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Effects of Ethanolic and Aqueous Extract of Morus alba Leaves on Blood Glucose and Cholesterol Levels in Experimental Rabbits

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Abstract
There has been an increase in cases of high cholesterol and high blood pressure in people worldwide. This is due to poor diets consisting of high processed sugar, fats and sugar content, as well as lifestyle that encourage little physical exercise. This study investigated the effects of ethanolic extract and aqueous extract of *Morus alba* leaves on blood glucose, cholesterol levels on twelve randomly selected rabbits which were fed on commercial rabbit pellets. The weights of rabbits were also analyzed to determine if they were affected by *M. alba* leaf extract. The rabbits were divided into four groups, Group I (Control) was not administered with any ethanolic extract of *M. alba* leaves, Group II was administered with ethanolic crude extract of *M. alba* leaves at a dose of 1ml/kg, Group III were administered with the normal saline dilution plus ethanolic crude extract of *M. alba* leaves and Group IV were administered with aqueous extract of *M. alba*. Oral administration of the extract was done for two consecutive days per week. The rabbit's weight, cholesterol and blood sugar was monitored every week during the study duration. The results indicates that the control increased the most weight (394.67±75.23g**)** while the rabbits treated with the aqueous extract increased the least (246.67±26.57g). For the control, cholesterol and blood glucose levels increased during the 8 weeks of study while the other groups cholesterol and blood glucose levels decreased. The rabbits treated with normal saline plus ethanolic extract had the most significant decrease in cholesterol levels (41.34±4.19mg/dl) while the rabbits treated with aqueous extract decreased the least (15.33±1.49mg/dl). The rabbits treated with the aqueous extract had the most significant decrease in blood glucose levels (8±1.93mg/dl) while the rabbits treated with the normal saline plus ethanolic extract decreased the least (4.03±0.79mg/dl). There is a notable difference in the weights (ANOVA, F2.95, 28(3) =6.01, P=0.05), cholesterol levels (ANOVA, F2.95, 28(3) =10.79, P=0.05) and blood glucose levels (ANOVA, F2.95, 28(3) =33.76, P=0.05) of the four groups of the experimental rabbits after the eight weeks of treatment.
Keywords: *Morus alba,* aqueous, cholesterol, ethanolic, extract.

Introduction

Plants for a long time have provided a wide range of foods and compounds of medicinal value. For instance *Aloe vera* is used to treat wounds and other skin diseases (Raina *et al*., 2008). *Azadirachta indica* has anti-fungal and antimalarial properties several eucalyptus species have antibacterial activities (Ahmad & Beg, 2001). *Morus alba* has antioxidant, hypoglycemic and antihyperlipidemic effects (Cai *et al*., 2004). There are several plant species that lowers cholesterol and blood glucose levels in mammals. *Terminalia Chebula, T. belerica, Emblica officinalis* reduces blood sugar levels (Sabu & Kuttan, 2002). *Cyamopsis tetragonoloba* and *Plantago ovata* has been shown to have cholesterol lowering effects due to their high fiber content (Brown *et al*., 1999). Herbal medicines show minimal, or no side effects, and are considered safe (Agrahari *et al*., 2012).

Morus alba belongs in the family *Moracea* and it is commonly known as White Mulberry. *Morus alba* has been used as a medicinal plant for centuries, especially in Asian countries such as China. It has been used to treat diarrhoea, asthma, cough, dyspepsia, eye problems, intestinal ulcers, headaches, haemoptysis, hepatopathy, lumbago, melancholia and splenopathy (Khan *et al*., 2012). It has also been reported that it has anticancer properties (Kim *et al*., 2000).

Morus alba has been shown to have hypotensive and hypolipidemic effects. A study involving feeding male rats 7.5% cocoa butter and 1.25% cholesterol to induce thickening of the arteries caused by fat deposits and high cholesterol levels was done. These rats were then fed on leaves from *Morus alba* for 14 weeks. The results indicated and increase in the diameter of the arteries and an improved lower level of plasma cholesterol and triglycerides in their blood (Lee *et al*., 2011).

