

Preparation and Pharmacological Evaluation of Potential Hypolipidemic Activity of Yemeni Activated Charcoal

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ABSTRACT

Background: Activated charcoal is a common form of carbon that is used to filter pollutants from water and air, among numerous other uses. It is a very useful adsorbent, due to their high surface area, pore structure, and high degree of surface reactivity. **Objectives:** To prepare and evaluate the potential hypolipidemic effect of the Yemeni activated charcoal. **Materials and Methods:** The present study was carried out *in vivo* by examination of blood lipid components for rabbit before and after therapy and calculating the difference to determine the effect of activated charcoal in comparison to the hypolipidemic standard drug (cholestyramine) and other commercial activated charcoal. In this study the lipid components (cholesterol, triglycerides, HDL, LDL and Risk Factor (RF)) were used as parameters for evaluating the activity of activated charcoal. **Results:** The prepared activated charcoal was the best product in reducing the lipid components (parameters) that must be reduced by therapy in comparing to standard and similar commercial products, the commercial product was the best in one parameter (HDL) but the worst in three parameters (cholesterol, triglycerides, and LDL). **Conclusion:** Based on the results obtained from this study, The prepared Yemeni activated charcoal was the best hypolipidemic in comparing to standard drug (Cholestyramine) in all lipid parameters (Cholesterol, triglycerides, HDL, LDL and RF) and also the prepared Yemeni activated charcoal was the best hypolipidemic in comparing to commercial activated charcoal product in four parameters (Cholesterol, triglycerides, LDL and RF).

Keywords: Activated charcoal, Cholesterol, Hypolipidemic, Yemeni, Ziziphus.

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INTRODUCTION

Activated charcoal (Carbon) is a common form of carbon which used to filter pollutants from air and water, amongst several other uses, its activation is carried out by processing to possess small, low-volume pores that leading to surface area increasing.^{1,2}

Activated charcoal is simply available in wood, coal, and coconut shells. There are different methods by which activated charcoal can be obtained. Diverse types of activated charcoals are used for diverse industrial purposes. The most common activated charcoals are granulated, pelletized, powdered, impregnated, and catalytically activated charcoals. <https://carbon2019.org/wp-content/uploads/2019/07/137-yamada.pdf>

Activated charcoal is a black, flavourless, odourless powder that has been used since ancient times to treat various ailments. Activated charcoal is charcoal treated with oxygen at very high temperatures to become more porous. This treatment led to increasing its surface area, changes its internal structure, and reducing its pores size.^{3,4} Moreover, activated charcoals are best economically adsorbents for many industries such as water purification, cosmetology, food grade products, industrial gas purification, automotive applications, petroleum and precious metal retrieval mostly for gold.

Hyperlipidemia is a state that includes several heritable and acquired troubles that describe the levels of elevated lipid within the human body. Hyperlipidaemia is very common, especially in the Western Hemisphere, but also worldwide. Instead, a more objective definition describes hyperlipidaemia as Low-Density Lipoprotein (LDL), total cholesterol, triglyceride levels, or lipoprotein levels greater than the 90th percentile compared to the general population, or an HDL level less than the 10th percentile in comparing him to the general population⁵ Lipids usually



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include cholesterol levels, lipoproteins, chylomicrons, VLDL, LDL, apolipoproteins, and HDL.

Hyperlipidemia is one of the major danger factors for atherosclerosis, which has been the leading cause of mortality globally.^{6,7} It is generally believed that an increase in plasma Low-Density Lipoprotein (LDL), Triglycerides (TG)-rich lipoproteins such as Chylomicron (CM), and Very-Low-Density Lipoprotein (VLDL) and/or a decrease in High-Density Lipoprotein (HDL) are associated with or cause atherosclerosis.⁸ For the study of human lipid disorders as well as for the development of therapeutic agents, it is essential to use an appropriate experimental animal.

Through the tries to confirm the benefit of activated charcoal in lowering the body lipid contents, several previous studies establish that the activated charcoal have preventing effect on obesity,⁹ preventive effect on HDL,¹⁰ inhibition effect on atherosclerosis,^{11,12} effect on cholesterol in hyperlipidaemia,¹³ effect in therapy of cholestasis on pregnancy,¹⁴ effect Versus Cholestyramine for Cholesterol Lowering,¹⁵ effect on uremic hyperlipidemia^{16,17} and reductive effect on hyperlipidemia in hemodialysis patients.¹⁸

This study was aimed to preparation and identification of the Yemeni activated charcoal and evaluate the effect of this form of activated charcoal on body lipid in comparing to antihyperlipidemic drug (cholestyramine) and other commercial activated charcoal product.

