

Chemical studies on protein profile in albino rats under the effect of aqueous extracts of *hibiscus rosa sinensis* leaves and pomegranate (*punica granatum*) peels

Mahmoud, Sh.S. El-Sayed

Agricultural Biochemistry Department, Faculty of Agriculture, Ain Shams University, P. O. Box: 68, Hadayek Shoubra 11241, Cairo, Egypt.

Abstract

Hibiscus rosa sinensis (HRS) leaves and pomegranate (*punica granatum*) peels (PP) have many medicinal applications. Effect of natural products (aqueous extract of *Hibiscus rosa sinensis* leaves (AEHRS leaves), pomegranate (*punica granatum*) peels (AEPP) and their mixture) on protein profile (total protein (TP), albumin (Alb) and albumin/globulin (A/G) ratio) concentrations at 7, 14 and 21 days of male albino rats was studied. Seventy two male albino rats were divided into twelve groups of six animals each (n=6). The first group served as the normal health control (I). Animals of the second group (II) were induced hyperglycemia (diabetic control group) by injection of 65 mg/kg bw of streptozotocin (STZ). Three groups (III, IV and V) were given *hibiscus* aqueous leaves extracts (AEHRS) orally daily, at doses of 250, 500 and 750 mg/kg bw respectively. Also, three groups (VI, VII and VIII) were given *P. granatum* aqueous peels extracts (AEPP) orally daily, at doses of 100, 200 and 300 mg/kg bw respectively. Finally, three groups those IX, X and XI were given mixture of AEHRS leaves and AEPP orally daily, at doses of (125/125, 225/225 and 350/350 mg/kg bw). In addition, one of streptozotocin (STZ) rats group was treated with AEHRS leaves and AEPP mixture 400/400 mg/kg bw (XII) respectively. Serum was separated for estimation of total protein (TP) and albumin (alb) as well as albumin/globulin (A/G) ratio was calculated after 7, 14 and 21 days. Protein profile contents results showed different changes at all experimental periods which sometimes observed some increased or decreased values comparing with normal health control group (I) only but highly significant increase was obtained when compared to diabetic control group (II) only. In view of these facts the improving and protective effect of AEHRS leaves, AEPP and their mixture against male albino rats protein profile was showed.

Key words: *Hibiscus rosa sinensis*, pomegranate (*punica granatum*) peels, aqueous extract, total protein (TP), albumin (alb), albumin/globulin ratio (A/G) ratio.

Introduction

The herb *Hibiscus rosa-sinensis* Linn (Malvaceae) is a glabrous shrub widely cultivated in the tropics as an ornamental plant and has several forms with varying colours of flowers. In medicine, however the red flowered variety is preferred (Adhirajan *et al.*, 2003). The leaves and flowers are observed to be promoters of hair growth and aid in healing of ulcers (Jadhav *et al.*, 2009). Flowers have been found to be effective in the treatment of arterial hypertension and to have significant antifertility effect (Sethi *et al.*, 1986). Flowers are considered as aphrodisiac, emollient and emmenagogue and the decoction of flowers is used in bronchial catarrh (Pullaiah, 2002) and diarrhoea (Kasture *et al.*, 2000). And also has calcium channel blocking action (Gilani *et al.*, 2005).

Previous studies have demonstrated that flavonoids have remarkable inhibiting effects on protein glycosylation (Asgary *et al.*, 1999 and 2002).

The level of protein in plasma was found to be reduced in diabetic animals when compared to control ones. The lowered level of protein, after the *H.rosa sinensis* treatment, increased approximated to control. The levels of albumin and albumin/globulin ratio in plasma were decreased in diabetic animals. These lowered levels of plasma albumin and

albumin/globulin ratio level were restored significantly in the *H. rosa sinensis*-treated diabetic rats (Mandade and Sreenivas, 2011).

Reduction in plasma total protein and albumin level was observed in diabetic rats and this is consistent with the results obtained by (Bakris, 1997) and (Tuvemo *et al.*, 1997). The decrease in protein and albumin may be due to microproteinuria and albuminuria which are important clinical markers of diabetic nephropathy (Mauer *et al.*, 1981) and/or may be due to increase protein catabolism (Almdal and Vilstrup, 1988). The results of this study demonstrated that the treatment of diabetic rats with the extract of *H. rosa sinensis* caused a noticeable elevation in the plasma total protein and albumin levels as compared with their normal levels (Safiyeh *et al.*, 2007). *H. rosa sinensis* extract could influence protein metabolism and marker enzymes in STZ-induced diabetic rats. The extract also protect liver and kidney from damage due to diabetes (Zanna *et al.*, 2008).

Goat lens treated with the ethanolic extracts of leaves of *hibiscus-rosa sinensis* (EEHRS) at concentrations of 1 mg, 1.5 mg and 2 mg showed significantly (P<0.05) increased total protein and water soluble protein levels respectively compared to the positive control (Aziz *et al.*, 2015).