The potential of mulberry foliage as a feed supplement was investigated in India. The study indicated how mulberry trees are easily cultivated and provides large yields of leaves rich in nutrients. The study presented the Mulberry leaves as a very good feed not only for rabbits but also for cattle, chicken and other livestock (Singh & Makkar, 2002).

In a study done in Nigeria, it indicated the mulberry leaves can support good feed intake, digestibility and satisfactory weight gain in rabbits, and could reduce reliance on and cost of expensive concentrate diets. However, some level of concentrate feeding is necessary to reach potential weight gains (Bomikole *et al*, 2005).

In another study to evaluate the hypoglycemic effects of *Morus alba*, rats were fed on extracts from *Morus*

alba for five weeks. Blood glucose, glycosylated haemoglobin, triglyceride, LDL, HDL, blood urea, cholesterol, number of β cells, and diameter of the islets of langerhans were measured at the beginning and at the end of the experiment. The results showed a drastic decrease in blood glucose, cholesterol and triglycerides (Mohammadi & Naik, 2008).

M. alba has several health benefits. It acts as an antioxidant, anti-inflammatory and it has antimicrobial properties. It provides protection to the liver and it boosts immunity (Scott, 2010). *M. alba* leaves contains certain phytosterols, such as sterols and stanols (Tushishvili & Kekelidze, 1979). These phytosterols are capable of lowering the total LDL blood cholesterol level by preventing cholesterol absorption from the intestines (Ustun & Bulam, 2010). They are able to do this as they are more hydrophobic and have a greater affinity for micelles than the cholesterol. Due to this the micellar cholesterol concentration will be reduced resulting in a decrease of cholesterol absorption (Arnoldi, 2004). *M. alba* has several other active secondary metabolites such as flavonoids, alkaloids and phenylpropanoids (Xi-Da *et al*., 2010). These secondary metabolites, especially flavonoids reduce blood-lipid and glucose levels in humans (Ghasemzadeh & Ghasemzadeh, 2011).

This study involves the evaluation of the effects of ethanolic and aqueous crude extract of *Morus alba* on the cholesterol and blood glucose levels in experimental rabbits.

Materials and Methods

Plant material and collection

The *M. alba* leaves were obtained for a small scale farm near Juja town, Nairobi, Kenya and authenticated by plant taxonomist at botany department of Jomo Kenyatta University of Agriculture and Technology (JKUAT). The leaves were collected and kept in the shade for 3 days to air dry. The dry leaves were grounded in to power form and the extract were made out of them.

Preparation of Plant Extract

Preparation of ethanolic extract

The dried leaf power (300g) were mixed with 900 ml of 90% ethanol at room temperature and mixture was allowed to settle for 48 hours, filtered using whatmann filter number 1. The filtrate was then concentrated under a vacuum at 78^{o}C in a Buchi rotavapor R-200 rotary evaporator for 2 hours. The extract was then stored at 4^{o}C until further analysis.

Preparation of Aqueous extracts

The 300g of the dried leaf power was boiled in 900ml of distilled water for 15minutes. It was then allowed to cool to room temperature. The supernatants were decanted and centrifuged at 5400rpm for 10 minutes. The extract was then decanted into a beaker and stored at 4^{o}C until further analysis.

Experimental animals and Design

The rabbits used in this study were of the chinchilla breed, aged between 3-4months old. They were obtained from animal house of the Department of Zoology, JKUAT. The rabbits were housed in a controlled animal house with 12hours light 12 hours darkness at 25^{o}C. They were kept individually in a cage and fed twice a day. All procedures involving handling of laboratory animals were followed in accordance with the international standards on animals ethics and regulations as made available in our laboratory.

The experiment was carried out with four groups with 3 rabbits in each group. The weight, cholesterol and blood glucose were tested. All rabbits were fed on 150g of commercial rabbit pellets a day (75g in the morning and 75g in the evening). Pellets were used to feed the rabbits because it was readily available and low in cost. The commercial pellets also contain all the important minerals, fats and carbohydrates. Rabbit pellets contain maize germ, pollard, bran, sunflower, fish meal, cotton seed cake and premix.

