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Biochemical Investigations on *Cocculus pendulus* Leaves Emphasizing its Utility for Medical use

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ABSTRACT

The present study aims to characterize the medicinal plant *Cocculus pendulus*, collected from three wadis in Gebel Elba South Eastern Egypt, chemically and biochemically. Also, the effect of the successive treatments with the alkaloidal extracts using animal model was assayed. The chemical components of *C. pendulus* leaves reveal that the moisture content varies within narrow limits, ash content is relatively high and its major and minor elements composition were measured. Total carbohydrates, proteins, total lipids and crude fiber were also determined. Amino acids levels in leaves showed that, the acidic (aspartic and glutamic) are the most predominant while the least abundant are the cyclic and basic (tyrosine and histidine) within and between wadis. To assess the safety use of plant for medicinal purposes aristolochic acid was extracted and determined, where leaves were found to contain trace amounts of aristolochic acid (0.80 ppm). The biochemical markers of liver and kidney functions were conducted on 60 male Swiss albino mice (divided equally into 5 groups GI, GII, GIII, GIV and a control group. The experimental groups were treated separately with four increasing concentrations of leaves alkaloidal extracts (20, 60, 96, 160 mg/ Kg B.W.) every 48 hours through intraperitonial injection for 16 days. All these concentrations gave biochemical data lie within the normal international ranges, indicating the safety use of *C. pendulus* alkaloidal extract in folk medicine. Also, *C. pendulus* alkaloidal extract can be used as a base for pharmaceutical drugs preparations for the treatment of many diseases.

Key words: *Cocculus pendulus, Gebel Elba,*aristolochic acid, alkaloidal extracts, biochemical markers.

Introduction

The genus *Cocculus* belongs to the family Menispermaceae which comprises about 35 species of shrubs or woody climbers. The members of this genus are used in the traditional system of medicine for various ailments(Kirtikar and Basu,1993). The leaves and stems of *C.pendulus* are possibly used in the indigenous system of medicine for the treatment of intermittent fever and biliousness, febrifuge, vermifuge, antipyretic, diuretic, cholagogue, menstrual cycle troubles, internal parasites antiblennorrhagic and rheumatic pain (Oliver-Bever,1986).

Due to the lack of pertinent information concerning the molecular and biochemical aspects of *C.pendulus* species in Egypt, Shadia *et al*. (2014)conducted the fingerprinting of this plant.

Since alkaloids are the most active ingredients, the current investigation was planned to give an insight on the plant from the biochemical and chemical aspects. Besides, the biochemical assays are undertaken to figure out the possible effects of extracted alkaloids using animal model, with special emphasis on liver and kidney functions.

The present study was accomplished to furnish a comprehensive knowledge on this plant species as data base and to highlight the importance and the safety of this medicinal plant species through using its active ingredients (alkaloids) in pharmacognostical aspects.

Materials and Methods

1. The study area:

Gebel Elba, in the Eastern Desert, (20-25 Km) from the Red Sea is among the notable mountains because of its unique attributes and is considered the most important area for flora and fauna of Egypt. It lies in the Halaib Triangle situated between Egypt and Sudan. The study area includes three of the most relevant wadis, Yahmeib, Acaw and Izab [Fig.1a] in which *C.pendulus* is grown intensively (Ayyad *et al*.,1993).

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2. Field work:

The concerned plant *C. pendulus* was identified and authenticated in February, 2009, then the leaves samples were collected to represent satisfactorily the prevailed environmental conditions of the three wadis in which the plant is grown [Fig.1b]. Individual samples were taken for laboratory analyses.

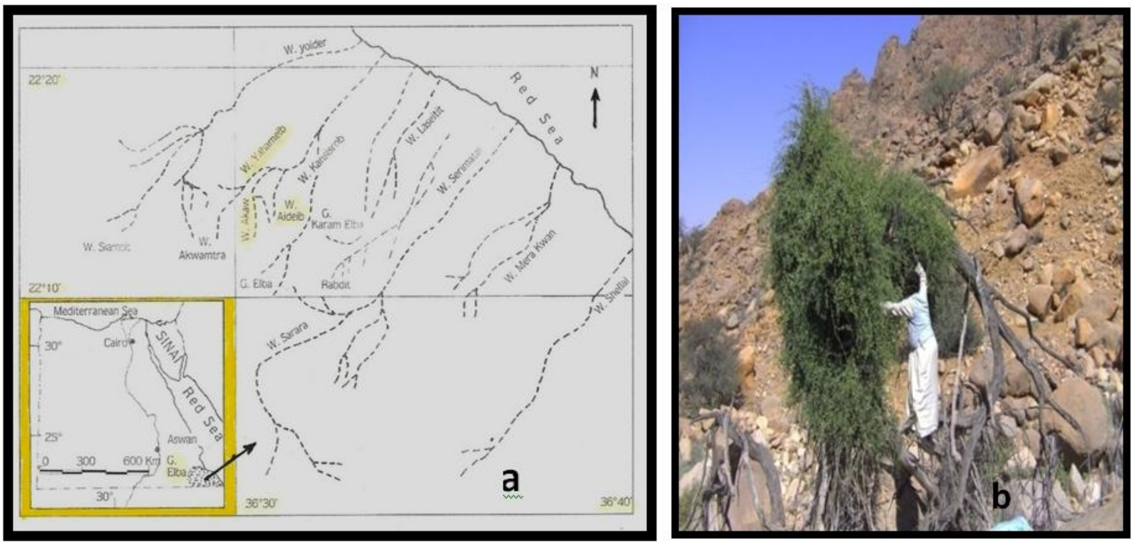

Fig. 1: (a) A map showing the sampling sites (wadi Yahmeib, wadiAcaw and wadi Izab) in Gebel Elba area, South Egypt, (b)Collection of *Cocculus pendulus* Leaves samples.