Total 50% ethanolic and benzene extracts of *hibiscus rosa-sinensis* treatments showed increase in protein content of the adult female rats markedly depending the dose and duration. The effect is more pronounced at 300 mg/kg/day level and for 18 days duration. Benzene extract is more potent than the ethanolic extract when given for 12 and 18 days (Prakash, 1979). Enhancement in protein contents of adult female rats treated with *hibiscus rosa sinensis* extracts may be due to the antiestrogenic nature of the extracts which has already been assayed by (Kholkute *et al.*, 1977). Feeding by *hibiscus rosa sinensis* flower extract (240 mg/kg bw) was more effective on reduction of serum albumin in hypercholesterolemic group may be due to formation of protein adduct, which leads to covalent modification of cellular target protein, cell death and organ damage (Janbaz *et al.*, 1998). The treatment of diabetic and normal rats with aqueous methanolic extract of *hibiscus rosa sinensis* leaves induced slightly and significant increase in concentration of plasma protein (Zaki *et al.*, 2017).

Pomegranate (*Punica granatum* L.), a fruit native of the Middle East, has gained widespread popularity as a functional food and nutraceutical source is gaining tremendous attention due to its powerful antioxidant properties (Johanningsmeier and Harris, 2011). The health effects of the whole fruit, as well as its juices and extracts, have been studied in relation to a variety of diseases. *Punica granatum* (pomegranate) is used as food or as medication in folk medicine for antiviral, anthelmintic, antifungal, antibacterial and antimicrobial activity (Amorim *et al.*, 2003). Studies have shown that pomegranate products prevent and/or reduced chemically cardiovascular disease and diabetes (Lansky and Newman, 2007), induced tumors in skin (Hora *et al.*, 2003), breast (Kim *et al.*, 2002), lung (Khan *et al.*, 2007) and colon (Kohno *et al.*, 2004) *in vivo* and *ex vivo* study. Studies showed pomegranate fruit and flower extracts to exhibit free-radicals scavenging properties with simultaneous potent hepatoprotection against chemically induced liver damage in rodents (Celik *et al.*, 2009 and Bishayee *et al.*, 2011).

The effect of pomegranate peel ethanolic extracts on serum proteins (total protein, albumin and globulin) were determined. In the hepatoprotective period, the CCl₄-treated group showed a significant reduction in the serum total protein content. Treatment with pomegranate peel ethanolic extracts during CCl₄ administration significantly increased the serum total protein content. When compared with CCl₄-treated group. In the curative period, the highest increase in serum total protein content was noticed in the rats treated with pomegranate peel ethanolic extracts and no significant difference was found when compared with normal group. From the obtained data in both hepatoprotective and curative periods, treated rats with carbon tetrachloride showed

a significant decrease in the serum albumin contents. Treatment rats with pomegranate peel ethanolic extracts significantly increase the serum albumin contents when compared with CCl₄-treated group. No significant effects were observed on the serum globulin contents between all groups and normal control in the hepatoprotective period. In the curative period, a relative high increase in serum globulin content was noticed in the rats treated with pomegranate peel ethanolic extracts. (Zafar and Ali, 1998 and Osman *et al.*, 2011).

Determining kidney damage can be carried out by checking biochemical parameter such as the examination of the levels of the total serum protein (Primarizky *et al.*, 2016). The administration of pomegranate extracts as a nephrotoxicity treatment in white rats can maintain the normal levels of total protein.

The induction of pomegranate peel extract PPE during the time course of the experimental period elicited no effect on the levels of total protein and albumin with respect to normal control group. The 15 days pre and 15 days co-administration of (PPE) with oxytetracycline (OTC) caused a well marked increase in serum total protein and albumin levels compared with OTC-treated group (El-sayed *et al.*, 2014).

The aim of this study is to evaluate the possible ameliorative effect of natural products (aqueous extract of *hibiscus rosa sinensis* leaves, aqueous extract of pomegranate (*punica granatum*) peels and their mixture on protein profile concentrations (total protein (TP), albumin (alb) and albumin/globulin ratio (A/G ratio)) which are very high important for examining kidney and liver damage of male albino rats in order to find new potential sources of natural antioxidants.

Materials and Methods

Plant material collection

The leaves of *Hibiscus rosa sinensis* were collected from Faculty of Agriculture, Ain Shams University garden, while the fruits of pomegranate (*Punica granatum*) were purchased from local market. All plant materials were identified and authenticated by Horticulture department, Faculty of Agriculture, Ain Shams University. The samples were washed with distilled water to remove any impurities and dried under shade. Then the dried plant materials were ground into powder with grinder mixture.

Extract preparation

Boiling distilled water (500ml) was added to 8 g pomegranate peels powder and the same conditions with 8 g powder of *Hibiscus rosa sinensis*, then left for 10 minutes and filtered. The filtrates were dried at 40-45°C.

