinal Plants(1,2).More than thirty years ago, the research studies focusing on flavonoids from medicinal plant species have increased

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DOI: 10.37200/IJPR/V24I5/PR2020226
Received: 14 Mar 2020 | Revised: 30 Mar 2020 | Accepted: 06 Apr 2020

considerably, because of their versatile benefits for human health(3), These includes: antioxidants, anti-inflammatory, anticancer, antibacterial, antiviral, anti-allergic, immune system promoting and also as detoxifying and pro-survival agents(4,5,6). So that the many researcher use different methods of isolation and identification of flavonoids from different medicinal plants and evaluate the pharmacological effect of the isolated flavonoids , such as; Al-Salihi et al, study the Hypolipidemic effect of isolated flavonoids from date palm pollen(7),while Al Samarraietal (8),isolated the flavonoids from Bay leaf Laurusnobilis L. And also study the hypolipidemic effect.

Arctium lappa (also known as burdock), one of the important plant in traditional medicine worldwide medicine, many studies have evaluate the biological activities of the different parts of plant, roots, seeds and leaves (9,10),including antioxidant activities(11), anti-inflammatory(12),anti-cancer(13) and anti-hepatotoxicity(14). Many researcher identified many secondary metabolites in different parts of the plant, which include: phenolic compounds, lignans, saponins, tannin, sterols, alkaloids and Flavonoids (15-17), the types of flavonoids which identified in *Arctium lappa* leaves include, luteolin, rutin, and quercetin and quercetinrhamno side. On the other hand Rajasekharanetal (18), identified quercetin 3-vicianoside and quercetin 3-O-glucuronide in the root of the plants, with no flavonoids in seeds. The present study aim to isolate and identify the flavonoids from *Arctium lappa* stem and study the protective effect of it against the liver damage induced by paracetamol (acetaminophen).

II. MATERIAL AND METHODS

Plant Materials: The dried stems of *Arctium lappa* were obtain from a local market in Samarra city, Salah Al-Din, Iraq. The stems were separated from other parts of the plant, kept in a dark container until used.

Methods

1. Preparation of Extracts

Crude extract from *Arctium Lappa Stems* E-ALS:45g of *Arctium Lappa Stems* powder was suspend in 180ml normal saline solution.

Isolation of total flavonoids from *Arctium Lappa Stems*: This Isolation was done according to (Chen et al, method) with some modification (19), the first step before extraction was remove fatty contents, 250g plant stems powder were extracted with 750ml diethyl ether, soxhlet apparatus for 3 hours, dried the defatted plant material at 35 C° in an air oven, the second step extracted flavonoids twice with 500ml (70%) ethanol solution at 90C° for 2h. The solution was filtered and centrifugation at 3000 rpm for 15 min. The solvent was evaporated and the extract was condensed under reduced pressure. The extract was collected, labeled as ITF and stored at 4C° until used.

2. Identification of Flavonoids by High-Performance Liquid Chromatography-HPLC

Identification of flavonoids in Arctium Lappa Stems and in isolated flavonoids were carried out according (20) method with some modification, In which 500mg of plant sample (crude, isolated flavonoids extract from stems) were dissolved in 20 ml of hexane to remove fat, 100 ml of 80:20 (methanol: water), the extract was subjected to ultra-sonication at 60% duty cycles for 25 min at 25o c followed by centrifugation at 7,500 rpm for 15min. The clear supernatant of each sample was subjected to charcoal treatment to remove pigments prior to evaporation under vacuum (Buchi Rotavapor Re Type). Dried samples were re-suspended in 1.0 ml HPLC grade methanol by

DOI: 10.37200/IJPR/V24I5/PR2020226
Received: 14 Mar 2020 | Revised: 30 Mar 2020 | Accepted: 06 Apr 2020

overtaxing, the mixture were passed through 2,5um disposable filter and stored at 4o C for further analysis, then 20 µl of the sample injected into HPLC system according the optimum condition. Five stander solutions (25µg/ml) were used (Rutin, Myricetin, Querecetin, Apigenin and Kaempferol).

The concentration of identified flavonoids was done according to the following equation:

Area of Sample

$$\text{Conc. of Flavonoids (µg/ml)} = \frac{\text{Area of Sample}}{\text{Area of standard}} \times C \times D$$

C=Conc. Of Standard solution

D=Dilution factor

3. Animals

Twenty four local adult male rabbits (1200-1550 g weight) were used in this study, Groups of rabbits were housed at room temperature with a lighting schedule of 12 hours light and 12 h dark. Animals had free access to a standard pellet diet. All animals were divided into four groups (6animal in each group) described as follow:

Control 1(C1): Orally1ml /kg/day administrated daily dose normal saline only.