3. Laboratory analyses:

Six plant leaves samples were collected from the three wadis in Gebel Elba area (Yahmeib, Acaw and Izab); the plant samples were air dried ground and subjected to appropriate analyses.

The *C.pendulus* leaves were dried at 70°C, ground in Willy mill and digested with H_2SO_4 and H_2O_2 according to Parkinson and Allen (1975). The digested samples were analyzed for macronutrients (N, P, K, Ca, and Mg), micronutrients (Fe, Mn, Cu, Zn, B, Mo and Ni) and heavy metals (Sr, Cd, Ba and Pb) according to the methods of Cottenie *et al.* (1982) and Willson *et al.*(1997) . Determination of total Nitrogen was carried out by Kjeldahl method Jackson (1958). Phosphorous and Potassium were determined by the colormetric method and flame photometer, respectively (Piper, 1950). Trace metals contents in leaves samples were measured by Thermo 6500 UK, inductively coupled plasma (ICP), after digestion of the plant samples with ternary acids mixture (HNO_3 ,H_2SO_4 and $HClO_4$ according to Zeng *et al*. (2002) and Stefania *et al*. (2006). Moisture and ash contents, proteins, lipids, fiber and total amino acids were done using the amino acid analyzer (Biochrom 30) as described by AOAC (2006). The total carbohydrates were determined according to Chaplin and Kennedy (1994), while aristolochic acid was carried out according to Rick *et al*. (2001)using the high performanceliquid chromatography (HPLC) with reference standard (Sigma, lot 071M1493V, labelled to contain aristolochic acid I).

The powdered leaves of *C.pendulus*, 500 gms, were used for the preparation of the plant alkaloidal extract with 80 % methanol in Soxhlet apparatus till exhaustion. The alcoholic extract was concentrated under vacuum till dryness. The concentrate was acidified with 2N hydrochloric acid, shaked and filtered; the filtrate obtained was extracted with chloroform to remove undesirable impurities(Woo *et al*., 1977).Ammonium hydroxide was added to the acidic aqueous layer to liberate the alkaloidal bases which were extracted with chloroform till exhaustion and detected with Dragendorf's reagent (Balbaa *et al*.,1981). The combined chloroform extracts were filtered over anhydrous sodium sulfate and evaporated under vacuum. The extracted alkaloids (1.33 g / 500g leaves) were solubilized in 5% Tween 80 and used to prepare the stock solutions for injection on basis of body weight.

Sixty male Swiss albino mice (30-35 gm) were divided equally (12 mice /cage) into a control group (C) and 4 experimental groups (GI, GII, GIII and GIV). Animals of each experimental group received a balanced pellet diet, water ad libitum and were maintained under standard temperature and humidity conditions. These animals were acclimatized to the laboratory conditions for 5 days before experimental work. Doses of leaves stock alkaloidal solutions (20, 60, 96 and 160 mg/Kg B.W.) were administered to the mice groups by intraperitonial (i.p) injection every 48 hours. These concentrations were recommended because there is no estimate of the substance's lethality is available(OECD,2008).Animals from GI, GII, GIII and GIV received the 1st treatment

(40, 120, 192 and 320mg/Kg B.W) were sacrificed after 48 hours and the same procedure was applied for the 2nd, 3rd and 4th treatments. Blood samples were collected after 4, 8, 12 and 16 days respectively from the beginning of the experiment, meanwhile, the control mice follow the same pattern of sacrifice. The plan for injections protocol is clarified in table (1).

The sera samples representing the increasing doses of injection were tested for serum levels of AST and ALT which were determined according to the methods described by Murray (1984) and Young (2001). Total proteins were measured by Biuret reagent, Tietz (1994a) and albumin was done as described by Tietz (1990b)then globulins were calculated. The standard methods of Tietz (1990b)and Tietz (1986c)were used for the determination of blood urea (BUN) and serum creatinine, respectively.

Table 1: The injection protocol of the four animal groups treated with *C. pendulus* leaves alkaloidal extracts.

Groups	Leavesalkaloidal extract			
No. of injections	Group I (20mg/Kg B.W.)	Group II (60mg/Kg B.W.)	Group III (96 mg/Kg B.W.)	Group IV (160 mg/Kg B.W.)
1st treatment X 2 injections	40 mg extract/Kg B.W. were injected i.p.	120 mg extract/Kg B.W. were injected i.p.	192 mg extract/Kg B.W. were injected i.p.	320 mg extract/Kg B.W. were injected i.p.
2nd treatment X 4 injections	80 mg extract/Kg B.W. were injected i.p.	240 mg extract/Kg B.W. were injected i.p.	384 mg extract/Kg B.W. were injected i.p.	640 mg extract/Kg B.W. were injected i.p.
3rd treatment X 6 injections	120 mg extract/Kg B.W. were injected i.p.	360 mg extract/Kg B.W. were injected i.p.	576 mg extract/Kg B.W. were injected i.p.	960 mg extract/Kg B.W. were injected i.p.
4th treatment X 8 injections	160 mg extract/Kg B.W. were injected i.p.	480 mg extract/Kg B.W. were injected i.p.	768 mg extract/Kg B.W. were injected i.p.	1280 mg extract/Kg B.W. were injected i.p.