Animals

Male albino rats weighing 120±10 g were obtained from experimental farmland animals unit, Ministry of Health, Helwan, Cairo, Egypt. The

animals were caged and provided with food and water *ad-libitum*. The rats were kept for one week to adapt to the laboratory conditions before starting of the experiment. The experimental rats (72 animals) adult male albino rats were divided into twelve groups (6 rats/each).

Experimental design

Three dose levels of each AEHRS leaves and AEPP beside their mixture at four doses, three for normal animals (125/125, 225/225 and 350/350) and one for diabetic rats treated with mixture of AEHRS leaves AEPP at dose of 400/400 mg/kg bw. The experiment rats were divided into the following groups.

- Group I: normal control rats
- Group II: diabetic control by a single intraperitoneal (i.p.) injection of 65 mg/kg of streptozotocin (STZ) only.
- Group III: normal rats daily received AEHRS leaves of 250 mg/kg bw.
- Group IV: normal rats daily received AEHRS leaves of 500 mg/kgbw.
- Group V: normal rats daily received AEHRS leaves of 750 mg/kg bw.
- Group VI: normal rats daily received AEPP of 100 mg/kg bw.
- Group VII: normal rats daily received AEPP of 200 mg/kg bw.
- Group VIII: normal rats daily received AEPP of 300 mg/kg bw.
- Group IX: normal rats daily received AEHRS leaves and AEPP mixture of 125/125 mg/kg bw.
- Group X: normal rats daily received AEHRS leaves and AEPP mixture of 225/225 mg/kg bw.
- Group XI: normal rats daily received AEHRS leaves and AEPP mixture of 350/350 mg/kg bw.
- Group XII: diabetic rats daily received AEHRS leaves and AEPP mixture of 400/400 mg/kg bw.

Duration of the experiment was extended for 21 days.

Collection of blood

The blood was withdrawn from the retro-orbital sinus puncture of the eye using mild ether anesthesia. The collected samples were centrifuged for 10 min. (Jadeja *et al.* 2009) at 7 days, 14 days and 21 days of the experimental period. The protein content in the serum was estimated by the method of Lowry *et al.*, (1951). The albumin content was estimated by the method described by Reinhold, (1980). The serum globulin was calculated by subtraction of serum total protein and serum albumin, as following:

serum globulin (g%) = serum total protein - serum albumin.

Albumin/globulin ratio was calculated by dividing each albumin value by its corresponding globulin value.

Chemical and equipments

All chemicals and equipments used were of analytical grade and purchased from El-Gomhoria Chemicals Co and electoscient chemicals Co.

Statistical analysis

All the data were expressed as mean \pm SE. Statistical analysis was carried out using student's t-test to analyze the significance between the groups. A value of $P < 0.05$ was considered to be significant Sendecor and Coebam (1969).

Results and Discussion

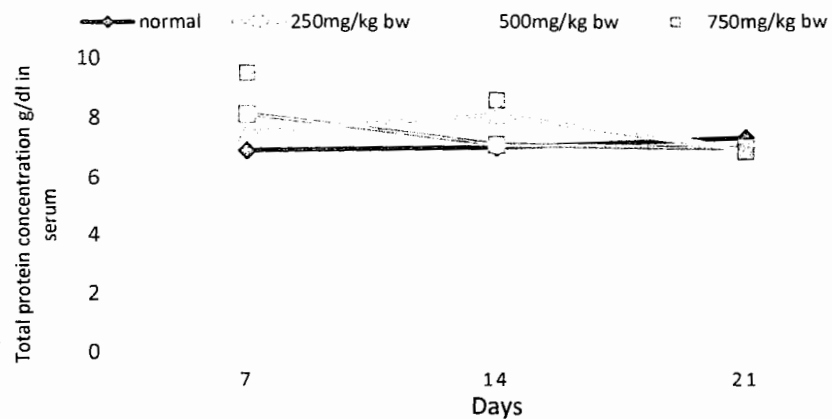
Results of serum total protein are presented in Table (1) and figure (1-a,b,c and d). Serum total protein values were 6.9 g/dl, 7.01 g/dl and 7.34 g/dl; 4.01 g/dl, 4.30 g/dl and 4.61 g/dl; 5.91 g/dl, 6.53 g/dl and 6.62 g/dl at 7, 14 and 21 days of the experimental period for normal control (I), diabetic control (II) and last group (diabetic rats treated with 400/400 - XII) respectively. Also, the results showed that different doses of natural products (AEHRS leaves and AEPP) significantly increased serum total protein in all groups after 7 days when compared to normal control, diabetic control and the curative diabetic group (XII). The results also indicated that increasing doses of AEHRS leaves, AEPP and their combination led to increase serum total protein when compared to the I, II and XII groups after 14 days. On the other hand, after 21 days in all groups the serum total protein decreased relative to normal control (7.34 \pm 0.39) only. This elevation of serum total protein after 7 and 14 days may be due to that different doses of natural product (AEHRS leaves and AEPP) effect on either the regulation of serum and liver proteins levels by stimulating the protein biosynthesis to produce specific enzymes to antagonize the harmful effect of free radicals and also by stimulating certain endocrine gland activity for excretion the hormones which regulated protein metabolism. The results obtained cleared that both AEHRS leaves and AEPP individually or in combination increased serum total protein which are in agreement with Biswas *et al.*, (2014) they reported that feeding of *hibiscus rosa sinensis* flower extract for four weeks to hypercholesterolaemic rats significantly ($P < 0.001$) increased in total protein (TP) levels compared to the hypercholesterolaemic control group. Abd El-Monem, (2014) found that pomegranate molasses (PM) supplementation together with Diazinon (DZN) improved the values of serum total protein (TP). This may be attributed to the powerful antioxidants such as polyphenol, total phenols and total flavonoids.

