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Some biochemical effects of *Aloe vera* leaves on tissues in normal mice

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Abstract

This research was carried out mainly to investigate the effects of the aqueous extract and proteinous fractions of *Aloe vera* leaves on cholesterol, acetycholinesterase in brain, glycogen, glutathione in liver and malonaldehyde levels in heart in normal male albino mice. The antioxidant properties and inhibition of acetylcholine-sterase in tissue were detected. Intraperitoneal administration of *Aloe vera* extract in concentration of 400 mg/kg significantly decreased the levels of AchE in brain by (-88.27%) and glutathione content in liver by (-35.48%), and increased the levels of glycogen in liver and malonaldehyde in heart by (22.60%, 85.50%) respectively. At a concentration of 300 mg/kg *Aloe vera* extract significantly increased the level of cholesterol in brain by (24.39%). These results clearly show the antioxidant property of the extract of *Aloe vera* leaves.

Keywords: *Aloe vera*; Aqueous extract; Brain; Liver.
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بعض التأثيرات الكيموحيوية لأوراق الصبار في أنسجة الفئران السليمة

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قسم الكيمياء، كلية العلوم، جامعة الموصل، الموصل، العراق

الخلاصة

تم دراسة تأثيرات المستخلص الخام والمركبات البروتينية المعزولة من أوراق نبات الصبار على مستوى الكوليسترول وانزيم الاستيل كولين استريز في الدماغ، ومستوى الكلايكوجين والكلوتاثايون في الكبد، ومستوى المالونلديهايد في قلب ذكور الفئران البيض السليمة. درست فعالية المستخلص كمضادات الأكسدة فضلا عن إمكانية تثبيط أنزيم الاستيل كولين استريز في الأنسجة. أشارت النتائج إلى ان الحقن في التجويف البريتوني للمستخلص الخام بجرعة (١٠٠ ملغم/كغم) يمتلك فعالية في خفض مستوى انزيم الكولين استريز في الدماغ بحدود -٨٨,٢٧% ومحتوى كلوتاثايون الكبد بحوالي -٣٥,٤٨%، وزيادة في محتوى كلايكوجين الكبد ومالونالديهايد القلب بحدود ٢٢,٦% و ٨٥,٥% على التوالي، كما ادت الجرعة (٣٠٠ ملغم/١٠مل) الى زيادة معنوية في مستوى كوليسترول الدماغ بحوالي ٢٤,٣٩%. توحي هذه النتائج بخصائص مضادات الأكسدة لمستخلص أوراق الصبار.

Introduction

Aloe vera is the gift of nature, it is a miracle been used medicinally for centuries. It belongs to the family (Liliaceae) (1). Botanically known as Aloe barbadensis, Mill (2).

The medicinal properties of various parts (Leaf, Aloe gel) are rich in minerals (magnesium, calcium, chromium, copper, iron,…etc), enzymes, amino acids, vitamins as antioxidants (3). *Aloe vera* plays an important role in many medical properties as a traditional medicine in treatment of many physiological disease and conditions such as Insomnia, Burns, Ulcerative colitis, Psoriasis, wound healing (4). Despite the fact that more than 200 plants are used around the world in the empirical control of antioxidant effect, most of them have not been pharmacologically and chemically investigated (5). Oxidative stress in cells and tissues results from the increased generation of reactive oxygen species and /or from decrease in antioxidant defense potential (6). The

antioxidant defense system represents a complex network with interactions, synergy and specific tasks for a given antioxidant (7). The plant contains substantial amounts of antioxidant including vitamin E, vitamin C, flavenoids, carotenoids and tannins (8).

The present investigation was carried out to study the effect of the aqueous extract and proteinous fractions of *aloe vera* on tissues glycogen, glutathione, malonaldehyde, acetylcholinesterase, cholesterol in experimental mice.

Material and methods

Plant

The plant was collected from the nursery of the College of Agriculture and Forests, University of Mosul and was classified according to plant taxonomy or plant classification references related to medicinal plants. Scientific name = (*Aloe vera*) also known (Aloe barbadensis) (9). Common name = True Aloe or Barbados Aloe. Family = Liliaceae (10).

Preparation of aqueous extract

The cold extract of the plant was prepared by grindingfresh leaves of *Aloe vera* for 10 minutes using a blender, then frozen in a deep freezer, sufficient amount of distilled water was added and the crude homogenate was stirred for additional one hour then filtered through glass wool. The mixture was centrifuged for 15 minutes at 4000xg. The filtrate Concentrated to one third volume by lyophilizer then kept until investigation.

Preparation of proteinous fractions

Proteinous precipitate was separated by full saturation of ammonium sulphate (75%) (11). Gradual addition of ammonium sulphate at 4ºC for 60 minutes accompanied with mixing by electrical mixer. The mixture was left at 0 ºC for 24 hours and then the proteinous precipitate was isolated by centrifugation for 20 minutes at 6000xg at 4 ºC. The precipitate was dried by lyophilizer then kept for gel filtration chromatography. Total protein concentration in each step was determined by modified –lowery method (12).