4. Statistical analysis:

The obtained results of the biochemical analyses were statistically analyzed using SPSS (statistical package for social science, Ver.17) following the methods of Snedecor and Cochran (1980). Results were calculated as mean ± standard deviation. The P values below 0.05 were considered significant.

Results and Discussion

Over 95 % of dry weight of plants is made up of carbon, hydrogen and oxygen taken from air and water. The remaining 5% of the plant dry weight is adsorbed from the soil where roots adsorb these elements from their surroundings. Among these elements, only 14 elements are necessary for plant growth, along with C, H and O are referred to as 17 essential inorganic nutrients, or elements. Within this number of essential elements, C, O, H, N, P, K, Ca, Mg and S are needed in larger amounts (macronutrients) whereas Fe, B, Zn, Mn, Cu, Mo, Ni and Cl are needed in lesser amounts (micronutrients). This doesn't exclude that some trace elements such as Si and Co are beneficial for certain plants but not essential for all plants. Almost all elements are used in a variety of ways, such as catalysts for enzymatic reaction (either as part of the enzyme structure or as regulators of activators), as regulators of the movement of water in or out of the cell and maintenance of turgor pressure, as regulators of membrane permeability, as structural components of the cell of electron receptors or as buffers maintaining the pH within cells (Bonner and Galston, 1952).

Chemical analysis:

Data depicted in table (2) revealed that *C. pendulus* dry leaves total nitrogen content ranges from 1.16 to 1.34 %. The highest N content is associated with *C. pendulus* grown in Acaw delta (sample 4) and Wadi Izab (sample 5). However, the common content (1.16–1.18%) is recorded in leaves of plants grown in other sampling sites. Data in table (2) showed that the phosphorous content of *C. pendulus* leaves is generally high (0.29 to 1.19 %). The highest P content is found in leaves of plants grown in the low lying bed of wadi Izab whereas the lowest P level is that found in plant leaves of wadi Yahmeib. Phosphorous is an essential nutrient plays an important role as a structural and regulatory element in plant growth and development (Sacala *et al.*, 2008). Adequate P nutrition may minimize and ameliorate negative effects of water stress (Shobhura *et al.*, 2004).

Table (2) postulates the total potassium content leaves varies from 1.31 to 1.88 %. The highest K content corresponds to leaves of plants grown in the wadi bed of Izab (site 3) while the lowest K content is that of leaves of plants grown in the elevated land of both wadi Acaw and wadi Izab (sites 3 and 6).K is an important nutrient and plays an essential role in water relation, osmotic adjustment, stomatal movement and finally plant resistance to drought. Decrease in K concentration was reported in many plant species under water deficient conditions, mainly due to membrane damage and disruption in ion homeostasis and K deficient plant has lower resistance to water stress (Lisar *et al.*, 2012). The macronutrient elements could be arranged according to their content in leaves in the descending order; K, N and P. Calcium content in leaves ranges from0.09-0.12% where the lowest Ca content is found in plants grown on the elevated land of Yahmeib (site 2).Like Ca, Magnesium constitutes almost the same high levels (0.01-0.02). The total iron content, as a micronutrient, in leaves ranges from 6.57 to 13.42 ppm.

Table 2: The elemental composition of *C.pendulus*leaves.

Location of sampling	Plant sample No.	Leaves											
		Macronutrients %					Micronutrients (ppm)						
		N	P	K	Ca	Mg	Fe	Mn	Cu	Zn	B	Mo	Ni
Wadi Yahmeib	1	1.17	0.35	1.41	0.12	0.01	9.91	2.04	0.24	0.70	3.19	0.04	0.03
	2	1.16	0.82	1.41	0.09	0.01	7.21	1.31	0.26	0.53	3.99	0.04	0.03
Wadi Acaw	3	1.18	0.29	1.31	0.12	0.02	9.54	1.61	0.23	0.59	4.45	0.02	0.03
	4	1.34	1.01	1.56	0.12	0.01	13.42	3.03	0.28	0.66	6.62	0.02	0.04
Wadi Izab	5	1.26	1.19	1.88	0.11	0.01	8.00	1.42	0.37	0.68	5.61	0.04	0.02
	6	1.16	0.50	1.31	0.12	0.01	6.57	1.38	0.30	0.61	6.13	0.03	0.03
Mean value ± SD		1.21± 0.07	0.69± 0.37	1.48± 0.22	0.11± 0.01	0.01± 0.00	9.11± 2.48	1.80± 0.66	0.28± 0.05	0.63± 0.06	5.00± 1.33	0.03± 0.01	0.03± 0.006

The highest Fe content is found in leaves of plants grown in delta wadi Acaw (site 4), while the lowest one is assigned to plants grown in the elevated land of wadi Izab (site 6). Manganese content in leaves ranges from 1.31 to 3.03 ppm. The highest content is recorded in wadi Acaw delta (site 4) whereas the lowest is confined to the elevated land of wadi Yahmeib (site 2).The total Copper content in leaves ranges from 0.23 to 0.37 ppm. Table (2) show that Zinc content in leaves varies from 0.53 to 0.70 ppm. The highest content characterizes the low lands of wadi Yahmeib (site 1), whereas the lowest is that of the elevated land of the same wadi (site 2).Moreover, in leaves Zn content is in all cases more than Cu content (twice or even more). Data in table (2) include the Boron content in leaves of *C.pendulus* indicated its high content ranged from 3.19 to 6.62 ppm. The highest content is recorded in leaves of plants grown in the wadi bed of Acaw (site 3) whereas the lowest is that of plants grown in the wadi bed of Yahmeib (site 1). Molybdenum content in leaves ranges from 0.02 to 0.04 ppm. The highest Mo content is recorded in 3 plant samples representing wadi Yahmeib (sites 1 and 2) and lowland of wadiIzab (site 5) whereas, the least content is recorded in plants grown in wadi Acaw (bed and delta). Nickel content of leaves is ranged from0.02-0.04 ppm. Based on the foregoing results, the micronutrient elements in leaves of *C. pendulus* could be ranked according to their contents descendingly; Fe, B, Mn, Zn, Cu, Mo and Ni.

