Blood Lipid Profile and Liver Lipid Peroxidation in Normal Rat Fed with Different Concentrations of *Acacia senegal* and *Acacia seyal*

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Abstract—The aim of the present study was to evaluate the blood lipid profile and liver lipid peroxidation in normal rat fed with different concentrations of *Acacia senegal* and *Acacia seyal*. Thirty six Sprague Dawley male rats each weighing between 180-200g were randomly divided into two groups. Each group contains eighteen rats and were divided into three groups of 6 rats per group. The rats were fed *ad libitum* with commercial rat's feed and tap water containing different concentrations of *Acacia senegal* and *Acacia seyal* (3 *%* and 6%) for 4 weeks. The results at 4 weeks showed that there was no significant difference (p≤0.05) in the total cholesterol (TC) and triglycerides (TG) between the control group and treated groups while the results for the high density lipoprotein (HDL-C) showed a significant decrease (P≥0.05) at the 3% and 6% of gum arabic treated groups compared to control group. There was a significant increase (P≥0.05) in low density lipoprotein (LDL-C) with 3% and 6% of gum Arabic (GA) groups compared to the control group. The study indicated that there was no significant (p≤0.05) effect on TC and TG but there was significant effect (P≥0.05) on HDL-C and LDL-C in blood lipid profile of normal rat. The results showed that after 4 weeks of treatment the malondialdehyde (MDA) value in rat fed with 6% of *A. seyal* group was significantly higher (P≥0.05) than control or other treated groups of *A. seyal and A. senegal* studied. Thus, the two species of gum arabic did not have beneficial effect on blood lipid profile and lipid peroxidation.

Keywords—*Acacia senegal*, *Acacia seyal*, lipid profile, lipid peroxidation, malondialdehyde (MDA).

I. INTRODUCTION

GUM acacia, also known as gum arabic (GA), is a natural gum harvested from the exterior of Acacia trees in the form of dried, hard nodules up to 50mm in diameter, and ranging from almost colorless to brown. Its unique properties endow it with a wide range of uses in food, beverage, confectionery, pharmaceutical, nutraceutical and industrial applications. The major producer of gum acacia is the Republic of Sudan. Other producer counties are Chad, Senegal and Nigeria [1].

Gum arabic is a dried exudate obtained from stems and branches of *Acacia senegal* or *Acacia seyal*. Gum arabic consists mainly of high-molecular weight polysaccharides and their calcium, magnesium and potassium salts, which on

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hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid. Items of commerce may contain extraneous materials such as sand and pieces of bark, which must be removed before use in food [1]-[4].

Gum acacia has been used in pharmaceutical applications for many years and it is specified in European and USA pharmacopoeias. The usage of gum acacia in pharmaceutical applications is based on its natural properties of emulsification, stabilization, demulcent action, adhesiveness and binding [1], [2], [5]. Gum acacia is a 100% water-soluble non-starch polysaccharide (NSP) which is resistant to hydrolysis by the digestive enzymes of humans. It contains excess of 85% total dietary fiber, a high molecular weight lipoprotein and low molecular weight heterogeneous gum polysaccharides [6]. Exudate gums are used in an overwhelming number of applications, mainly situated in the food area. Gum arabic is being widely used for industrial purposes such as a stabilizer, a thickener, an emulsifier and an encapsulating in the food industry, and to a lesser extent in textiles, ceramics, lithography, cosmetic, and pharmaceutical industry. In the food industry, the gum arabic is primarily used in confectionery, bakery, dairy, beverage, and as microencapsulating agent [7].

Gum arabic readily dissolves in cold and hot water in concentrations up to 50%. Because of the compact, branched structure and therefore small hydrodynamic volume, gum arabic solutions are characterized by a low viscosity, allowing the use of high gum concentrations in various applications. Solutions exhibit Newtonian behavior at concentrations up to 40% and become pseudo plastic at higher concentrations [8]. Chemically, gum arabic is a complex mixture of macromolecules of different size and composition (mainly carbohydrates and proteins).

A lipid profile is a blood test that measures the amount of lipids or fats in the blood. When levels of these lipids are abnormal, there is an increased risk of heart attack and stroke [9].

