Effect of Crude Extract of Saponin of Seeds Sesbania Sesbon on Cholesterol Level in Blood of Mice

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Abstract

The current Study was carried out to determine the effect of different concentrations (0.03, 0.06, 0.12, 0.25) of crude extract of saponin seeds Sesbania sesbon (L) Merrill on the haemolytic activity of saponin on red blood corpuscles under laboratory conditions. Results indicated that percentage of haemolysis was 100% at concentrations 0.12 and 0.25 of saponin but the activity of saponin blocked by adding different concentrations (0.2, 0.4, 0.6, 0.8, 1) % of cholesterol. Thus, negative relationship was found between haemolytic activity of saponin and cholesterol concentration. No observable lysis was found at 1% of cholesterol. The effect of sesban saponin extract on the cholesterol level of mice blood at different time intervals (4, 8, 12 hours) post injection showed decrease in cholesterol level when increased saponin concentration with decreasing the period of injection. The lowest level of cholesterol observed in mice that injected with 0.12 and 0.25 of saponin after 4 and 8 hour of injection.

Keywords: Saponin, cholesterol, haemolysis.

تأثير المستخلص الخام لسابونين بذور نبات السيسبان على معدل الكوليسترول في دم الفئران البيض

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الخلاصة :

تم دراسة تأثير تراكيز (0.03 ، 0.06 ، 0.12 ، 0.25) للمستخلص الخام للسابونين من بذور نبات السيسبان Merrill (L) *Sesbania sesbon* على الفعالية التحليلة لكريات الدم الحمراء .أظهرت النتائج ان نسبة التحلل كانت 100% في التراكيز (0.12 ، 0.25) ، في حين ثُبط تأثير السابونين بأضافة الكوليسترول بتراكيز مختلفة (1 ,0.8 ,0.6 ,0.4 ,0.2)% فوجدت علاقة عكسية بين فعالية السابونين التحللية من جهه ونسب الكوليسترول من جهه أخرى، فكانت نسة التحلل صفر % بتركيز 1% كوليسترول، اما تأثير السابونين على معدل الكوليسترول في دم الفئران بعد (4, 8, 12)ساعة من الحقن فقد لوحظت زيادة واضحة في أنخفاض مستوى الكوليسترول كلما زاد تركيز السابونين المستخدم وقلة فترة الحقن فبلغ أوطا معدل للكوليسترول في التراكيز (0.12 ، 0.25) للسابونين بعد (8,4) ساعات من الحقن .

الكلمات المفتاحية :سابونين ، كوليسترول ، تحلل

Introduction

Steroid sapogenins have till now been found only in higher plants which named as secondary product [1]. These compound were named because they have no obvious metabolic function and their very diversity of structure and distribution among living organisms [2]. The aqueous solution of saponin which form foam like soap[3], saponin has a more or less strong haemolysing effect on blood erythrocytes in vitro [4]. The effect was showed that after diet containing well soybean saponin were fed rats and mice nor saponin could be found in the blood stream of the test animals, this showed that no saponin had been absorber through digestive tract [5]. Cholesterol is widely distributed in all cells of the body but particularly in nervous tissue, it is the parent compound of all steroids synthesized in the body. It occurs in animal fats but not in plant fats [6]. Many investigators have demonstrated a correlation between raised serum lipid levels and the incidents of coronary heart disease and atherosclerosis in human [7], of the serum lipids. Cholesterol has been the one most often singled out as being chiefly concerned in the relationship of the factor that lower blood cholesterol, the substitution in the diet of poly unsaturated fatty acid for some of the saturated fatty acids has been the most intensely studied, naturally occurring oils are beneficial in lowering plasma cholesterol include pear rut cotton seed, corn and soybean [8].

Material and methods

Sesbania saponin was extracted (SSE) from seed of sesbania sesban (L.) Merrill. It was prepared from ether extracted sesbania flour [9]. haemolytic activity of saponin on red blood corpuscles (RBC) has been studied by dissolving saponin extract in physiological saline (0.85% Nacl) to give a concentration of 10 mg/ml, the erythrocytes were prepared by centrifuging mice blood at 6,000 g for 5 min