In the leaves of *C. pendulus* the contents of non-essential elements and heavy metals (Table 3) were detected, clarifying that the plant attains considerable amounts of some heavy metals like Aluminum (8.7 and 20.61 ppm), Strantium (4.85-6.35 ppm). The presence of considerable amounts of Al in plant parts is mainly rendered to the nature of soil on which this plant is grown since it is mainly composed of alumino-silicates together with Al components that stimulate the uptake of Al by grown plants. Chromium, Cobalt, Vanadium, Cadmium, Barium and Lead constitute traceable amounts in leaves. Commenting on the above mentioned results one can conclude that the studied plant is not polluted by heavy metals. The presence of an element in plant doesn't necessarily indicate its essentiality. The accumulated and determined elements in *C.pendulus* reflect, in a way, the status of these elements in soils and surrounding environment that stimulates or contradicts their absorption and uptake by grown plants.

Table 3: Non-essential elements and heavy metals in *C.pendulus* leaves.

Location of sampling	Plant sample No.	Leaves							
		Non-essential elements and heavy metals(ppm)							
		Al	Cr	Co	V	Sr	Cd	Ba	Pb
Wadi Yahmeib	1	15.92	0.06	0.004	0.03	6.34	0.001	0.84	0.01
	2	10.62	0.07	0.004	0.03	4.85	0.001	0.43	0.01
Wadi Acaw	3	13.33	0.06	0.005	0.03	5.74	0.001	0.50	0.04
	4	20.61	0.05	0.006	0.04	5.22	0.001	0.85	0.01
Wadi Izab	5	12.08	0.03	0.003	0.03	6.35	0.001	0.95	0.02
	6	8.70	0.03	0.003	0.02	5.88	0.001	0.38	0.004
Mean value ± SD		13.54± 4.24	0.05± 0.02	0.004± 0.001	0.03± 0.01	5.73± 0.60	0.001±0 .00	0.66±0 .25	0.02± 0.01

Table (4) presented the contents of moisture, total protein, total carbohydrates, total lipids and fibers in the plants leaves collected from the different wadis in Gebel Elba area. The moisture content of leaves varies from 5.80-6.80 %. This is expected since the plant is grown in arid desert environment and sandy soils impoverished in water and nutritive elements with a common shortage of water essential for plant growth. The ash content of leaves is ranged from10.40-14.29 %.The total carbohydrates content in leaves ranges from 64.97-68.99%.In this respect, Strogonove *et al.*(1970) and Kramer,(1983)reported the accumulation of carbohydrates in plants as a response to salinity or drought, despite a significant decrease in net CO_2 assimilation rate. This is also confirmed by global gene expression studies which showed a reduction in the expression level of most genes encoding chloroplast enzymes involved in carbon fixation, while genes encoding cytoplasmic and vascular enzymes in the pathways leading to glucose, fructose and fructan production were up-regulated under drought stress, Xue *et al.*(2008), suggesting a coordination in the regulation of transcripts of key enzyme genes involved in carbon fixation and carbohydrate accumulation. Carbohydrates act as nutrient and signaling molecules, modulating the expression of a large number of genes and they are also involved in the response to abiotic stresses. The minute variations express the prevailing aridity (hyper arid conditions) and open unique environments that contribute to photosynthesis in the same way and magnitude (Osuna *et al*., 2007). Table (4) showed the total lipids contents in leaves of *C.pendulus* where they ranged from 0.50 – 1.00 %. Lipid peroxidation is the well known effect of drought and many other environmental stresses via oxidative damage. This behavior was explained by Monteiro de Paula *et al*. (1993) and Matos *et al.*(2001)who correlated the decrease in membrane lipid content under water stress to an inhibition of lipid biosynthesis and a stimulation of lipolytic and peroxidative activities. The leaves crude fiber content varies widely from 9.00 -15.00 % while the total protein displayed little variations within leaves (12.52 – 13.85 %). In this regard Nour El-Din and Ahmed,(2004)found that the increase in soil moisture stress may remarkably increase the assimilation and accumulation of nitrogenous compounds. Align with them, Campllans *et al.*(1999)showed evidence that protein residues may be altered during drought stress and some proteins are irreversibly damaged by the effect of drought stress and degraded by proteases. They further suggested that proteases mobilize amino acids from proteins to the synthesis of compatible osmolytes.

Table 4: The biochemical profile moisture and ash contents of *Cocculus pendulus* leaves.