Reactive oxygen species (ROS) are highly reactive and in the absence of any protective mechanism they can disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids [10], [11].

Peroxidation of lipids is a binding process connected with the formation of aldehydes and one of them is malondialdehyde (MDA). Thibarbituric acid (TBA) assay is the most common method to be used to measure MDA activity [12]. It is known that the quantity of MDA is an intensity

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index of peroxidation process of polyunsaturated fatty acids (PUFAs) contained in food [13]. Malondialdehyde is one of the final products of polyunsaturated fatty acids peroxidation in the cells, increase in free radicals causes overproduction of MDA. The MDA level is commonly known as a marker of oxidative stress and the antioxidant status [14], [15]. Mohammad et al. reported that the level of lipid peroxidation can be determined by measuring the level of MDA which is a stable lipid peroxidation product [16]. Therefore, the main objective of this work was to compare the effect of different concentrations of *Acacia senegal* and *Acacia seyal* on blood lipid profile and liver lipid peroxidation in normal rat for 4 weeks of growth.

II. MATERIALS AND METHODS

A. Instruments

The following instruments were used in this study: (i) High-speed homogenizer (DI18 basic, IKA, Germany) (ii) centrifuge (Kubota 2010, Malaysia) (iii) UV-Visible spectrophotometer (Hitachj U-1800 single, Germany), (iv) Reflotron (ROCHE, 10007908, Germany).

B. Chemicals

Potassium chloride (KCl), thiobarbituric acid ($C_4H_4N_2O_2S$), tetraeethoxypropane (TEP), acetic acid ($C_2H_4O_2$), and butanol ($C_4H_{10}O$) were obtained from Sigma (USA) while sodium dodecyl sulfate ($C_{12}H_{25}O_4S$) was obtained from Sigma-Aldrich, Japan. Pyridine (C_5H_5N) was from Hopkin and Williams Chemicals Company.

C. Animals and Experimental Diets

Thirty six Sprague Dawley male rats each weighing between 180-200g and approximately 80 days old were obtained from the animal house of the Faculty of Science and Technology, Universiti Kebangsaan Malaysia. They were randomly divided into two groups. Each group contained eighteen rats and were divided into three groups of 6 rats per group. The rats were fed *ad libitum* with commercial rat's food and tap water containing different concentrations of *Acacia Senegal* and *Acacia seyal* (3% and 6%) for 4 weeks. At the end of the experiment, (after 4 weeks of treatment) the feeding of rats was stopped and rats were fasted for 18 hours. They were anesthetized using chloroform. All procedures were reviewed and approved by the Universiti Kebangsaan Malaysia Animal Ethics committee (FST/SBB/2010/HALIMAH/24-AUGST/322).

D. Lipid Analysis of the Blood

Total Cholesterol (TC) and Triglyceride (TG) were measured by strips with Reflotron machine using 32 μL whole blood. High Density Lipoprotein Cholesterol (HDL-C) was determined by strips (Roche, Germany) with Reflotron machine using 32 μL plasma blood. Plasma blood was prepared using a centrifuge (KUBOTA 2010, Malaysia) with speed 3000 rpm at room temperature for 10 min to remove red blood cells and recover plasma. Low Density Lipoprotein Cholesterol (LDL-C) was calculated from TC, HDL-C and TG

values using the Fried Wald equation [17]:

LDL (mg/dL) = Total Cholesterol-HDL Cholesterol-Triglycerides/5

All analyses were completed within 24 h of sample collection.

E. MDA Standard

The MDA standard used was tetraethoxypropane (TEP) as a 20 mM stock solution. For a standard curve, we pipetted the volumes shown in Table I to give a total of 200 μL of standard. [18]. Malondialdehyde was calculated using the following equation:

$$[MDA (\mu mol/g)]= A_{532} x V_T/V_s,$$

where: V= Total value; Vs= Sample value.