Group I: (Control) Rabbits were fed on the commercial rabbit feeds alone.

Group II: Rabbits were fed on the pellets as well as the ethanolic crude extract at a dose of 1ml/kg,

Group III: Rabbits were fed pellets as well as the ethanolic extract that has been diluted with normal saline (50:50).

Group IV: Rabbits were fed on pellets as well as the aqueous extract.

The administration of the ethanolic crude, aqueous extracts and diluted extracts was done for two consecutive days a week. This was done for 8 weeks. Every week, the rabbits' weight, blood glucose and cholesterol levels were measured.

Administration of the extracts of *Morus alba*

The rabbits were first weighed and then extracts were given orally in a dose of 1ml/kg. Syringes were used to feed the rabbits the extract to ensure they take the entire dose. This was done for 2 consecutive days per week for 8 weeks.

Testing blood glucose and cholesterol levels

The rabbit's ear, at the area where the marginal vein is visible, was first sterilized with methylated spirit. This area was then carefully shaved with a sterile scalpel. A small prick was made on the marginal vein, if the marginal vein is not easily seen, the ear was tapped gently to dilate the vein. A little blood was drawn and placed on the cholesterol testing strip and the blood glucose testing strip. The ear was then sterilized with a little methylated spirit and held on tightly to stop any bleeding. The results obtained from the glucose and cholesterol meter were noted.

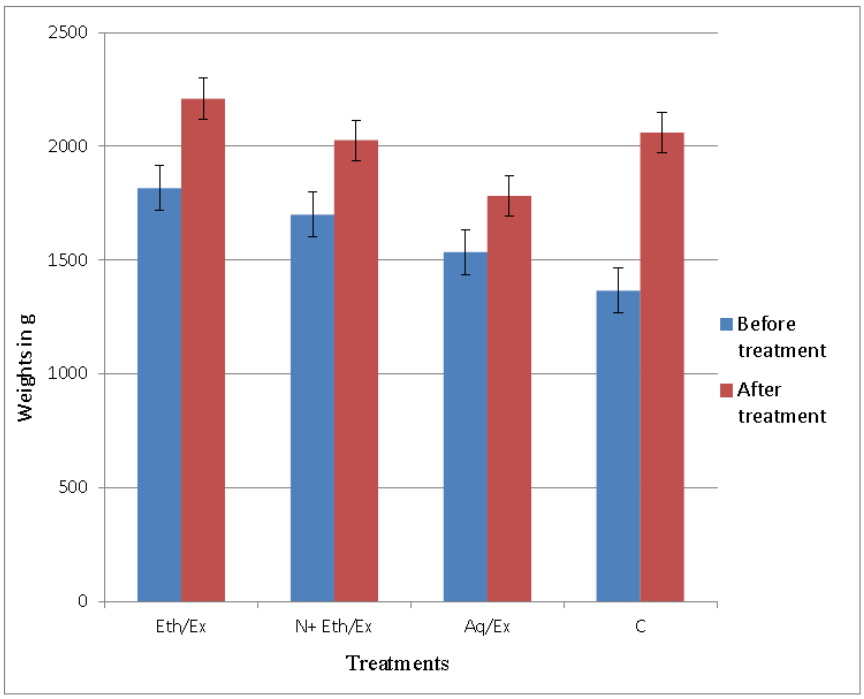
Statistical analysis

Data was presented as mean ± standard error of mean (mean ± SEM). One way analysis of variance (ANOVA) was used for the comparison of relative expression levels for different groups.

Results

Weights of the four groups of the experimental rabbits before and after 8 weeks of study.

There was an increase in the weights of the four groups of rabbits after the 8 weeks of treatment as shown in figure 1. There was a significant difference in the mean weights of the four groups of rabbits (ANOVA, F2.95, 28(3) =6.01, P=0.05). The rabbits treated with aqueous extract showed the lowest increase of 246.67±26.57g. The rabbits treated with normal saline plus ethanolic extract had a weight increase of 328±36.70g while the rabbits treated with the ethanolic extract had a weight increase of 394.67±45.71g. The control had greatest increase of 694.67±75.23g.