Sampling site	Plant sample No.	Moisture content (%)	Ash content (%)	Total carbohydrates (%)	Total lipids (%)	Fiber content (%)	Total protein (%)
Wadi Yahmeib	1	6.45	14.12	65.48	0.50	12.2	13.45
	2	5.80	13.36	68.32	1.00	13.2	12.52
Wadi Acaw	3	6.43	11.30	67.52	0.90	12.6	13.85
	4	6.80	14.29	64.97	0.90	**15**.0	13.04
Wadi Izab	5	6.69	10.40	68.80	1.00	10.2	13.11
	6	6.33	11.06	68.99	0.90	9.0	12.72
Range		5.80-6.80	10.40-14.29	64.97-68.99	0.50-1.00	9.00-15.00	12.52-13.85
Mean value± SD		6.41± 0.35	12.42±1.70	67.35±1.73	0.87±0.19	12.03±2.15	13.12±0.48

Since the concerned plant (*C.pendulus*) is actually grown in Egyptians desert areas of arid to hyperarid climatic conditions with edaphic and abiotic factors controlling the prevailed environment, it is of interest to shed light on the protein amino acids in the aerial parts of such plant. Table (5) depicts the protein amino acid composition in the leaves of *C. pendulus* plants grown in Gebel Elba three wadis. In this respect, 15 amino acids in a combined and free forms were determined. Examination of the obtained data reveals variations in amino acids concentrations in plants grown in different wadis and even within the same wadi.

Table 5: Total amino acids percentages in *Cocculus pendulus* leaves.

Amino acid %		Leaves						
		Wadi Yahmeib		Wadi Acaw		Wadi Izab		Mean±SD
		1	2	3	4	5	6	
Aliphatic	Glycine	0.55	0.42	0.57	0.44	0.42	0.42	0.47 ±0.07
	Alanine	0.63	0.52	0.66	0.55	0.54	0.54	0.57 ±0.06
	Valine	0.62	0.50	0.63	0.54	0.51	0.51	0.55 ±0.06
	Leucine	0.95	0.70	0.99	0.80	0.77	0.77	0.83 ±0.11
	Isoleucine	0.61	0.44	0.61	0.52	0.49	0.49	0.52 ±0.07
	Threonine	0.54	0.37	0.57	0.39	0.38	0.38	0.43 ±0.09
	Serine	0.49	0.34	0.51	0.37	0.33	0.34	0.40 ±0.08
Acidic	Glutamic	1.02	0.78	1.06	0.85	0.78	0.81	0.88 ±0.12
	Aspartic	1.07	0.77	1.10	0.87	0.79	0.81	0.90 ±0.15
Basic	Lysine	0.62	0.46	0.63	0.48	0.46	0.46	0.52 ±0.08
	Histidine	0.44	0.21	0.32	0.23	0.22	0.22	0.27 ±0.09
	Argenine	0.63	0.43	0.63	0.51	0.47	0.49	0.53 ±0.08
Cyclic	Proline	0.60	0.38	0.63	0.50	0.41	0.49	0.50 ±0.10
	Phenylalanine	0.65	0.45	0.70	0.47	0.46	0.45	0.53 ±0.11
	Tyrosine	0.35	0.20	0.37	0.21	0.21	0.20	0.26 ±0.08

Data presented in table (5) show clearly that, the most predominant amino acids in leaves are aspartic and glutamic (concentration ranged from 0.77- 1.10 % and 0.78- 1.06 %, respectively) followed by leucine (concentration ranged from 0.70 to 0.99 %).The lowest concentrations were correlated with wadi Yahmeib while the highest concentrations were correlated with wadi Acaw, respectively. In contrast, the least abundantamino acids were tyrosineand histidine in the three wadis. Other amino acids are detected in concentrations that lie in between those extremes. The identified amino acids could be arranged descendingly:

aspartic, glutamic, leucine, alanine, valine, argenine and phenylalanine, lysine and isoleucine, proline, glycine, threonine, serine, histidine and tyrosine.

Noteworthy to mention that, there was a relation between the amino acids concentrations and location within and between wadis. The highest concentrations of each amino acid which are strictly confined to the leaves of plants grown in the downstream of wadi Acaw (low waterstress) whereas the lowest concentrations of each amino acid are associated with plants grown upstream of wadi Yahmeib (high water stress).Numerous studies have appraised the metabolic adjustments of nitrogen metabolism in plants subjected to water deficit and plant survival either during or after the period of stress. They also clarified that marked differences have been found in the amino acids pattern under stress conditions. Some other studies have delineated certain amino acids as indicators for drought resistance and defense(Stewart and Larcher 1980; Hanson and Hitz 1982 ; Navari-Izzo *et al.*, 1990).

In our study, when amino acids are grouped according to their chemical composition, one may state that acidic amino acids are the most predominant followed by aliphatic amino acids while basic and cyclic amino acids are the least components irrespective of location on which plants are grown.

We conclude that this specific pattern of amino acid groups may indicate that the synthesis of protein types rich in acidic amino acids(glutamic and aspartic) may be the key to survival of *C.pendulus* plant species under the prevailing climatic (drought stress) or edaphic conditions. Although proline is known by its usefulness in adaptive response and helping plants to withstand stress effect, Matysik *et al.*(2002), our results showed that *C. pendulus* leaves accounted only low concentrations of proline (0.38 to 0.63%). This means that it is not able to produce considerable amounts of proline and therefore has a lower drought stress tolerance. Xin & Browse (1998) and Nanjo *et al.*(1999)stated that transgenic plants which are not able to produce proline, have a significantly lower stress tolerance. On contrary, *C.pendulus* is a drought tolerant plant grown under hyper arid conditions, we can explain its proline lower magnitude and glutamate high content according to Yang and Kao (1999), Kenklies *et al.* (1999) and Matysik *et al.*(2002)who showed the importance of proline in drought stress defense, through its biosynthesis indirectly from glutamate or alternatively synthesized forming the glutamic acid semi aldehyde as a precursor of proline.