Table (1) and figure (1-a,b,c and d): Effect of different concentrations of natural products (aqueous extract of *hibiscus rosa-sinensis* leaves –(AEHRS), aqueous extract of pomegranate (*punica granatum*) peels (AEPP)) and their mixture on serum total protein (g/dl) in male albino rats

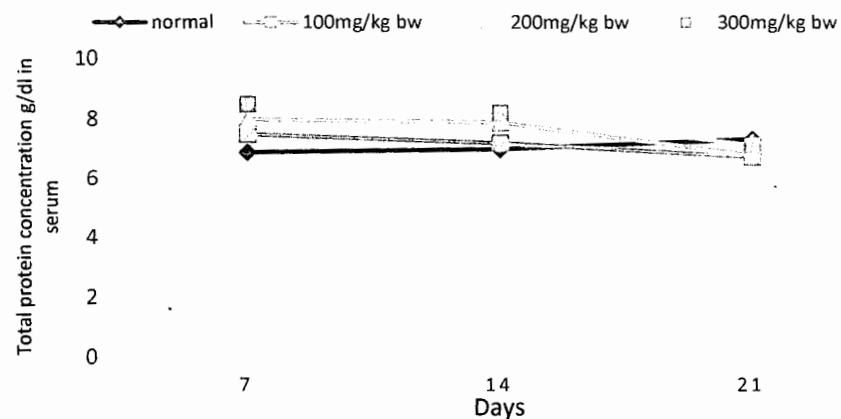
Treatments	Normal control	Diabetic control	AEHRS leaves (mg/kg bw)			AEPP (mg/kg bw)			AEHRS leaves and AEPP mixture (mg/kg bw)			Diabetic rats + 400 / 400
			250	500	750	100	200	300	125 / 125	225 / 225	350 / 350	
Days												
7	6.91±0.85	4.01±0.77	8.13±0.31*	7.51±0.37	9.51±0.79*	7.51±0.55	7.99±0.99	8.50±0.58	9.11±0.88*	8.55±0.44	9.69±0.39*	5.91±0.42
14	7.01±0.55	4.30±0.88	7.11±0.91	8.10±0.95	8.59±0.96	7.21±0.76	7.91±0.78	8.22±0.73	8.21±0.66	8.51±0.33	9.75±0.93*	6.53±0.55
21	7.34±0.39	4.61±0.91	6.99±0.37	6.91±0.93	6.87±0.88	6.73±0.70	6.80±0.44	7.13±0.40	7.01±0.55	7.21±0.11	7.20±0.39	6.62±0.33

Values are expressed as mean ± SE. n=6

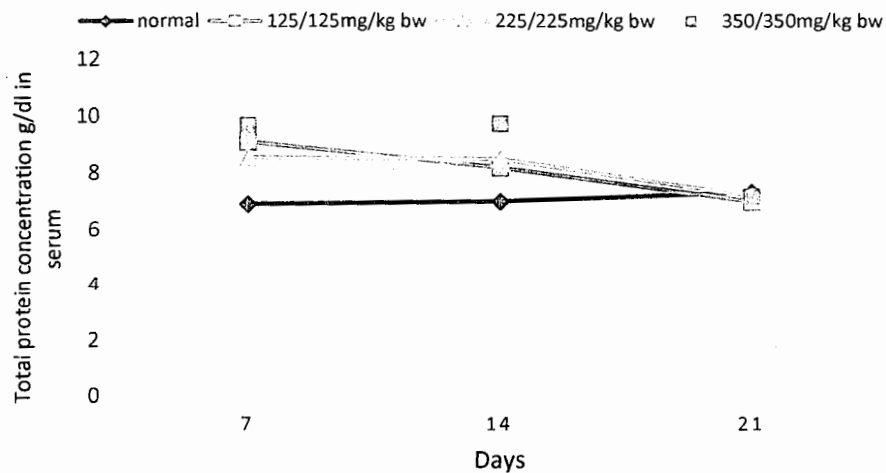
significant difference (P<0.05) compared with normal control and diabetic control groups