Gel filtration chromatography

Total protein was fractionated by gel filtration chromatography using sephadex G-100 in column (2×88 cm). Distilled water was used as an eluent solution. The same technique was used to determine the comparative molecular weights of proteinous fractions (13).

The molecular weight of each proteinous compound (comparative) was obtained from its elution volume under the same conditions of known molecular weights such as (Blue dextran, Bovine serum albumin, Eggs albumin, Trypsin, Insulin, Tryptophan).

Animals

Forty eight male albino mice were obtained from the animal house of College of Education, University of Mosul, at 2-2.5 months of age, weighing 22.5±2 g. They were housed in polypropylene cages under standard conditions at temperature 27 ºC and had free access to food and water.

The mice were divided randomly into twelve groups (4mice/group). The first group received distilled water and served as control. Groups (two, three and four) were administered orally with gavages needle in increasing concentration (300, 400 and 500) mg/kg of body weight of crude extract of the plant (14). Groups (five and six) were administered orally with dose of (40 mg/kg) of body weight of proteinous peak I, II respectively after fasting for (16) hours (15). Another set of animals (group eight, nine and ten) were administered intraperitoneally in increasing concentration (300, 400 and 500) mg/kg of body weight of crude extract. Group (eleven and twelve) were administered intraperitoneally with dose of (40mg/kg) of body weight of proteinous peak I, II respectively after fasting (16) hours. While the group seven was kept as control and administered intraperitoneally with distilled water. After two hours of administration, blood samples were collected by orbital sinus puncture technique after ether anesthesia (16). The liver, heart and brain of these mice were immediately removed after sacrification washed with ice-cold saline and kept frozen (-10 °C) until glycogen, glutathione, malonaldehyde, cholesterol and acetyl cholinesterase contents were estimated.

Parameters assayed

UV-visible CECIL CE 1021 single beam spectrophotometer were used to determine all parameters. Serum was separated by centrifugation at (4000xg). Serum and brain cholesterol was determined using (BLOLABO SA. Maizy, France) (17). Glycogen content in liver was determined by Colorimetric method (18). Glutathione content in liver was determined and quantitated by modified Ellman method (19). Malonaldehyde content in heart was determined by spectrophotometric method (20). Acetyl cholinesterase assayed using spectrophotometric method after slight modification (21) using acetylthiocholine iodide (AschI) as a substrate.

Statistical analysis

The statistical methods used to analize data and estimate mean and standard error. Student's-T-test was used to compare between control and experimental mice was at significance of level ($P<0.05$) (22).

Results

Mean values of blood cholesterol, brain cholesterol and acetylcholinesterase, liver glycogen and glutathione, heart

malonaldehyde for control and different doses of *Aloe vera* (oral administered) for normal mice shown in (Table 1). Mean values of blood cholesterol and other parameters in tissues for control and different doses of *Aloe vera* (Intraperitoneal administered) for normal mice shown in (Table 2).

Table 1: Mean blood serum cholesterol,liver glycogen and glutathione content, heart malonaldehyde content, brain cholesterol, acetyl cholinesterase levels in mg/dl±SE after oral administration of different doses of crude extract and proteinous materials of *Aloe vera* in normal fasted mice.

Group No.	Treatment	Glutathione content liver nmol/g±SE	Change %	Malonal- dehyde heart µmol/L ±SE	Change %	Glycogen content liver mg/g±SE	Change %	AchE brain µmol/min/ml ±SE	Change %	Cholesterol brain mg/dl±SE	Change %	Serum Cholesterol level mg/dl ±SE	Change %
1	Control	33.19 ± 1.14	---	14.04 ± 0.38	---	2.11 ± 5.7	---	0.37 ± 0.14	---	152.02 ± 8.51	---	173.69 ± 6.00	---
2	Crude extract 300mg/10ml	35.70 ± 0.97	5.66	18.86 ± 1.22	34.35	2.12 ± 6.5	0.47	0.27 ± 0.11	-27.37	181.16 ±	19.16	200.23 ± 5.84	15.28
3	Crude extract 400mg/10ml	31.19 ± 0.82	-6.02	21.60 ± 0.88	49.67	2.29 ± 9.4	8.53	0.53 ± 0.16	42.28	168.11 ± 7.27	10.58	209.88 ± 16.76	20.84
4	Crude extract 500mg/10ml	32.33 ± 0.83	-2.59	19.46 ± 0.94	38.70	2.22 ± 9.5	5.21	0.19 ± 0.12	46.34	170.0 ± 4.95	11.82	131.10 ± 10.43	-24.5
5	Proteinous Frc.I 40mg/10 ml	34.22 ± 0.93	3.10	20.27 ± 1.04	44.39	2.05 ± 3.3	-2.84	0.30 ± 0.02	12.68	156.55 ± 7.19	2.97	199.10 ± 7.69	14.63
6	Proteinous Frc.II 40mg/10 ml	33.89 ± 1.01	2.10	12.64 ± 1.23	-9.85	2.25 ± 3.3	6.63	0.33 ± 0.1	11.65	172.71 ± 7.08	13.61	211.36 ± 6.95	21.69

The values are mean ± SE of 4 mice each group.