Control 2(C2): Orally 1ml /kg/day of paracetamol (300mg/kg- normal saline was used as solvent) administrated daily for 7 days.

Group 1(G1): Orally 1ml /kg/day of paracetamol(300mg/kg- normal saline was used as solvent) administrated daily for 7 days + Orally 1ml /kg/day of *Arctium Lappa Stems* extract(250mg/kg- normal saline was used as solvent) administrated daily for 4 weeks.

Group 2(G2): Orally 1ml /kg/day of paracetamol(300mg/kg-normal saline was used as solvent) administrated daily for 7 days + Orally 1ml /kg/day of flavonoids isolated from *Arctium Lappa Stems* (50mg/kg- normal saline was used as solvent) administrated daily for 4 weeks.

4. Collection of Blood Samples

After 4 weeks, serum samples were collected by heart puncture from fasting rabbits for 12 hours. Determination of serum levels of Glutathione-GSH (21,22), Glutathione peroxidase-GPX (23), Glutathione-Transferase-GST(24), alanine aminotransferase-ALT and Aspartate aminotransferase-AST(25) by using standard methods.

5. Statistical Analysis

Results were analyzed statistically by using (analysis of variance test-ANOVA the statistical program Minitab).Averages were compared to calculations of the characteristics of the application Duncan's Multiple Range Test by probability level $P \leq 0.05$.

III. RESULTS AND DISCUSSION

Part 1: Phytochemical Study

The HPLC analysis of flavonoids were done firstly by using five standard flavonoids, Table 1 showed the

DOI: 10.37200/IJPR/V24I5/PR2020226
Received: 14 Mar 2020 | Revised: 30 Mar 2020 | Accepted: 06 Apr 2020

Retention Times and Area under Curves for Standard Flavonoids

Table 1: Retention Times and Area under Curves for Standard Flavonoids

Standard Flavonoids	Retention time (min)	Area under curve µvolt
Rutin	2.473	256193
Myricetin	3.742	261362
Quercetin	4.80	276233
Apigenin	5.877	240672
Kaempferol	6.64	249241

The HPLC analysis of the crude *Arctium lappa* stem extract showed seven pecks with different Rt (1.987, 2.51, 3.807, 4.883, 5.763, 6.038, 6.823) min [Fig1], while the area under curve were (186616, 406614, 387611, 58062, 404152) µvolt [Table2].

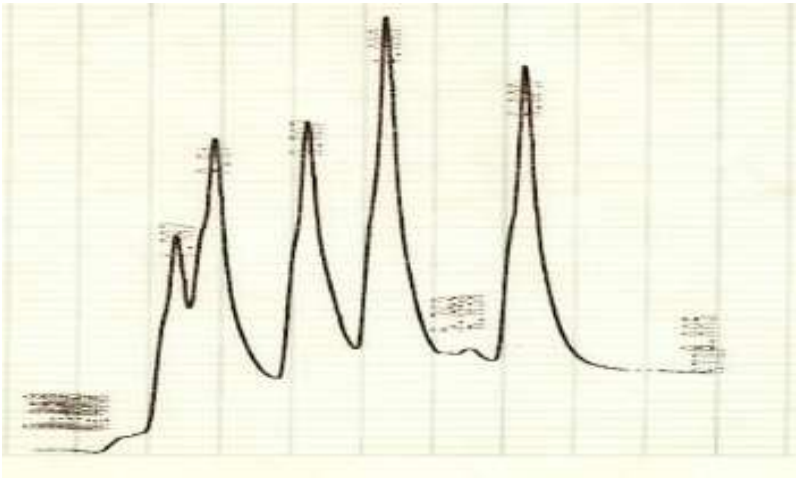

Fig. 1: HPLC Analyses of Flavonoids in *Arctium Lappa* Stems

The types of flavonoids in *Arctium Lappa Stems* were identified by comparing the Rt obtained from chromatograms of crude stems extract with Rt in chromatogram of standard flavonoids, and then the concentration of the identified flavonoids was done by using the values of the area under curve for stem extract and for each standard. The results indicate that the crude plants contain 793.56 µg/g of rutin, 741.33 µg/g of Myricetin, 866.31 µg/g of Quercetin, 44.80 µg/g Apigenin and 810.76 µg/g Kaempferol with two unknown pecks [Table 2].