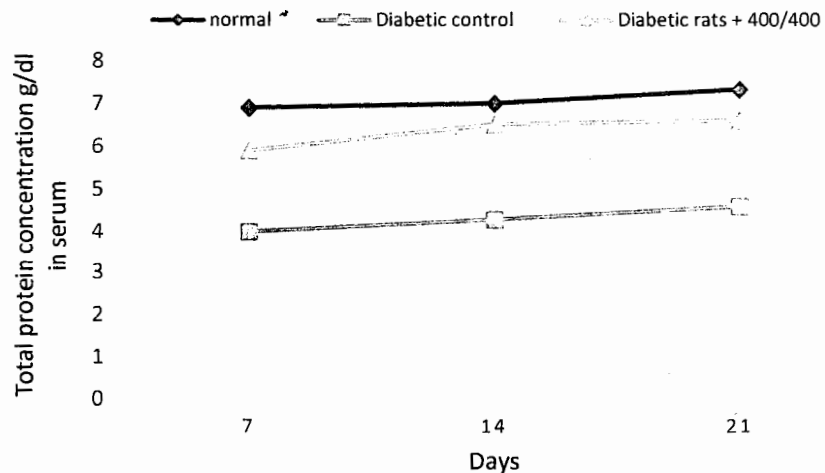
(a)Effect of different concentrations of AEHRS leaves on serum total protein g/dl



(b)Effect of different concentrations of AEPP on serum total protein g/dl



(c)Effect of different concentrations of AEHRS leaves and AEPP mixture on serum total protein g/dl



(d)Effect of AEHRS leaves and AEPP mixture on serum total protein g/dl in Diabetic rats

Data are represented in **Table (2) and figure (2-a,b,c and d)** indicated that different doses of natural products either in single dose or in mixtures led to elevation of serum albumin in rats after 7 days of experiment comparing to I, II and XII groups except low dose of AEHRS leaves which showed slightly decrease of this value which (3.89 g/dl) when compared with normal control (I) (4.08 g/dl) only. These findings are coincided with **Mandade and Sreenivas, (2011)** who noticed that administration of *hibiscus rosa sinensis* (500 mg/kg) aqueous extract to diabetic rats for four weeks significantly elevated plasma albumin. Also, the present results agree with **Abd El-Monem, (2014)** results who found that serum albumin (Alb) was increased in male rats which given 0.5 ml pomegranate molasses plus 0.5 ml distilled water/day for 10 days when compared to normal control. On the other hand, respect to serum albumin after 14 days data revealed significant increase in this value in all groups when compared to I, II and XII groups except the groups of rats which received medium dose of AEHRS leaves and AEPP which showed lowering in serum albumin when compared to normal control (I).

Regarding to this parameter, after 21 days the present results showed reduction in serum albumin of rats orally daily administrated with AEHRS leaves when compared to normal control (I). Meanwhile a remarkable increase was shown in

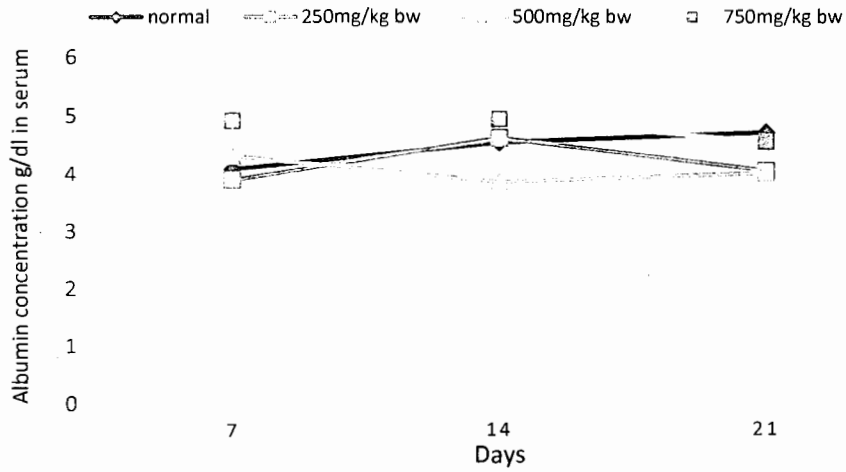
serum albumin in rats received AEPP in low or high dose .The values were 5.31 g/dl and 5.61 g/dl comparing to 4.69 g/dl, 1.76 g/dl and 3.78 g/dl in normal control, diabetic control and last group (XII) respectively. In contrary medium dose of AEPP showed lowering serum albumin when compared to normal control only. In addition, the groups of rats orally administrated daily mixture of AEHRS leaves and AEPP showed some increasing in serum albumin comparing to I, II and XII groups except the rats orally administrated daily mixture of AEHRS leaves and AEPP with dose 350/350 mg/kg bw (group XI) which showed lowering in serum albumin when compared to normal control (4.69±0.63 g/dl). It could be saying that AEPP was more effective in increasing serum albumin compared with AEHRS leaves which cleared after 21 days. Also, the mixture dose generally increased serum albumin and this raising cleared too after 21 days especially in groups IX and X when compared with normal control, diabetic control and the last group (XII). These results are in accordance with **Salim et al., (2014)** who mentioned that groups treated with pomegranate seeds alone or pomegranate seeds supplemented to dimethoate treated group caused very significant improved ($P<0.05$) in serum albumin level.