For aristolochic acids (AAs) (AAI and AAII), a family of structurally related nitrophenanthrene carboxylic acids, are found naturally in medicinal plants. Many countries including UK, Canada, Australia, and Germany have announced banning to restrict the importation, sale and use of AA- containing medicines. AA was incriminated in the outbreak of theso-called Chinese herbs nephropathy (CHN), severe tubulointerstitional nephritis (Vanherweghem *et al*., 1993). Also CHN is complicated by tumor transformations in the urothelium (Cosyns,2003).

Moreover, in 2001 the FDA in USA issued an important alert that herbal products are unsafe if they contain or are suspected to contain AA (Gold and Slone,2003). Among the plant species suspected to contain AAs, *C. pendulus*, National Toxicology Program(NTP,2008).

In this study 6 plant samples (leaves) were extracted and analyzed for the determination of AAI. The obtained results (not included) revealed that, only three leaves samples contain variable trace amounts (1.85, 0.61 and 0.01 ppm in wadi Yahmeib, Acaw and Izab, respectively) whereas; the other 3 samples are devoid of any AA.

Our results coincide well with those of NTP (2008)who mentioned that AA content of plants or botanical preparations varies depending on the plant species, where it was grown, the time of year and the part of the plant sampled (root, leaves, flowers, seeds, etc.).

Due to the importance of *C. pendulus* as a folk herb or traditional medicine for curing a number of diseases, it is of scientific scrutiny to find out its side effects and safety uses. Of major importance, is the possible effect on liver and kidney whose vital functions are to neutralize and eliminates toxic substances from the body (Boorman *et al*.,1990 and Effendy *et al*.,2006).

In the present study, and after 16 days of treatment (Fig.2a), a non significant change in AST levels in all groups was observed compared to control (53.8 ± 12.5IU/L). Also, the obtained results showed that applying 6 consequent doses in GI (120 mg/Kg BW) led to a decrease(40.64 IU/L) in AST compared to the control. On the other hand, applying the same dose partitioned in two consequent doses (GII,120mg /Kg BW) led to an increase (55.87IU/L) in AST compared to control. In other words, the partitioned administration into small doses doesn't lead to the same response of AST if compared to the larger doses. The fluctuating levels of AST in all the treated groups were not significantly increased or decreased ($P>0.05$) compared to control. The highest levels of AST were always found at the final highest dose in all groups compared to control. On the other hand, ALT (Fig. 2b) showed significant increase in GI, GII and GIII ($P<0.05$) and a non significant increase in GIV, over the control. The rise in ALT and AST in liver hepatitis is expected since these enzymes are located in the hepatocytes and they are an index of liver injury(Johnston,1999). Since aminotransaminases are ubiquitous in their cellular distribution, serum elevations may occur with a variety of nonhepatobiliary disorders. Since the concentration of ALT is significantly less than AST in all cells except hepatic cytosol, ALT serum elevations are less common in nonhepatic disorders Vroon and Israili (1990)& Katzung (2002) showed that the increase in

ALT hepatic cell injury may reach from 50 to 100 times the highest normal value. Moreover, the treatment with some plant extracts cause serum aminotransferase activity to increase due to destruction of hepatocytes (Muchtaromah *et al*., 2011). In our study, the AST levels increase or decrease were insignificantly different from the control and didn't reach such an increase. Also data presented in Fig. (2b) showed that most of the applied doses of leaves alkaloidal extract led to a decrease in ALT level, whereas some doses [40 mg/Kg BW; GI, 480 mg/Kg BW; GII, 576 and 768 mg/Kg BW; GIII] have resulted in an increase in ALT (21.44, 25.28, 22.23 and 22.78, IU/L) compared to control. For instance, though the least dose (40 mg/Kg BW) of leaves alkaloidal extract has resulted in an increase of ALT, most increases are strictly confined to the highest doses in all groups except for GI (160 mg/Kg BW). This means that the changes in ALT are not corresponding to increasing concentrations of doses in most cases. This is clearly evident if the increase of ALT at 40 mg/Kg BW (GI) is compared with ALT at the highest concentration 1280 mg/Kg BW (GIV). In conclusion, one can figure out that ALT values vary widely between 13.71 and 25.28 IU/L overall groups. Needless to mention that the increases or decreases in this enzyme are notproportional to increasing doses and this behavior dictates that the use of this alkaloidal plant extract doesn't affect the status of ALT in a right way. The results obtained for both AST and ALT reveal that mice received the different doses are healthy and no signs of liver dysfunction.

Serum total protein is a biochemical test for measuring total amounts of protein in blood plasma or serum (Pagana and Pagana 2010). Data in Fig. (2c) reveal that the increases or decreases in serum protein don't express clear correspondence to increasing alkaloidal extract concentrations of leaves. For convenience, the highest serum protein (8.61g/dl) corresponds to 2nd dose (384 mg/Kg BW) in GIII, while the lowest serum protein among all groups (4.84g/dl) corresponds to 2nd dose (640 mg/Kg BW) G IV and even increasing the dose to 1280 mg/Kg BW (G IV) led to a small increase in serum protein level (6.82g/dl). These patterns of serum protein in all treatments indicate that the increase or decrease in serum protein depends on the metabolic process in mice rather than the increasing concentration of administered alkaloid extract. These levels of serum protein which are actually within the normal range, (3.5-7.2 g/dl) dictated that the alkaloid extract is safe irrespective of its concentration.