Table 2: Retention Time, Area under Curve and Concentration of Identified Flavonoids in Arctium Lappa Stems

Identified compounds	Retention time (min)	Area µvolt	Concentration µg /g
UnKnown	1.987	186616	……..
Rutin	2.51	406614	793.56
Myricetin	3.807	387510	741.33
Quercetin	4.883	478611	866.31
Apigenin	5.763	21568	44.80
Kaempferol	6.038	58062	810.76
UnKnown	6.823	404150	……..

The HPLC analysis of flavonoids in isolated flavonoids from plant stem showed six pecks with different Rt (2.843, 2.490, 3.828, 4.98, 6.088, 6.852) and area under curve were(121378, 311395, 189935, 397924, 241849,

DOI: 10.37200/IJPR/V24I5/PR2020226
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318015)Fig.2 and Table 3.

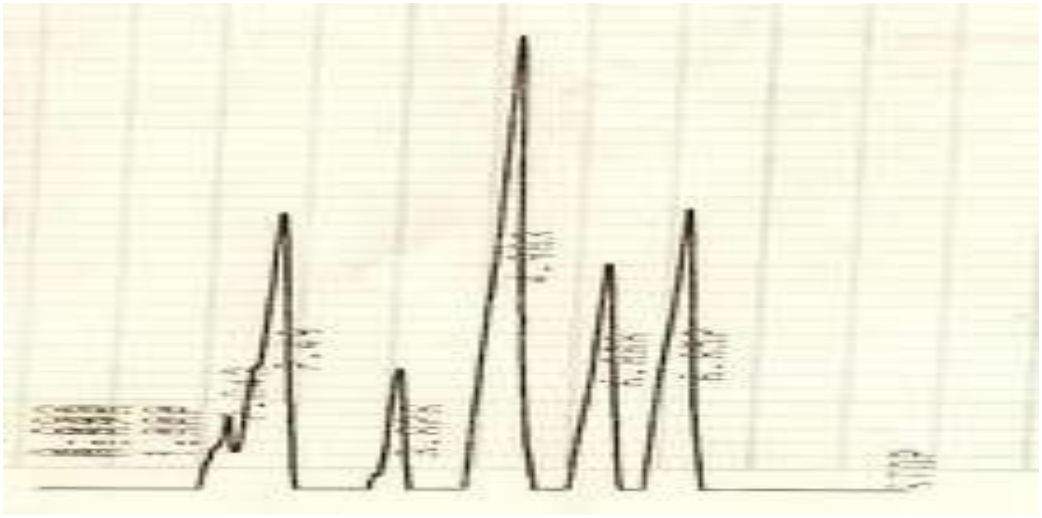

Fig. 2: HPLC Analyses of Isolated Flavonoids Extract from *Arctium Lappa Stems*

The result indicate that the types of flavonoids in isolated flavonoids fraction from the plant stem indicate 607.73 µg/g of rutin, 363.35 µg/g of Myrecetin, 720.26µg/g Quercetin, 502.44µg/g Apigenin and 637.96 µg/g Kaempferol, with two unknown pecks[table3].

Table 3: Retention Time, Area under Curve and Concentration of Identified Flavonoids in Isolated Flavonoids from Arctium Lappa Stems

Identified compounds	**Retention time (min)**	**Area µvolt**	**Concentration µg /g**
UnKnown	2.843	121378	**……..**
Rutin	2.49	311395	607.73
Myricetin	3.828	189935	363.35
Quercetin	4.983	397924	720.26
Apigenin	6.888	241849	502.44
Kaempferol	6.852	318015	637.96

Part 2: Biochemical Study

The hepato protective effect of crude extract and isolated flavonoids from *Arctium Lappa Stems* as an antioxidant on liver damage induced by acetaminophen were investigate in the present study, the results indicate that the levels of GSH,GPX and GST were significantly ($P \leq 0.05$) decreased in control-2 treated with paracetamol as comparing with control-1 group with significant($P \leq 0.05$) elevation of ALT activity and non-significant effect on AST, while the level of GSH and GPX were significantly($P \leq 0.05$) elevated in G1 and G2 treated with crude stem extract and isolated flavonoids respectively as compared with C2, in which the levels of GSH and GPX are less than the levels in C1 and G1.

Otherwise the levels of GST, ALT and AST significantly ($P \leq 0.05$) decreased in G1 and G2 as compared with C2 [Table 4].

DOI: 10.37200/IJPR/V24I5/PR2020226
Received: 14 Mar 2020 | Revised: 30 Mar 2020 | Accepted: 06 Apr 2020

Table 4: Mean ± Standard Deviation of Antioxidant Levels and Liver Enzymes Activity in Sera of Groups under Investigation.