Table (2) and figure (2-a, b, c and d): Effect of different concentrations of natural products (aqueous extract of *hibiscus rosa-sinensis* leaves (AEHRS) , aqueous extract of pomegranate (*punica granatum*) peels (AEPP)) and their mixture on serum albumin (g/dl) in male albino rats

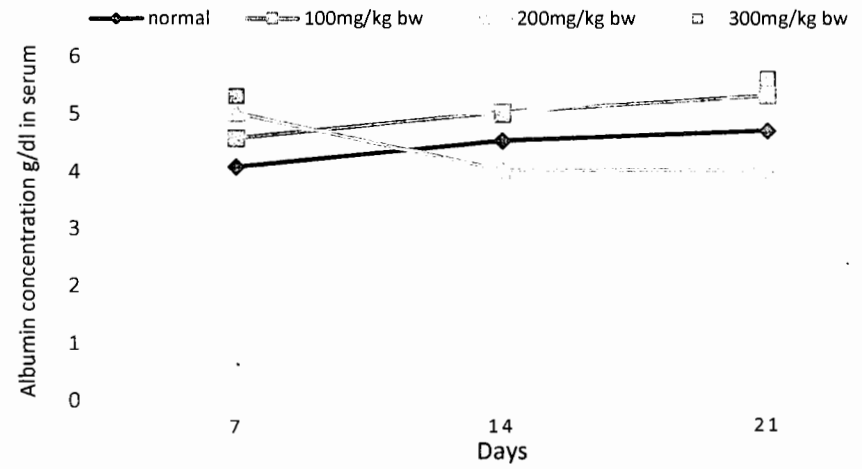
Treatments	Treatments		AEHRS leaves (mg/kg bw)			AEPP (mg/kg bw)			AEHRS leaves and AEPP mixture (mg/kg bw)			Diabetic rats + 400 / 400
	Normal control	Diabetic control	250	500	750	100	200	300	125 / 125	225 / 225	350 / 350	
Days												
7	4.08±0.43	1.52±0.23	3.89±0.91	4.31±0.37	4.91±0.93	4.58±0.31	5.01±0.77	5.31±0.44	4.78±0.78	4.89±0.88	5.31±0.55	3.40±0.25
14	4.53±0.50	1.65±0.44	4.61±0.57	3.84±0.99*	4.93±0.77	5.02±0.58*	4.01±0.56*	4.98±0.88	5.81±0.99*	4.97±0.93	5.06±0.78*	3.51±0.37
21	4.69±0.63	1.76±0.78	4.03±0.89	3.99±0.95	4.53±0.55	5.31±0.49*	4.00±0.59	5.61±0.47*	4.93±0.55	4.88±0.66	4.13±0.73	3.78±0.93

Values are expressed as mean ± SE. n=6

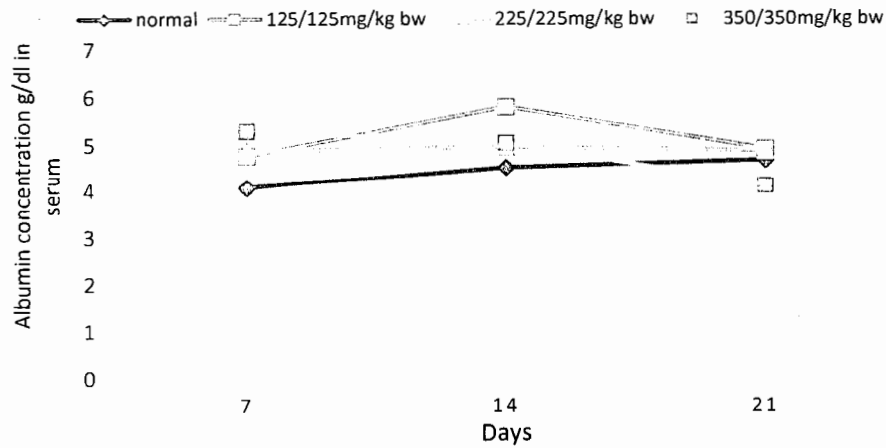
significant difference (P<0.05) compared with normal control and diabetic control groups