TABLE I
STANDARD CURVE DILUTION VOLUMES

Target concentration of standard (μM)	0	0.25	0.5	1.0	2.0	4.0
Volume of 20μM standard (μL)	0	25	50	100	150	200
Volume of water, μL	200	175	150	100	50	0

The clear supernatant was transferred to a cuvette and was absorbent at 586 nm. A plot of A_{586} nm vs. [MDA] for the standards was constructed Fig. 1.
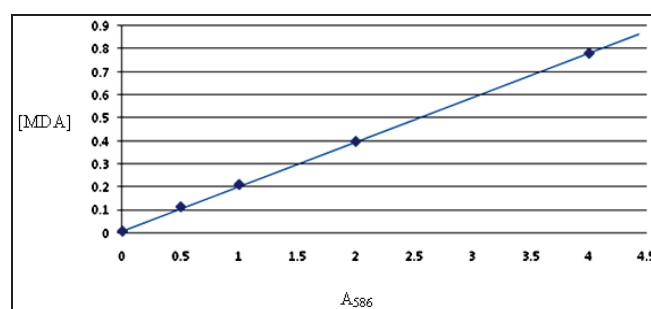

Fig. 1 Malondialdehyde Standard curve

F. Determination of Lipid Peroxidation

Liver sample for MDA was prepared by 1 g of liver cut to small pieces. Tissue was suspended in 9 ml of 1.15% KCl, and was homogenized using a mixer at top speed for 3 min. Thiobarbituric acid reactive substances (TBARS) was measured by the modified spectrophotometric assay [19]. The reaction mixture contained 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid solution, 1.5 ml of 0.8% aqueous solution of TBA, and homogenate (10%, 0.1 ml). The mixture was finally made up to 4.0 ml with distilled water, and heated at 95 °C for 60 min. After cooling with tap water, 1.0 ml of distilled water was added and the red pigment produced was extracted with 5.0 ml of the mixture of n-butanol and pyridine (15:1, v/v), and the mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the absorbance of the organic layer (upper layer) was measured at 532 nm.

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G. Statistical Analysis

Results were expressed as mean values ± SD (n = 6). Means of six samples were compared by analysis of variance (ANOVA). Significant differences between means were determined by Tukey's least different significant difference (P ≤ 0.05). The software used was MINI-TAB.

III. RESULTS AND DISCUSSION

A. Body Weight

Tables II and III show the effect of different concentrations of gum arabic on body weight of rat. There was no significance difference (P≤0.05) between treatment groups and control group but there was significant increased (P≥0.05) in body weight of rats after treatment period compared to the body weight before the treatment period with gum arabic. Samia et al. reported that the New Zealand rabbits fed on the diet include gam arabic showed higher body weight throughout the experiment period, which is significant after first and fourth week of treatment [21].

TABLE II
THE MEAN ± SD VALUES OF RAT BODY WEIGHT (N=6) AFTER 4 WEEKS
FEED WITH DIFFERENT CONCENTRATIONS (0%, 3% AND 6%) OF *A. SENEGAL*

Group	Before	After
	Mean±SD	Mean±SD
Control	222.04±8.5b	311.96±23.2a
3%	228.03±17.6b	323.17±19.2a
6%	229.52±22.4b	290.89±18.7a

Different alphabet within each row indicated significant difference (P≤0.05)

TABLE III
THE MEAN ± SD VALUES OF RAT BODY WEIGHT (N=6) AFTER 4 WEEKS
FEED WITH DIFFERENT CONCENTRATIONS (0%, 3% AND 6%) OF *A. SEYAL*

Group	Before	After
	Mean±SD	Mean±SD
Control	226.38±18.9b	321.88±19.7a
3%	211.36±7.3b	316.06±27.3a
6%	225.29±15.3b	319.34±36.5a

Different alphabet within each row indicated significant difference (P≤0.05)

B. Lipid Profile

Figs. 2 and 3 show the results of TC in blood samples of rats that were treated with 0%, 3% and 6% of *A. senegal* and *A. seyal* respectively. After 4 weeks of treatment the results of total cholesterol values were within the normal range. There was no significant difference (P≥0.05) between control group and groups fed with different concentration groups (3% and 6%).

Figs. 4 and 5 show the results of TG in different concentrations (0%, 3% and 6%) of *A. Senegal* and *A. seyal* respectively. After 4 weeks of treatment the results in this group had triglyceride values within the normal range.