This study was carried out on rabbits by using blood lipid components (cholesterol, triglycerides, LDL, HDL and Risk Factor (RF)) as study parameters.

MATERIALS AND METHODS

Materials

Different types of woods from different trees were collected from different regions in Yemen. (Al-Salam, al-Kahlab trees from Bajil-Al-Hodeida and Al-Talh, Al-Sider trees from Al-A'aroq-region- Taiz) as shown in Table 1, Cholestyramine (Chepla. Pharm. Arzneimittel GmbH, Egypt) was purchased from the market. Activated Charcoal Eucarbon tablet (Alpha Pharm. Com, Austria) were purchased from the market. Cholesterols (HPFC, India) was purchased from the market. Methylene Blue (Umco, British), Calcium chloride (Uni. Chem, USA), Acetic acid 66% (BHD, British), Distilled water (Umco Pharma, Yemen) and Methanol (99.5%), were obtained from UMS.

Instrumentations

UV spectrophotometer (Shimadzu, Japan), Electric balance (Radwag, Poland), Mixture (JJ-1mixer, China), Water bath (HH-4, China), Centrifuge (China), Hot Oven (Labline Stock Centre) India, Morter and Pestle, Sieves No. 80 with pore of 0.1 mm, Vacuum (Labline Stock Centre) India, Mixer (Panasonic, Japan), and Filter paper (AU 480, Beckman Coulter, American).

Methods

Collection and preparation of different types of charcoal

Four types of wood were collected from two Regions Bajil -Al-Hodieda and Ala'arooq-Taiz, these wood burned at 300-400°C to produce the four types of charcoal.

Preparation of activated Charcoal

Preparation of pure Charcoal

The cold burned charcoal was transferred to a clean container and rinsed with cool distilled water to remove ash and any remaining debris, then the water was drained. The charcoal types were weighed separately and dried in oven at 100°C for 2 hr. The dried four charcoal types were weighed separately and grinded firstly by electrical grinder then by mortar and pestle. Finally, all types of charcoal were sieved separately by sieve No. 80.

Activation of charcoal

Addition of 100 mg of calcium chloride dissolved in 300 mL of distilled water to the four types of charcoal and mixed separately until pastes were formed, then the pastes were left to dried for 24 hr. Then, the Pastes were dried more in oven at 100°C for 1.5 hr. After that, the dried paste were grinded by mortar and sieved on sieve No. 80.

Identification of activated charcoal

Methylene blue test

A quantity, equivalent to 1200 mg of pure dye was dissolved to 1000 mL in a volumetric flask. The solution was allowed to stand for several hours (overnight).¹⁹ The solution was checked by diluting 5.0 mL with 0.25% (v/v) acetic acid to 11 mL in a volumetric flask and the absorbance of solution was measured at 620 nm for 1 cm. The obtained absorbance must be 0.840 or near the value. Then 100 mg of charcoal was added to three solutions of 25 mL, 5 mL and 1 mL of methylene blue. The solutions were allowed to stand for 10 min, after that the solutions were filtrated, this experiment was carried out for all types of charcoal, when the color of methylene blue of filtrate disappeared this indicate that the charcoal was activated. https://activatedcarbon.org/images/Test_method_for_Activated_Carbon_86.pdf.

Study the Potential therapeutic effect (hypolipidemic) of activated Charcoal

Ideal animal models for human hyperlipidemia should possess several important characteristics:²⁰ they should be easy to induce hyperlipidemia by diet intervention or genetic manipulation, they should have similar lipoprotein profiles as humans, they should be easy to handle and be of the proper size to allow for all anticipated experimental manipulations, they should be easy to acquire and maintain at a reasonable cost.²¹