to remove the supernatant, after washing the collected (RBC), 20 ml RBC was added to 80 ml saline to give 2% erythrocyte suspension. To determine haemolytic activity five test tubes were used, for rep (0.25) ml dissolved extract was added to tube 1, tubes 2-5 that containing 0.25 ml physiological saline to tube 2 added 0.25 ml dissolved saponin extract. Series dilutions were made by transferring 0.25 ml of the dilution to tube 3, mixed well and then transferring (0.25) ml to tube 4 and the last tube was used as a control without added saponin extract. To all test tubes 0.25 ml of mice red corpuscles was added. As a result of the dilutions these concentra-tions of saponin per ml of water existed in tubes 1-5 (0.25, 0.12, 0.06, 0.03, 0 %). Test tubes were shaken to mix the contents, incubated at room tempera-ture (1 hour) then observed for haemolysis .When complete haemolysisoc-curred the tubes showed a uniform cherry-red from the hemoglobin released from disrupted red blood corpuscles. In partial haemolysis, the saline ap-peared pink and unlysis corpuscles had settled to the bottom of the tubes, and the saline was clear. To study the ability of cholesterol for depressing the effect of SSE for haemolytic activity could be overcome by adding cholesterol to blood at concentrations (0.2, 0.4, 0.6, 0.8, 1%) to each concentration of saponin.

Animals and Experimental design

SSE and cholesterol were given to five groups of white mice (albino mice - Musmusculus /Balb 1/C strain) weights ranged between 25-28 gm each group consist 4 boxes distributed in the form of square (4 x4), three mice (28-31 day old) and the same weight were put in summarized in table 2. From the table, it can be seen that the reversal relationship between SSE and cholesterol which appear each box, the boxes were placed in a constant temperature of room (23°±1°) the tails of mice were injected with SSE at concentrations (0.03, 0.06, 0.12, 0.25%) were anaesthetized by chloroform and wrapped in a saran net and the blood was taken by cutting the tail. The blood of every three mice of the same cage was put in one test tube and the level of cholesterol was determined after 4, 8, 12 hours.

Results and Discussion

Haemolysis of erythrocyte has been used to demonstrate the haemolytic activity of saponins with or without addition of cholesterol. The data in table 1 indicated that SSE at zero% of cholesterol showed the highest haemolysis at concentrations 0.12 and 0.25% of saponin, while addition of cholesterol at 0.4% caused partial haemolysis at concentrations of 0.12 and 0.25% of SSE, thus, haemolytic activity decreased gradually with increased the cholesterol concentration to reach zero % at 1% concentrations of cholesterol. Thus, haemolyticactivity of saponins on red blood corpuscles are probably caused by lipoid-protein constituents penetrating the membrane surface [10]. Activity of saponins may dissolve fatty materials or may denature proteins in the cells surfaces, leaving holes sufficiently large for hemoglobin molecules [11]. This is also supported by [12], recognized that certain chemicals such as benzene, toluene and saponin, which are fat – solvents may act on the red corpuscles membrane and disrupting the lipid components. The effect of SSE on the cholesterol level of mice blood was

decrease after 4 and 8 hours of injection, while the effect disappear after 12 hour. The lowest percentage of cholesterol reach at

0.25% concentration of SSE after (4,8) hour were 129.84 and 133.73 mg/100 ml respectively. While, at 0.03% of SSE, the percentage of cholesterol relatively equal to control 157.01, 156.90, 160 mg/100 ml at 4, 8, 12 hour, respectively. Highly signification differences could be found between control groups and SSE concentrations at 0.12 and 0.25% and period of injection on cholesterol level in plasma of mice , while no significant found in interaction effect. In fact, decrease of cholesterol level due to complex formed with SSE this is supported directly by previous study [13], that showed saponin has the ability to form complex with lipids [14], this study showed that saponin from an aqueous phase could penetrate into and complex with amber of surface absorbed lipids particularly cholesterol to form very insoluble complexes. The involvement of saponin with cholesterol may lead also to possibility of an interference with other function so a reduction in blood plasma cholesterol occurred when injection of saponin in mice, but organisms sensitive to saponin could react to one or more of the fraction [15]. Further studies are necessary to complete the work.

Table 1. The effect of cholesterol on haemolytic activity of ses ban saponin in vitro.

% Concentration of saponin					
% cholesterol	0	0.03	0.06	0.12	0.25
0	-	±	±	±	+
0.2	-	±	±	±	+
0.4	-	±	±	±	±
0.6	-	-	-	±	±
0.8	-	-	-	-	±
1	-	-	-	-	-

- = 0% haemolysis (no observable lysis)

± = partial haemolysis .

+ = 100% haemolysis .

Table 2. Cholesterol level in plasma of mice

Cholesterol level in plasma of mice mg/100 ml			
% Concentration of saponin	After 4 hour	After 8 hour	After 12 hour
0	158.17	157.43	160.01
0.03	157.01	156.90	160
0.06	139.46	143.63	158.80
0.12	131.30**	136.35**	154.01
0.25	129.84**	133.73**	153.22

** These figures are statistically different at $P<0.01$ for concentration of SSE and the period after in action

No significant difference in interaction

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