Discussion

In the present study, the activity of cholinergic neurotransmission following previous administration of increasing doses of crude extract and proteinous fractions I, II of *aloe vera* plant were investigated by assaying brain AchE activity. Results depicted in Tables 1 and 2 showed (group 9) inhibition of brain cholinesterase activity compared to the values of control group. The percentage of inhibition suppression of activity of AchE was (-88.27%). The inhibitory effect of curde extract (400 mg/kg) of *aloe vera* may be due to inhibition at the anionic site of AchE (23). On the other hand, many reports suggest that such inhibition is due to a decrease in the internal microviscosity of phospholipids leading to change in the fluidity of microsomal membranes of the brain (24).

The results in (Tables 1 and 2) also indicate that there is significant increase the brain cholesterol levels after intraperitoneal administration of curde extract (300 mg/kg) by (24.39%) in normal mice. The high fiber content of *Aloe vera* had been shown to exert beneficial effects on activation of the hydroxy methyl glutaryl-CoA reductase (HMG-CoA reductase), this enzyme is necessary for cholesterol biosynthesis (25).

The mean values of heart malonaldehyde (MAD) in control and *Aloe vera* extracts treated normal mice are shown in tables (1 and 2). The results illustrate a significant increase of MAD in group 9 at ($P<0.05$) by intraperitoneal administration of curde extract (400 mg/kg) by (85.5%) in normal mice compared to control group.

The increase of MAD may be due to increase of lipid peroxidation, so the higher level of glucose in blood due to

elevation of proliferative free radical as a result of, this due to increased lipid peroxidation (26).

Mice treated with *Aloe vera* extract at a dose of 400 mg/kg showed significant increase in liver glycogen contents at a level of 22.6% compared with control value, this may be to that the extract can activate glycogen synthytase leading to synthesize of glycogen and inhibition of glycogenolysis leading in turn to gluconeogensis or release insulin from β-cells in pancrease (27).

Glutathione is tripeptide normally present at high concentrations intracellularly, and constitutes the major reducing capacity of the cytoplasm. Glutathione is known to protect the cellular system against toxic effect of lipid peroxidation (28). The extract of *Aloe Vera* at 400 mg/kg orally administered indicate significant decrease of GsH level by (35.48%) compared to control value. This decrease may be due to decline in Glutathione peroxidase (GPx) activity (29) or activity of GsH to prevent oxidation in oxidative stress through elimination of free radical so the level of GsH is decreased (30).

Table 2: Mean blood serum cholesterol,liver glycogen and glutathione content, heart malonaldehyde content, brain cholesterol, acetyl cholinesterase levels in mg/dl±SE after intraperitoneal administration of different doses of crude extract and proteinous materials of *Aloe vera* in normal mice.

Group No.	Treatment	Glutathione content liver nmol/g±SE	Change %	Malonal- dehyde heart µmol/L ±SE	Change %	Glycogen content liver mg/g±SE	Change %	AchE brain µmol/min/ml ±SE	Change %	Cholesterol brain mg/dl±SE	Change %	Serm Cholesterol level mg/dl ±SE	Change %
7	Control	35.87 ± 0.82	---	24.71 ± 0.62	---	2.87 ± 11.2	---	0.32 ± 0.12	---	148.04 ± 11.96	---	173.68 ± 13.30	---
8	Crude extract 300mg/10ml	32.75 ± 0.67	-8.69	31.05 ± 1.44	25.65	2.65 ± 6.9	-7.66	0.26 ± 0.15	-17.28	*184.1 ± 6.71	24.39	196.64 ± 8.01	13.21
9	Crude extract 400mg/10ml	*23.1 ± 0.91	-35.4	*45.8 ± 2.23	85.50	*3.5 ± 8.6	22.6	*0.04 ± 0.03	-88.27	115.58 ± 7.32	-21.9	203.66 ± 17.89	17.26
10	Crude extract 500mg/10ml	33.27 ± 0.48	-7.24	33.55 ± 2.45	35.77	3.04 ± 2.9	5.92	0.22 ± 0.12	-29.93	170.95 ± 4.76	15.47	*244.4 ± 12.39	40.72
11	Proteinous Frc.I 40mg/10 ml	28.67 ± 0.85	-20.0	22.29 ± 0.97	-9.79	2.93 ± 8.0	2.09	0.22 ± 0.22	-31.48	162.22 ± 5.11	9.57	138.97 ± 5.93	-19.9
12	Proteinous Frc.II 40mg/10 ml	29.14 ± 0.71	-18.7	35.39 ± 1.10	43.26	2.89 ± 3.9	0.67	0.20 ± 0.13	-36.72	143.88 ± 5.12	-2.81	173.40 ± 13.84	-0.16

* Refers significance at P< 0.05 compared with control group.
The values are mean ± SE of 4 mice each group.

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