Because serum total protein represents the sum of albumin and globulins, it is of importance to know which protein function is high or low than what is the total protein. Therefore, a typical blood panel provides four different measurements; total protein, albumin, globulins and albumin/globulin ratios. Accordingly, the current study involves these four measurements for serum proteins of mice treated with increasing concentrations of leaves alkaloidal extract.

Albumin is synthesized by liver; data presented in Fig. (2d)clarify the effect of increasing concentrations of leaves alkaloidal extract on the albumin levels in treated mice groups. This effect varies from one group to another and also within each group. Both GI and GIV showed a non significant change in albumins levels as compared to control indicating no liver dysfunction. While in GIII there was a gradual increase in albumin levels with the gradual increase in doses ($P<0.05$).Only the highest doses injected in both GII and GIVled to an increase (3.72 and 3.64 g/dl, respectively) in albumin levels over control. This behavior dictates that the albumin levels are either decreased or increased regardless of increasing alkaloids concentrations. This test can help determine if the animal has liver or kidney disease or if the body is not absorbing enough proteins(Berk and Korenblat, 2011). The albumin level in all the experimental groups were about 50% of the total protein which agrees with the previous findings of Alderson *et al.*(2004) and Medline Plus Medical Encyclopedia (2010).

In the present study, data presented in Fig. (2e)revealed a normal globulin levels in all the studied groups. This indicates the absence of liver or kidney dysfunction. The A/G ratio in the treated mice with leaves alkaloidal extract may provide a clue as to the cause of the change in protein levels. This ratio isdependenton the albumin and total globulin levels and is normally used as a rough guide, due to the variability in albumin and globulin levels. The globulin portion of the ratio is considered to have the most impact and is therefore the most clinically relevant (Dicken and Scott, 2002). The obtained results as shown in Fig. (2f) indicate that A/G ratios are in most cases slightly higher than one or around one. In general, the highest A/G ratio usually corresponds to the highest concentrations in each group while the lower A/G ratios correspond to any other lower concentration, Therefore, the effect of increasing doses is not proportional to A/G ratio and one can expect that A/G ratio indicate normal response regardless of concentration. In addition, even the lower A/G ratio can't be explained on basis of liver or kidney dysfunction or disorders.

Urea is a byproduct of protein metabolism, formed in liver and excreted in the urine by the kidneys. The blood urea nitrogen (BUN) test measures the amount of nitrogen contained in the urea and high BUN levels can indicate kidney dysfunction. BUN is also affected by protein intake and liver function, the test is usually done together with a serum creatinine as a more specific indicator of kidney function. Serum creatinine is an important indicator of renal health because it is an easily measured byproduct of muscle metabolism (Inker and Perrone, 2013).

In our present study, Fig. (3a, b) shows that increasing dose of alkaloidal extract in all the tested animal groups has resulted in non proportional increase of blood urea or creatinine. The obtained values for blood urea and creatinine lie in the normal range of our control, indicating no effect of alkaloidal concentration on kidney function.

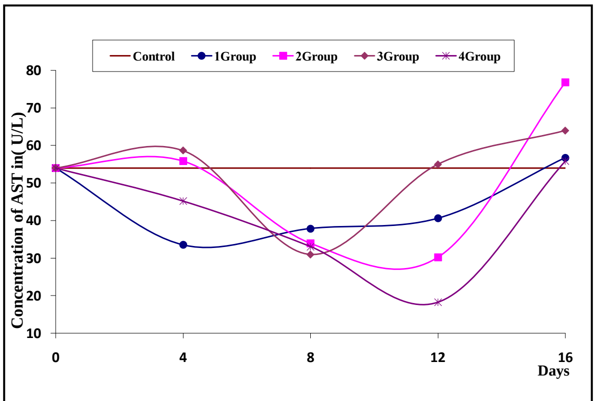

Fig. (2a) Effect of *C.pendulus* leaves alkaloids extract on serum AST of the tested animal groups.

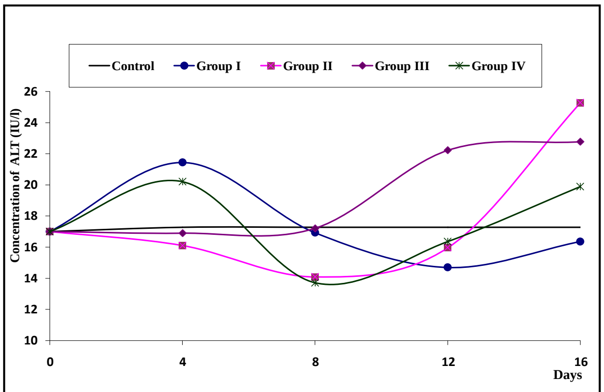

Fig. (2b) Effect of *C.pendulus* leaves alkaloidal extract on serum ALT of the tested animal groups.