Until now, several animal models have been used for the study of hyperlipidemia, including rats, mice, rabbits, guinea pigs, hamsters, pigs, and nonhuman primates. Unfortunately, there is no single animal model that meets all the requirements. Although each animal model has its advantages and limitations with respect to plasma lipoprotein profiles, handling, reproducibility, and cost, rabbits possess several unique advantages for the study of lipid metabolism. Due to their high susceptibility to a cholesterol diet, it is easy to induce hyperlipidemia and atherosclerosis in wild-type rabbits,²² which is different from most wild rat strains. Mouse models of hyperlipidemia have been established by targeting genes, such as apolipoprotein (apo) E receptor and LDL Receptor (LDLR).^{23,24} Nevertheless, there are a number of features that make rabbits a suitable model for human hyperlipidemia study.²⁰

Animal model study: 20 Male Rabbits in weight rang 1200-1600 g

Animal experimentation

Twenty normal healthy Rabbits of male sex weighing between 1200-1600 g were selected for the experiment from an inbred colony maintained under the controlled conditions of temperature ($32\pm 2^\circ$), humidity ($50\pm 5\%$) and light (10 and 14 hr of light and darkness, respectively). The animals were fed normal nutrition and all rabbits were fed with cholesterol-diet to increase lipid percent in their blood (Niimi *et al.*, 2020), the animals were divided to four groups the first one (-ev control) group only fed with cholesterol-diet and the Second group (+ev control) were fed with cholesterol-diet and administered two doses from (standard drug) cholestyramine, the third group (Test1) were fed with cholesterol-diet and administered two doses from our activated

charcoal, and fourth group (Test2) were fed with cholesterol-diet and administered two doses of commercial activated charcoal product. This procedure was continued for 15 days. The study was approved by Institutional Animal Care and use Committee (AL-Razi U-IACUC) AL-Razi University.

Feeding Rabbits with cholesterol

Group 1 (-VE control group; coded as -C): five rabbits were fed with 1 mL of 0.3% cholesterol twice daily for 15 days. Group 2 (+VE control group; coded as +C): five rabbits were fed with cholesterol in addition to the standard antihyperlipidemic drug cholestyramine twice daily for 15 days. Group 3 (Test 1 group coded as T1): five rabbits were fed with cholesterol in addition to our activated charcoal. Group 4 (Test 2 group coded as T2): five rabbits were fed with cholesterol in addition to a commercial activated charcoal product twice daily for 15 days.

Dosing

The following doses were applied for 15 days (therapy duration): Cholesterol: Oral feeding in all groups were 1 mL of 0.3% cholesterol solution every 12 hr. Activated Charcoal (Both our product and the commercial one): 350 mg/m² body mass index every 12 hr. Cholestyramine: 650 mg/m² body mass index every 12 hr.

The doses were calculated based as follows:

$$\text{Animal dose (mg)} = \text{Human dose} \times \text{BMI}_{\text{rabbit}} / \text{BMI}_{\text{human}}$$

Where BMI_{rabbit} BMI_{human} are 12 and 37 m² and the usual daily doses of activated charcoal orally in human is 25000 mg and that of cholestyramine is 4000 mg and both are calculated as per 37 m² human body mass index.²⁵

Table 1: Scientific name, Locality (coordinates and altitude), Date of Collection, Vernacular, and Herbarium Number of Plant specimens (Talh, Sider, Salam and Kahlab).

Sl. No.	Scientific name	Location	Coordinates		Altitude	Date of collection	Vernacular name	Herbarium No.
			Longitude	Latitude				
1	<i>Acacia origena</i>	Taiz governorate (Al-A'aroq District)	44°01' 15.28" E	13°34' 46.27" N	1400 m asl.	18 th Feb/2023	Talh	BHSS: 728
2	<i>Ziziphus spina-christi</i>	Taiz governorate (Al-A'aroq District)	44°01' 15.28" E	13°34' 46.27" N	1400 m asl.	18 th Feb/2023	Dawm, Sider	BHSS: 729
3	<i>Acacia ehrenbergiana</i>	Al-Hodeida governorate (Bajil Distinct)	42°57' 2.39" E	14°48' 4.79" N	000 m asl.	22 th Feb/2023	Salam	BHSS: 731
4	<i>Acacia origena</i>	Al-Hodeida governorate (Bajil Distinct)	42°57' 2.39" E	14°48' 4.79" N	000 m asl.	22 th Feb/2023	Kahlab	BHSS: 732

Procedure

The body lipids parameters were examined for the four groups of experimented rabbits before feeding, then the cholesterol feeding and administration of standard drug, our activated charcoal and commercial activated charcoal were continued for 15 days. After that, the body lipids were examined another time, from the examined results, the results were calculated.