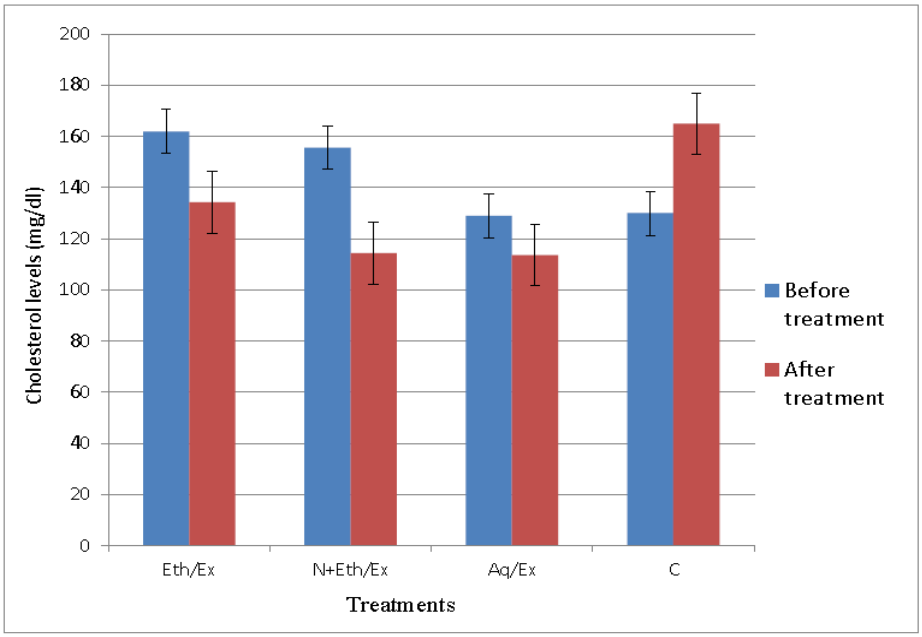


Key: EthyEx- Ethanolic Extract, Aq/Ex:-Aqueous Extract, C- Control
N+ EthyEx-Normal saline plus Ethanolic Extract

Figure 1: Weights (g) of the 4 groups of experimental rabbits

Cholesterol levels of the 4 groups of experimental rabbits before and after the 8 weeks of treatment.

There was a decrease in the cholesterol levels of three groups of rabbits, those treated with ethanolic extract, the normal saline plus ethanolic extract and the aqueous extract, after the 8 weeks of treatment, while in the control the cholesterol levels increased by 35±3.44mg/ml after the same 8 weeks of study as shown in figure 2. There was a significant difference in the mean cholesterol of the three groups treated with the *M. alba* leaf extracts and the control (ANOVA, F2.95, 28(3) =10.79, P=0.05). The rabbits treated with normal saline plus ethanolic extract showed the most prominent decrease 41.34±4.19mg/dl. The rabbits treated with ethanolic extract had a decrease of 27.67±3.36mg/dl. The rabbits treated with aqueous extract showed the least decrease of 15.33±1.49mg/dl.