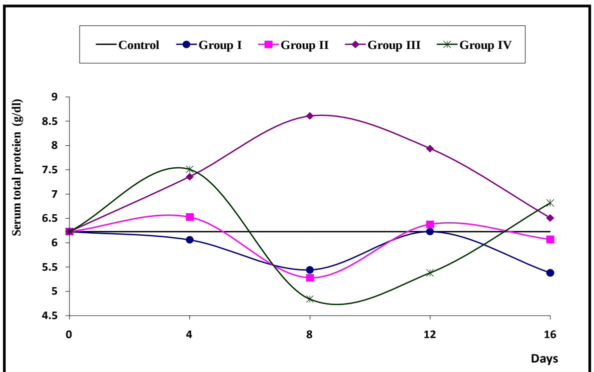

Fig. (2c) Effect of *C. pendulus* leaves extract on serum total protein in the tested animal groups.

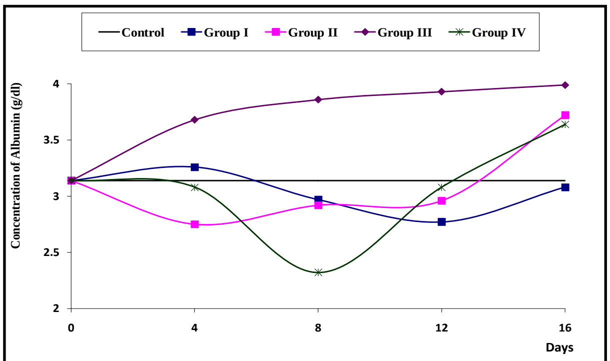

Fig. (2d): Effect of *C.pendulus* leaves extract on Albumin in the tested animal groups.

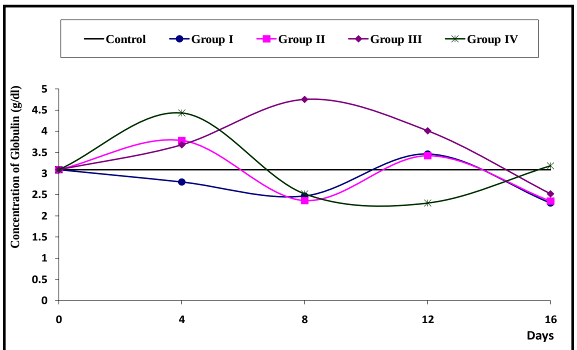

Fig. (2e) Effect of *C.pendulus* leaves extract on Globulin in the tested animal groups.

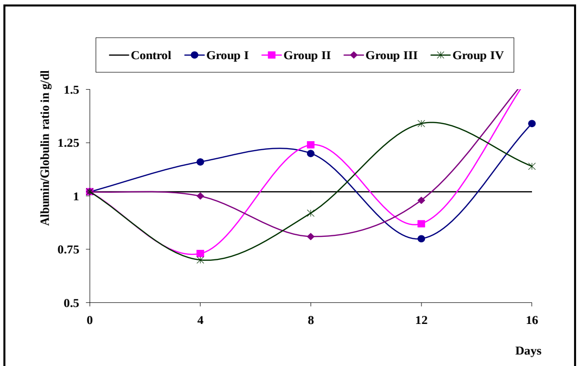

Fig. (2f): Effect of *C.pendulus* leaves extract on Albumin / Globulin ratio of the tested animal groups.

Fig. 2: Effect of ***C. pendulus*** alkaloidal leaves extract on liverfunctions in the tested animal groups, **a)** AST, **b)**ALT, **c)**Serum total protein, **d)**Albumin, **e)**Globulin, and **f)**Albumin/Globulin ratio.

Based on the foregoing studies concerning the chemical and biochemical aspects of *C. pendulus* leaves, one can conclude that the impotance and safety of this plant from the medicinal view point is assured and verified with no toxic hazards on mice. Nevertheless, further biochemical and pharmacological investigations should be conducted to findout the best way for its implementation and usage in medicinal and pharmacological approaches.

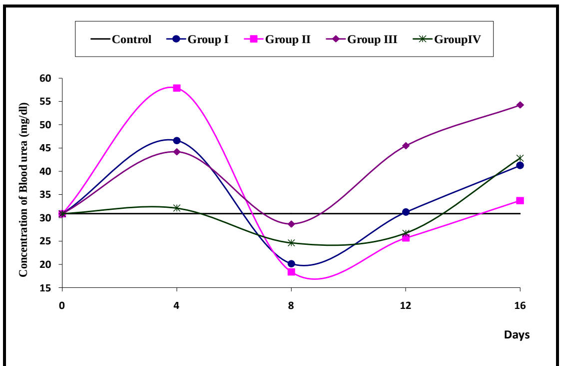

Fig. (3a): Effect of *C.pendulus* leaves extract on blood urea of the tested animal groups.

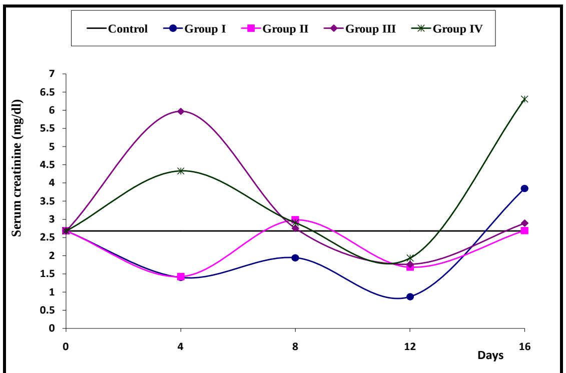

Fig. (3b): Effect of *C.pendulus* leaves extract on serum creatinine of the tested animal groups.

Fig. 3: Effect of *C. pendulus* alkaloidal leaves extract on kidneyfunctions in the tested animal groups, **a)** Blood urea and **b)** Serum creatinine.

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