RESULTS AND DISCUSSION

From the present study, the yield percents of charcoal produced from different sources of woods (trees) were 95%, 100%, 90% and 83.5% for al-Salam, al-Kahlab, al-Talh and al-Sider respectively (Table 2). The activation of all charcoal types was assessed following identification. The al-Sider charcoal demonstrated the highest activation level based on its methylene blue adsorption capacity, as shown in Table 2.

The activated charcoal from al-Sider tree was selected to complete the experiment of the activity of activated charcoal as hypolipidemic agent in comparison to standard cholestyramine and commercial activated charcoal. From previous studies that reveal the using of activated charcoal in some conditions of hypercholesterolemia and hyperlipidemia.^{7,9,11-14,18,20,22} Where, the results of cholesterol were reduction in their levels in all groups but more group T1 was more reduction in cholesterol level, this

means the prepared activated charcoal was the more activity than others as shown in Table 3.

The results of triglycerides were reduction in their levels in three groups (-ev Control, +ev Control, Test1) but the group T1 was more reduction in triglycerides level, so this means the prepared activated charcoal was the more activity than others, while T2 group was less activity (Table 3).

The results of LDL were reduction in their levels in all groups but group T1 was more reduction in LDL level, this means the prepared activated charcoal was the more activity than others (Table 3).

The results of the risk factor were reduction in their levels in two groups only (T1 and T2) where, group T1 was more reduction in RF level, this means the prepared activated charcoal was the more activity than others (Table 4).

Finally, the results for HDL were increasing in their level for two groups where, the commercial activated charcoal more activity (Table 4).

So, the prepared activated charcoal was the more activity in four important parameters (cholesterol, triglycerides, LDL and RF). All study results were demonstrated in Table 5 in addition to statistical analysis in which the *p* value was less than 0.05 that indicates there is significant or no significant difference between the results.

Table 2: Results of the yields and activation of charcoals from different trees (al-Salam, al-Kahlab, al-Talh, al-Sider).

Sample No.	Plant name	Site of collection	Weight of charcoal before washing	Weight of charcoal after washing and before drying	Weight of charcoal after washing and drying	Yield percentage	Amount of methylene blue solution (mL)		
							25 mL	5 mL	1 mL
Sample 1	<i>Acacia origena</i>	Bajel (Al-Hodeida)	200 g	345 g	190 g	95.00%	No effect (Still color)	Low adsorption	Full adsorption No color
Sample 2	<i>Ziziphus spina-christi</i>	Bajel (Al-Hodeida)	200 g	335 g	200 g	100.00%	No effect (Still color)	Low adsorption	Full adsorption No color
Sample 3	<i>Acacia ehrenbergiana</i>	Ala'arooq-Taiz	200 g	375 g	180 g	90.00%	No effect (Still color)	Low adsorption	Full adsorption No color
Sample 4	<i>Acacia origena</i>	Ala'arooq-Taiz	200 g	390 g	167 g	83.50%	No effect (Still color)	Full adsorption (no color)	Full adsorption No color

Table 3: Results of cholesterol, triglycerides and LDL levels before and after therapy for four groups.

Triglycerides mg/dL Normal: upto 160 mg/dL					Lipid Contents	Cholesterol mg/dL Normal: < 200 mg/dL				LDL mg/dL Normal: < 100 mg/dL						
Before		After		Reduction		Before		After		Group	Before		After		Reduction	Reduction
SD	Average	SD	Average			%	SD	Average	SD		Average	SD	Average	SD		
±16.0	109.5	±11.5	19	22.5	±1.5	25.3	±6.9	(-C)	41.5	±15.5	45	±7.0	8.4	92	12.6	
0	41.5	±3.5	-30.8	14	±1.0	9.5	±3.5	(+C)	27	±2.0	26	±7.0	-3.7	60	-32.1	
±12.9	37.7	±8.2	-61.7	44	±0.0	10.3	±2.3	T1	42	±2.9	30	±9.6	-28.6	98.3	-76.5	
±5.1	62	±22.0	10.7	19.7	±3.9	16	±3.3	T2	36	±1.4	35	±2.5	-2.8	56	-18.6	

The negative (-) indicates to increase in reduction %. SD: Standard Deviation.