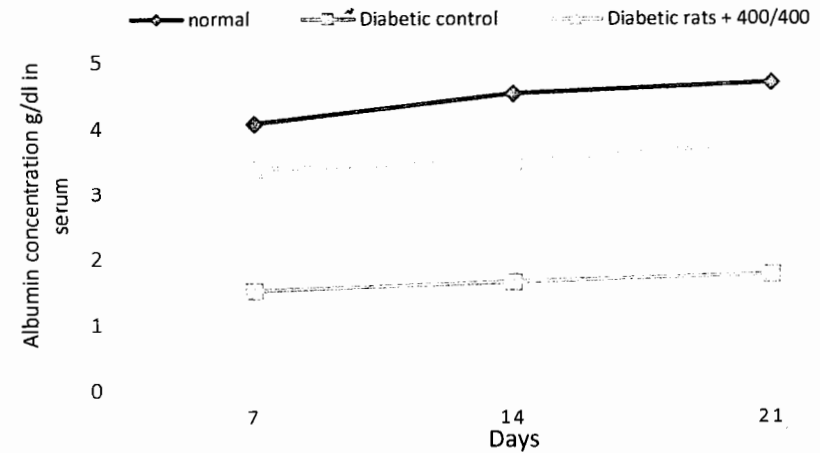
(a) Effect of different concentrations of AEHRS leaves on serum Albumin g/dl



(b) Effect of different concentrations of AEPP on serum Albumin g/dl



(c) Effect of different concentrations of AEHRS leaves and AEPP mixture on serum Albumin g/dl



(d) Effect of AEHRS leaves and AEPP mixture on serum Albumin g/dl in Diabetic rats

Results obtained in **Table (3) and figure (3)** represented the calculated ratio of serum albumin to globulin (A/G ratio) in male albino rats under investigation. The ratio showed an elevation in this value through 7 and 14 days (1.44 ± 0.17 and 1.82 ± 0.09 respectively) then return to reduction after 21 days (1.76 ± 0.08) in normal control rats (I). Also, the same trend was observed in diabetic control group (II).

On the other hand, opposite trend was cleared in last group (XII) (diabetic rats + 400/400). Meanwhile, after 7 days observed in all groups lowering of serum albumin / globulin ratio except low, medium and high doses of AEPP which showed an elevation in A/G ratio which reached to (1.56, 1.68 and 1.66 respectively) when compared to normal control (I) (1.44) and the last group (XII) (1.35). However, data represented in Table (3) indicated that all different doses of natural products

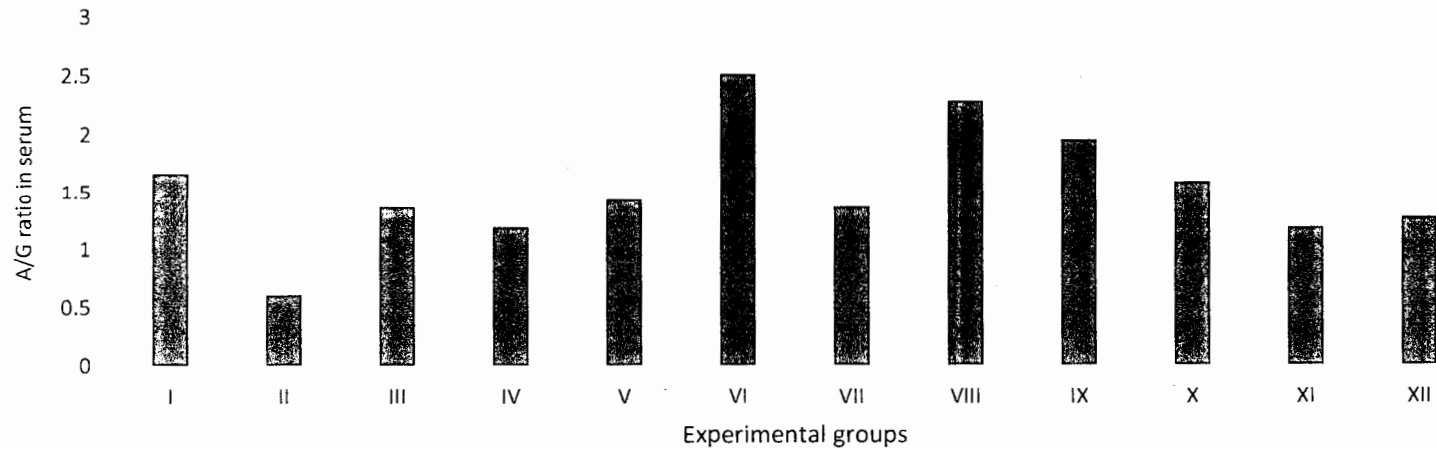
(AEHRS leaves and AEPP) decreased serum albumin/globulin ratio in rats after 14 days except low doses of AEHRS leaves, AEPP and their mixture which showed increase of A/G ratio (1.84, 2.29 and 2.42 respectively) when compared to normal control (I) (1.82). These results are in agreement with **Mandade and Sreenivas, (2011)**. A/G ratio at 7, 14 and 21 days revealed that this parameter was increased in all groups under study with a single dose or a mixture doses of AEHRS leaves and AEPP when compared to diabetic control (II). Meanwhile, the ratio indicated that different changes after 14 days which sometimes showed some increase or decrease comparing with the same value after 7 and 21 days but in all time this value was given the highest ratio with lowest dose of AEPP after 21 days. However, our data are in good agreement with stated by **Abd El-Monem, (2014)**.