Groups	GSH (µ mol/L)	GPX (U/L)	GST (UlL)	ALT (U/L)	AST (U/L)
C_1	1.010 ± 0.245b	0.597± 0.058c	349.067±45.728a	3.500 ± 1.369 e	254.37± 12.73 a
C_2	0.656 ± 0.179d	0.426± 0.137d	290±59.228b	21.91 ± 3.28 b	240.03 ± 7.21 a
G1	1.151 ± 0.196a	0.714± 0.191b	112.5± 10.828 d	4.733± 1.555 e	12.04 ± 3.36d
G2	0.930 ± 0.202b	0.576 ± 0.021c	81.2±47.814d	12.92 ± 2.82 c	180.1 ± 49.0b

Discussion

Arctium lappa one of the common medicinal plant in china. The fresh or dried roots, ripe seed and leaf were used medicinally(26), with no information related to use of plant stem in traditional medicine, in spite of the Arctium lappa stem may be eaten stewed or raw as a snack(27). Kim etal(28), indicate that the methanolic extract from leaves and stem of Arctium lappa have anti-inflammatory effect. In recent years, the drug derived from natural sources have been given much attentions more than the chemical drugs, in addition to study the chemical composition and biochemical effect, The Arctium lappa contain lignin especially arct in(lower concentration than other part of the plant)(29), no more information were available about the chemical composition of the Arctium lappa stem or about the protective effect of it against the liver damage induced by paracetamol.

The results of the present study identified five types of flavonoids for the first time in plant stem(Rutin, Myricetin, Quercetin, Apigenin, Kaempferol) with quantified determination for the concentration of each type of flavonoids by HPLC, isolated the stem flavonoids and identified the same five flavonoids and then study the hepatoprotective and antioxidant effect of the crude stem and its isolated flavonoids.

The results of the present study indicate that quercetin and Kaempferol concentration in the crude extract and in isolated flavonoid were more than the concentration of other types of flavonoids, so the hepatoprotective effect of crude extracts and isolated flavonoids may be due to the present of those two compounds. Barrosetal(30)provides invaluable insights into the therapeutic efficacy of quercetin in acetaminophen induced toxic liver damage, which suggest that the reduction in serum ALT and AST levels due to the treatment with quercetin, which is useful for prevention of liver damage caused by paracetamol, this results agree with the result of the present study, whereas the treatment with crude extract of stem and isolated flavonoids significantly ($P \leq 0.05$) redacted the activity of AST and ALT in serum of rabbit in G1 and G2, on the other hand Wangetal(31) indicate that treatment with different doses of kaempferol decrease the oxidative stress, lipid peroxidation and increase the antioxidant defense activity, So this findings show the protective effect of kaempferol against liver injury.

The treatment with high dose of paracetamol in C1 cause significant high ($P \leq 0.05$) elevation in serum liver enzymes(AST and ALT) and reduction of antioxidant parameters levels(GSH,GPX,GST), this depression in the level of GSH may be due to the effect of toxic metabolite N-acetyl-p-benzoquinone imine –NAPQI, which produced by hepatic cytochrome p450- CYP450 system which oxidize the excess paracetamol, and the normal detoxification of the toxic metabolite (NAPQI) by the thiol group in GSH cause consume it(32-34), resulting in accumulation of NAPQI which then binds covalently -SH groups of proteins in hepatocytes forming NAPQI-protein adducts(35,36)This effect cause release of reactive oxygen species-ROS that affect the cellular membrane of the

DOI: 10.37200/IJPR/V24I5/PR2020226
Received: 14 Mar 2020 | Revised: 30 Mar 2020 | Accepted: 06 Apr 2020

liver and induce lipid peroxidation and also cause hepatic necrosis (33,37), the injury cause leaking of hepato-cellular enzymes(ALT and AST) into the blood stream, So the antioxidant So the antioxidant capacity of flavonoids maybe prevent the consuming glutathione molecules, block releasing of ROS, and prevent the hepatocyte injury and detoxified the toxic metabolite (NAPQI).

IV. CONCLUSIONS

1. *Arctium Lappa* Stem (E-ALS) and isolated total flavonoids (ITF) have hepatoprotective properties against liver injury induced by paracetamol.
2. *Arctium Lappa* Stem (E-ALS) and isolated total flavonoids (ITF) have the protective role of anti-oxidative defense system.

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DOI: 10.37200/IJPR/V24I5/PR2020226
Received: 14 Mar 2020 | Revised: 30 Mar 2020 | Accepted: 06 Apr 2020