Table 4: Results of risk factor, and HDL levels before and after therapy for four groups.

Risk Factor Normal: < 3.0					Lipid Contents	HDL mg/dl Normal: > 40 mg/dL					
Before		After		Reduction %		Before		After		Group	Induction %
SD	Average	SD	Average			SD	Average	SD	Average		
±0.2	2	±0.9	90.5	20	±10.0	11.3	±5.9	(-C)	1.1	-43.3	
±0.1	0.8	±0.1	6.3	17.5	±0.5	12	±4.1	(+C)	0.8	-31.4	
±0.1	0.6	±0.2	-50	15.3	±10.9	16.7	±0.9	T1	1.2	8.7	
±0.4	0.9	±0.3	-25.7	17.7	±2.5	20	±3.3	T2	1.2	13.2	

The negative (-) indicates to increase in reduction %. SD: Standard Deviation.

Table 5: Statistical analysis between the results of all lipid (Cholesterol, Triglycerides, HDL, LDL and Risk factor) levels for four groups.

Parameters	(-C)	(+C)	T1	T2
Cholesterol	8.43	-3.70	-28.57	-2.78
TG	19.02	-30.83	-61.69	10.71
HDL	-43.33	-31.43	8.69	13.21
LDL	12.59	-32.14	-76.52	-18.64
R. F	90.48	6.25	-50.00	-25.71
P	0.058798*			

The negative value (-) indicates to increase in reduction % in result of cholesterol, TG, LDL and R.F while indicates to decrease in induction% in results of HDL. *ANOVA-single way-test between hypolipidemic effects of studied animal groups [control group without treatment (-C), control group with standard cholestyramine (+C), group with Yemeni prepared activated charcoal product (T1) and group with commercial activated charcoal product (T2)] that indicated no sig variation ($p < 0.05$).

Statistical study

The Statistical Package for Social Sciences (IBM SPSS) version 27.0 was used to perform statistical analysis. Single-way Analysis of Variance (ANOVA) was used for comparison of the reduction percentage profiles for cholesterol, TG, LDL and R.F and the induction percentage profile for HDL. The results were considered statistically no significant, because the p -values were not less than 0.05.

CONCLUSION

Based on the results obtained from the present study, the best prepared activated charcoal was obtained from the wood of al-Sider trees. The results of therapy on the blood lipid contents levels: The prepared Yemeni activated charcoal was the best

hypolipidemic in comparing to standard drug (Cholestyramine) in all lipid parameters (Cholesterol, triglycerides, HDL, LDL and RF). The prepared Yemeni activated charcoal was the best hypolipidemic in comparing to commercial activated charcoal product in four parameters (Cholesterol, triglycerides, LDL and RF). The commercial activated charcoal was the best in one parameter (HDL) but was the worst among the three treated rabbit groups in three parameters (Cholesterol, triglycerides and LDL).

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ABBREVIATIONS

BMI: Body Mass Index; **CM:** Chylomicron; **HDL:** High Density Lipoprotein; **LDL:** Low Density Lipoprotein; **LDLR:** Low Density Lipoprotein Receptors; **RF:** Risk Factor; **SD:** Standard Deviation; **TG:** Triglycerides; **USM:** University of Moderne Sciences; **VLDL:** Very Low-Density Lipoprotein.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

Ahmed Mohammed Al-Ghani carried out experiments, interpreted results and participated in writing manuscript.

Other authors participated in discussing the results and contributing to the final manuscript.

SUMMARY

The study aimed to prepare and evaluate the potential hypolipidemic effect of the Yemeni activated charcoal, the activated charcoal was prepared from different types of trees, the activation of charcoal was carried out through the study, the most activated was used for completing the present study. The potential hypolipidemic effect of the most activated charcoal was carried out *in vivo* by examination of blood lipid components for rabbit before and after therapy and calculating the difference to determine the effect of activated charcoal in comparing to the hypolipidemic standard drug (cholestyramine) and other commercial activated charcoal. In this study the lipid components (cholesterol, triglycerides, HDL, LDL and Risk factor (RF)) were used as parameters for evaluating the activity of activated charcoal.

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