In the current study, there was no change in levels of total cholesterol and triglycerides. This could reflect the short time period over which the study took place or the gum arabic should be mixed with another fiber or protein as reported in previous studies [22]-[25].
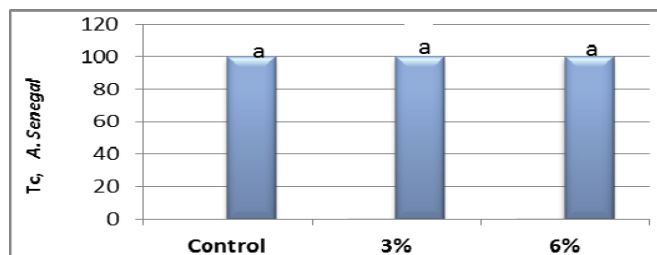

Fig. 2 The total cholesterol (TC) in blood of rats fed with different concentrations of *A. Senegal* (0%, 3% and 6%) for 4 weeks.
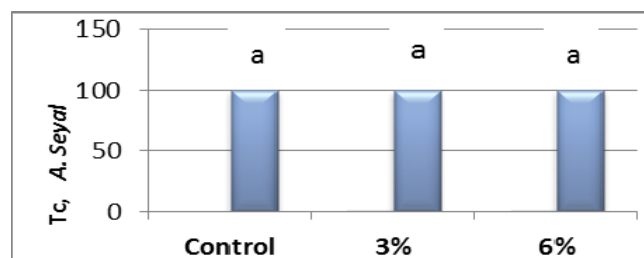

Fig. 3 The total cholesterol (TC) in blood of rats fed with different concentrations of *A. seyal* (0%, 3% and 6%) for 4 weeks.
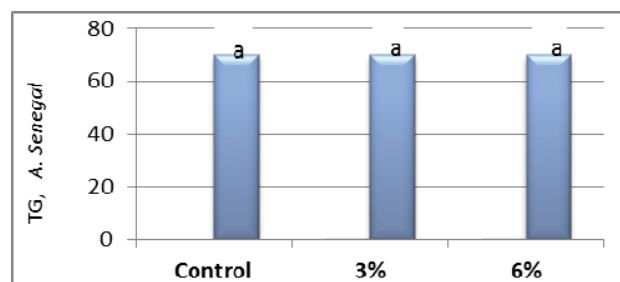

Fig. 4 The triglyceride (TG) in blood of rats fed with different concentrations of *A. senegal* (0%, 3% and 6%) for 4 weeks
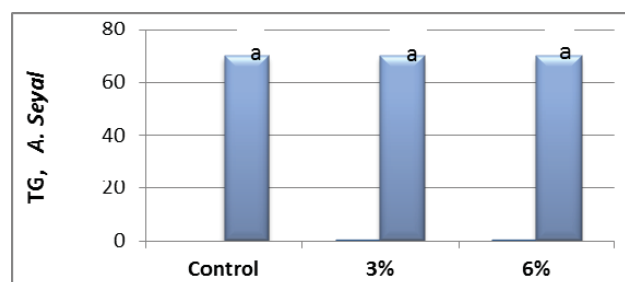

Fig. 5 The triglyceride (TG) in blood of rats fed with different concentrations of *A. seyal* (0%, 3% and 6%) for 4 weeks

Acacia gum (Gum arabic) alone did not alter serum lipids; however, the combination supplement lowered total cholesterol and LDL cholesterol [26].

The results of HDL-C levels of rat blood with 0%, 3% and 6% of *A. senegal* and *A. seyal* for 4 weeks of treatment are summarized in Figs. 6 and 7. The results showed that after 4 weeks of treatment with gum arabic there was significant decrease (P≤0.05) in treated groups compared to the control group. The results of LDL-C levels of rat blood with 0%, 3% and 6% of *A. senegal* and *A. seyal* for 4 weeks of treatment are