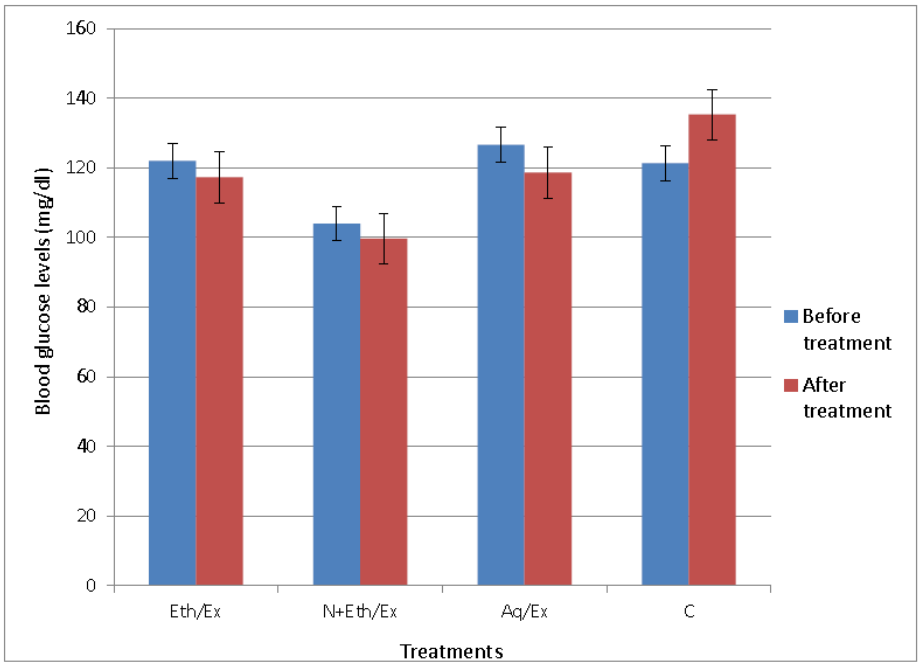


Key: EthyEx- Ethanolic Extract, Aq/Ex: Aqueous Extract, C- Control
N+ EthyEx-Normal saline plus Ethanolic Extract

Figure 2: Cholesterol levels (mg/dl) of the 4 groups of experimental rabbits

Blood glucose levels in the four groups of experimental rabbits before and after the 8 weeks of study.

There was a decrease in the blood glucose levels of three groups of rabbits, those treated with ethanolic extract, the normal saline plus ethanolic extract and the aqueous extract, after the 8 weeks of treatment, while in the control, the blood glucose levels increased by 14±0.83mg/ml after the same 8 weeks of study, as shown in figure 3. There was a significant difference in the mean blood glucose levels of the three groups treated with the *M. alba* leaf extracts and the control (ANOVA, F2.95, 28(3) =33.76, P=0.05). The rabbits treated with aqueous extract showed the most substantial decrease of 8±1.93mg/dl. The rabbits treated with ethanolic extract had a decrease of 4.67±0.79mg/dl while the rabbits treated with normal saline plus ethanolic extract showed the least decrease of 4.03±0.79mg/dl.



Key: EthyEx- Ethanolic Extract, Aq/Ex-Aqueous Extract, C- Control,
N+ EthyEx-Normal saline plus Ethanolic Extract

Figure 3: Blood Glucose levels (mg/dl) in the 4 groups of experimental rabbits

Discussion

The results indicates that the weights of the four groups of experimental rabbits increased during the 8 weeks of study. The control experienced the greatest increase in weight (694.67±75.23g) while the aqueous extract had the lowest increase of 246.67±26.57g. The rabbits treated with normal saline plus ethanolic extract had a weight increase of 328±36.70g while the rabbits treated with the ethanolic extract had a weight increase of 394.67±45.71g. This could be due to the rabbits in the control group developing more fat in their body tissues as their cholesterol levels rise (Bahrami *et al*., 2011).

The rabbits treated with the ethanolic extract, normal saline plus ethanolic extract and aqueous extract had the most significant decrease in the cholesterol levels (ANOVA, F2.95, 28(3) =10.79, P=0.05). This could be due to the leaves of *Morus alba* containing certain plant sterols referred to as 24-sterol-C-methyltransferase (SMT) which interfere with the absorption of dietary cholesterol in the small intestines. These plant sterols appear to decrease the solubility of cholesterol in the oil and micellar phases, thus displacing cholesterol from bile salt micelles and interfering with its absorption in the small intestines (Nguyen, 1999). *M. alba* leaves also have β carotene, polyphenols and phytonutrients which are capable of modulating the LDL oxidation (Andallu *et al*., 2009).