Table (3) and figure (3): Effect of different concentrations of natural products (aqueous extract of *hibiscus rosa-sinensis* leaves (AEHRS), aqueous extract of pomegranate (*punica granatum*) peels (AEPP)) and their mixture on serum albumin/globulin ratio in male albino rats

Treatments Days	Normal control	Diabetic control	AEHRS leaves (mg/kg bw)			AEPP (mg/kg bw)			AEHRS leaves and AEPP mixture (mg/kg bw)			Diabetic rats + 400 / 400
			250	500	750	100	200	300	125 / 125	225 / 225	350 / 350	
7	1.44±0.17	0.61±0.04	0.91±0.09	1.34±0.08	1.06±0.08	1.56±0.09	1.68±0.04	1.66±0.03	1.10±0.14	1.33±0.09	1.21±0.12	1.35±0.09
14	1.82±0.09	0.62±0.05	1.84±0.03	0.90±0.05	1.34±0.09	2.29±0.29	1.02±0.05	1.53±0.42	2.42±0.31	1.40±0.03	1.07±0.18	1.16±0.08
21	1.76±0.08	0.61±0.07	1.36±0.05	1.36±0.08	1.93±0.15	3.73±0.28	1.42±0.02	3.69±0.47	2.37±0.22	2.09±0.07	1.34±0.09	1.33±0.07

Values are expressed as mean ± SE. n=6

significant difference (P<0.05) compared with normal control and diabetic control groups



Conclusion

Results therefore, show that, the oral administration of natural products (aqueous extract of *hibiscus rosa sinensis* leaves, aqueous extract of pomegranate (*punica granatum*) peels and their mixture) increased total protein, albumin and albumin/globulin ratio when compared to diabetic control group (II) in albino rats. The use of the plants may have a pleasant effect on the kidney.

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دراسات كيميائية على مستويات بروتينات الدم في الجرذان البيضاء تحت تأثير المستخلصات المائية لأوراق نبات الهيبسكس روزا سينسس وقشور الرمان

محمود شكرى صادق السيد

قسم الكيمياء الحيوية - كلية الزراعة - جامعة عين شمس - القاهرة - مصر

أوراق الهيبسكس روزا سينسس وقشور الرمان لها تطبيقات طبية عديدة. حيث تم دراسة تأثيرهذة المنتجات الطبيعية (مستخلص أوراق نبات الهيبسكس روزا سينسس وقشور الرمان ومخلوط منهما) على تركيزات بروتينات الدم (البروتين الكلى والاليومين ونسبة الألبومين / جلوبيولين) عند ٧ و ١٤ و ٢١ يوم في ذكور الجرذان البيضاء و استخدم لتلك الدراسة (٧٢) ذكر جرذ أبيض قسموا إلى ١٢ مجموعة وكل (رفع بها مستوى السكر في الدم وأصبحت مصابة II) كتنترول عادى , حيوانات المجموعة الثانية (مجموعة بها ٦ حيوانات. المجموعة الأولى) أعطيت المستخلص المائى V, VII, IV, المرض السكر بواسطة الحقن بجرعة ٦٥ ملليجرام/كيلوجرام مادة الستريتوسوتوسين. ثلاث مجاميع عادية (لأوراق نبات الهيبسكس روزا سينسس يومياً فمياً جرعات ٢٥٠، ٥٠٠، ٧٥٠ ملليجرام لكل كيلوجرام من وزن الجسم بالترتيب) وأيضاً ثلاث مجاميع أعطيت مستخلص مائى لقشور الرمان يومياً فمياً بجرعات ١٠٠ و ٢٠٠ و ٣٠٠ ملليجرام لكل كيلو جرام من وزن الجسم VIII, VII, VI عادية (أعطيت مخاليط أوراق نبات الهيبسكس روزا سينسس وقشور الرمان يومياً فمياً X, X and XI بالترتيب) وأخيراً ثلاث مجاميع عادية (بالجرعات ١٢٥/١٢٥، ٢٢٥/٢٢٥، ٣٥٠/٣٥٠ ملليجرام لكل كيلو جرام من وزن الجسم) بالإضافة إلى مجموعة الفئران المعاملة بمادة الستريتوسوتوسين ومخلوط المستخلصات (الهيبسكس والرمان) وهى المجموعة الثانية عشر والأخيرة على الترتيب. تم فصل السيرم لتقدير البروتينات الكلية والألبومين ونسبة الألبومين / الجلوبيولين بعد ٧، ١٤، ٢١ يوم. نتائج محتويات بروتينات الدم تشير إلى تغيرات مختلفة فى كل فترات التجربة والتي أحياناً يلاحظ بعض الزيادات والنقص مقارنة بالكنترول العادى فقط ولكن زيادة معنوية كبيرة تم الحصول عليها مقارنة (و فى ظل هذه الحقائق يتضح التأثير الوقائى والتحسينى لمستخلص أوراق الهيبسكس روزا سينسس وقشور الرمان بالمجموعة المصابة) ومخيلطها ضد تلف الكبد والكلى فى ذكور الفئران البيضاء.