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summarized in Figs. 8 and 9. It showed that after 4 weeks of treatment with gum arabic there was significant increase (P≤0.05) in treated groups compared to the control group.
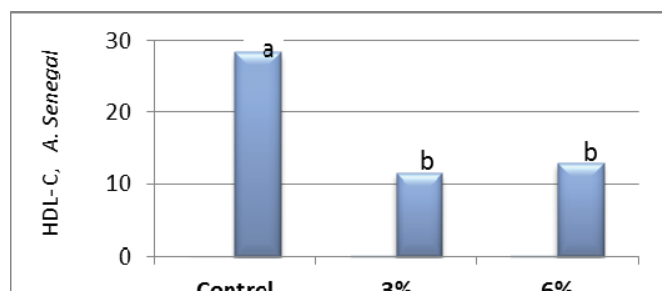

Fig. 6 High density lipoprotein cholesterol (HDL-C) (mg/dl) in rats fed with different concentrations of *A. senegal* (0%, 3% and 6%) for 4 weeks
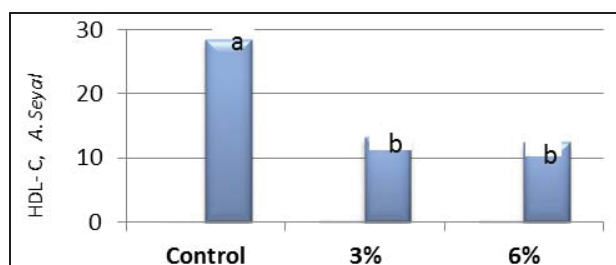

Fig. 7 High density lipoprotein cholesterol (HDL-C) (mg/dl) in rats fed with different concentrations of *A. seyal* (0%, 3% and 6%) for 4 weeks
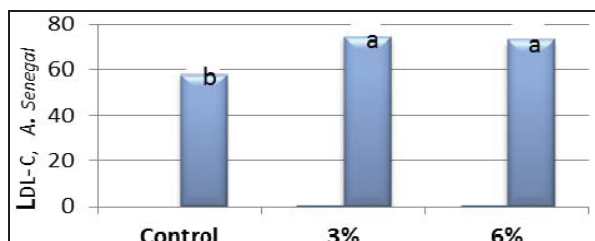

Fig. 8 Low density lipoprotein cholesterol (LDL-C) (mg/dl) in rats fed with different concentrations of *A. senegal* (0%, 3% and 6%) for 4 weeks
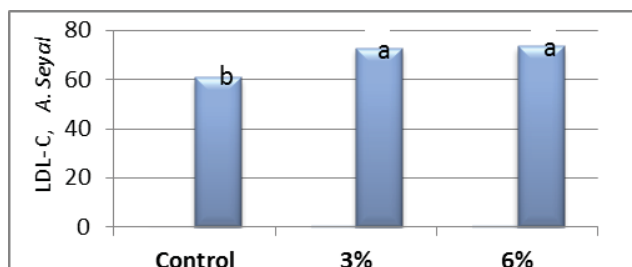

Fig. 9 Low density lipoprotein cholesterol (LDL-C) (mg/dl) in rats fed with different concentrations of *A. seyal* (0%, 3% and 6%) for 4 weeks

These results do not support the notion of a salutary effect of gum arabic in experimental lipid profile as was reported in some previous studies. The reason for the discrepancy may be related to a species difference or to other causes. This may be different from the conditions under which results claiming salutary effects of gum arabic [20]. However, in a longer term study, statistically significant sustained reductions in both total cholesterol and LDL cholesterol were seen in a group taking a mixture of psyllium, pectin, guar gum and locust bean gum, compared to the control group that was given acacia gum [26].

Geoffrey et al. reported that the acacia gums are non-starch polysaccharides (NSP) and consequently may be regarded as components of dietary fiber. Gum arabic is unusual among commercially important NSP in that there are more studies of its effects on lipid metabolism in humans than there are in animal models such as the rat and the consumption of gum arabic (15 g/d) for 4 weeks was found to have no significant effect on plasma lipids in either normal or control group [27]. In contrast, Mee and Gee carried out a 12 weeks cross over study to determine the combined effect of apple fiber and gum arabic on blood cholesterol levels in men with mild hypercholesterolemia [28]. The findings of the study indicated that the serum total cholesterol as well as serum LDL-C declined significantly (10% and 14% respectively) from baseline levels to post treatment levels. However, triglycerides and HDL-C concentrations were not significantly altered by the fiber supplementation [25], [29].