The results obtained in this study reveals that the extract had a reducing effect on the cholesterol levels of those groups of rabbits treated with the ethanolic and aqueous extract of *M. alba* leaves. The normal saline plus ethanolic extract was the most effective in lowering the cholesterol levels in the study groups. Along with the effect of the sterols in *M. alba,* salt solution affects the osmotic effect within the small intestines, thereby affecting the absorption of cholesterol. Increased salt intake will reduce absorption of cholesterol and increase its elimination through fecal bile, thereby reducing its levels in the blood (Schoppen *et al*., 2004).

The ethanolic extract of *M. alba* leaves was the second most effective in lowering cholesterol. Also with regards to the effects of the sterols present, the alcohol has an ability to increase the HDL in the blood. The HDL is able to bind on the LDL in the blood and tissue and then transport it back to the liver where it is broken down to by eliminated from the body. Alcohol however has no effect on the absorption of cholesterol in the small intestines (Crouse & Grundy, 1984). This can be compared with a previous study done on the hypotensive and hypolipidemic effects of *M. alba* leaves. A study involving feeding male rats 7.5% cocoa butter and 1.25% cholesterol to induce thickening of the arteries caused by fat deposits and high cholesterol levels was done. These rats were then fed on leaves from *Morus alba* for 14 weeks. The results indicated and increase in the diameter of the arteries and an improved lower level of plasma cholesterol and triglycerides in their blood, there for indicating a reduction in overall cholesterol and triglyceride levels in the mice. Another study done on plant sterols indicates that sitosterols (≥10g/d) lowered the serum cholesterols by 10-20% (Lees *et al*., 1977).

The extracts of *M. alba* leaves also had a notable effect on the blood glucose levels in the four groups of rabbits during the 8 week study (ANOVA, F2.95, 28(3) =33.76, P=0.05). The rabbits treated with aqueous extract showed the greatest decrease of 8±1.93mg/dl. The rabbits treated with the ethanolic extract had a decrease of 4.67±0.79mg/dl while the group of rabbits treated with normal saline plus ethanolic extract had the least decrease of 4.03±0.79mg/dl while in the control the blood glucose levels increased by 14±0.83mg/ml. this could be due to *M. alba* leaves containing plant alkaloids called trigonelline and moranoline (Mohammadi & Naik, 2008). Trigonelline stimulates the β cells to secrete insulin thereby reducing the blood glucose levels (Zhou & Zhou, 2012). Moranoline inhibits the enzyme α-glucosidase which is involved in the digestion of carbohydrates (Sahelian, 2006). As a result the carbohydrates are not broken down into glucose; hence blood glucose levels will decrease. Thus this concurs with the result of aqueous extract of *M. alba* leaves being the most effective in reducing the blood glucose levels.

Ethanolic extract of the *M. alba* leaves also had a clear effect on the blood glucose levels in the rabbits, however this was not as profound as the effects of the aqueous extract. Ethanol is broken down in the liver, as this occurs it inhibits a process called gluconeogenesis where glucose molecules are formed from alanine and glycerol, especially during a fasting state. This results in reduced levels of blood glucose (Emanuele *et al*., 1998).

The rabbits treated with normal saline plus ethanolic extract has the lowest decrease in the blood glucose levels. This could be due to the salt solution having no effect on the glucose levels in the blood. This can be compared with a previous study involving the hypoglycemic effects of the septum of walnut shell. The study showed that the blood glucose levels of the diabetic rats treated with this extract reduced from 322mg/dl on the 14th day of study to 220mg/dl on the 42nd day of study. The cholesterol levels and blood glucose levels of the control rabbits increased due to the commercial pellets being rich in starch and cellulose (Xiangmei, 2008).

Conclusion

The extracts of the *M. alba* leaves show a prominent difference in the cholesterol when administered to the experimental rabbits. The normal saline plus ethanolic extract showed to be the most effective in reducing the cholesterol levels while the aqueous extract showed to be the least effective in lowering the cholesterol levels.

The *M. alba* extracts also showed a significance difference in the blood glucose levels in the same

experimental rabbits. The aqueous extract showed to be the most effective in reducing the blood glucose levels while the normal saline plus ethanolic extract was the least effective. The weights of all the experimental rabbits increased during the study period.

Conflict of interest: There is no conflict of interest among authors in this work.

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