Studies using animals [30] and humans [31] have suggested that viscosity is an important contributor to the lipid-lowering potential NSP. The two gums used in the present study were of low viscosity and on that basis would not have been expected to lower plasma cholesterol or triacylglycerols. However, it must be considered that the role of viscosity is controversial.

One study has shown that viscosity relates to a lowering of plasma triacylglycerols [38], whereas others suggested that this property has no relationship to plasma lipids [27], [33], [34].

Ross et al. and Sharma found that the daily intake of 25 and 30 g of GA for 21 to 30 days reduced total cholesterol. The decrease was limited only to LDL and VLDL, with no effect on HDL and triglycerides [35], [36]. However, Topping et al. reported that plasma cholesterol concentrations were not affected by the supply of GA, but triglyceride concentration in plasma was significantly lower than in controls. Various mechanisms have been proposed to explain the hypocholesterolemic effect of GA and some studies have suggested that the viscosity of fermentable dietary fiber contributes substantially to the reduction of lipids in animals and humans [30], [32], [37]. However, other studies suggested that this property is not related to plasma lipids [7], [33].

Animal studies have given equally conflicting results. In rats fed gum arabic, plasma cholesterol concentrations were unaffected but plasma triglycerols were significantly lower than in controls [27]. In the present study, none of the two *Acacia* gums had any good effect on plasma cholesterol or liver MDA.

C. Lipid Peroxidation

The results of malondialdehyde levels in rat liver with 0%,

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3% and 6% of *A. senegal* and *A. seyal* for 4 weeks of treatment are summarized in Table IV. The results showed that after 4 weeks of treatment with gum arabic there was significant increase ($P \geq 0.05$) in 6% of *A. seyal* compared to the control group and another treated groups while no significant difference ($P \leq 0.05$) was found in the treated groups compared to the control group. This finding seems to suggest that there is no evidence that GA has a strong antioxidant action. This finding seems to suggest that there is no evidence that GA has a strong antioxidant action. It is difficult to explain the claimed hepato-, nephro- and cardio-palliative and protective effects of GA reported by Al-Majed et al. through an antioxidant mechanism, if GA lacks an appreciable antioxidant action [38].

TABLE IV
THE MEAN ± SEM VALUES OF RAT MALONDIALEHYDE (N=6) AFTER 4 WEEKS
FEED WITH DIFFERENT CONCENTRATIONS (0%, 3% AND 6%) OF *A. SENEGAL*

Malondialdehyde (MDA)µmol/g		
Group	*A. Senegal*	*A. Seyal*
	Mean±SD	Mean±SD
Control	59.77 ±10.68 a	59.33 ±7.58 a
3%	58.32 ±7.34 a	84.1 ±17.17 a
6%	66.95 ±18.42 a	99.18 ±0.3 b

Different alphabet indicated significant different ($P \leq 0.05$)

Other experimental designs have shown a potentially protective effect of Gum Arabic against gentamicin nephrotoxicity in rats [38], [39] possibly in part through inhibition of the production of oxygen free radicals that cause lipid peroxidation. The renoprotective effect of gum arabic seems to be independent of any effects that it may have on faecal bacterial ammonium [20], [39].

One of the major problems with gum arabic is that most commercially available forms have an inherently variable mass due to the fact that it is a natural product [40].

Al-Asaaf et al. showed that in 67 commercially available samples there was an extensive variation between individual samples, despite the fact that they were all marketed as "gum arabic". Multi angle laser light scattering was used to determine the average molecular mass of the samples which varied from 4.6 to 10.2×10^5 [41]. This wide variation in properties of different types of GA could be of major importance when carrying out a clinical study, as the biochemical properties of the different products may well vary. Therefore, a product with a consistent molecular make up is required [40].

IV. CONCLUSION

This study concludes that the gam arabic showed higher body weight after 4 weeks of treatment. The two species of gum arabic (*A. Senegal* and *A. seyal*) did not have any beneficial effect on blood lipid profile and lipid peroxidation. Possibly to get the effect of gum arabic on blood lipid profile the study need to be extended for a period of more than 4 weeks of treatment and mix the gum arabic with another type of fiber.

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