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Original Research Article

Historestorative effects of *Warbugia ugandensis* on high fat diet induced atherosclerosis in New Zealand rabbits

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ABSTRACT

Background: Historestorative feature is a vital component that any organ can acquire secondary to its damage. Most of the time damage arises from traumatic injury or toxification by toxic agents. In the current context the aortic intima lumen significantly histologically changed secondary to administration of *Warbugia ugandensis* thus increasing blood supply to vital organs.
Methods: Posttest only true experimental study design was used with 33 male New Zeeland rabbits considered for this study. In grouping of animals, Systematic sampling method was used to assign them as control and experimental groups. *W. ugandensis* extract was obtained after which phytochemical analysis and acute oral toxicity were conducted to determine safe dose. The animals were fed on high fat diet to induce atherosclerosis.
Results: The mean fraction of restorative group reduced significantly (p=0.0001) relative to vehicle control group. There was no significant difference in mean area fraction of *W. ugandensis* restorative group when compared with negative control group (p=1.000). On histological features, restorative group had a smaller lesion as compared to vehicle group. The lipid core was smaller in size with large fibrous cap around it. The endothelial cells surrounded the lesion as opposed to vehicle control group make it more stable.
Conclusions: Therefore, it can be concluded that *W. ugandensis* has historestorative benefits portrayed by reduction of atherosclerotic lesion with a lipid core covered by a large fibrous cap.

Keywords: Antioxidant, Anti-inflammatory, Atorvastatin, Historestorative, Phytochemicals

INTRODUCTION

Historestorative is generally the ability of an organ to attain its histological make secondary to toxicity or damage. It's science that involves causing damage using a toxic substance and thereafter administering another substance that possess restorative abilities.[1] Atherosclerosis is typical the development of a plaque within a lumen of a blood vessel after a high fat or cholesterol diet which initiates inflammation and oxidation. historestorative changes in atherosclerosis can be mapped by changes in histological make of blood vessel wall, and size of aortic intima. Plants with high phytochemical components have high antioxidative and anti-inflammatory benefits thus can highly reverse the changes caused. According to the presence of polyphenolic acid slowed the progression of an atherosclerotic lesion since it had a wide range of biological effects, including antiplatelets, antioxidants, endothelium protection qualities, and smooth muscle cell proliferation.[2] Alkaloids, flavonoids, phenolics, sugar alcohols, and unsaturated fatty acids, particularly linoleic

acid, were also found in W ugandensis active chemical components and hence this explains the historestorative might of *W. ugandensis*.[3] Indeed, it has been documented that active principles originating from plants, such as polyphenols, tannins, flavonoids, alkaloids, and so on, decrease the formation of cholesterol in the liver and impede the actions of enzymes that synthesize cholesterol.[4] They also reported that the histopathological changes were also reverted to normalcy and reports that the levels of lipid profile markers significantly reduced signifying restoration.

The objective of this study was to evaluate historestorative effects of *Warbugia ugandensis* on high fat diet induced atherosclerosis in New Zeeland rabbits.

METHODS

Experimental design and animals

This was a posttest only true experimental study design which involved use of 30 male New Zeeland rabbits.

Systematic sampling method was used in recruiting and assigning the animals into control and experimental groups.

The study was conducted at the University of Nairobi Biology Animal house for a period of 17 weeks which included 2 weeks of acclimatization and 15 weeks of study. The study started from March to August 2023.

Inclusion and exclusion criteria of animals

Inclusion criteria

All healthy New Zealand rabbits that had attained the desired weight at time of study were included.

Exclusion criteria

New Zealand rabbits that showed signs of sickness and were weak were excluded.

Grouping of animals

Systematic sampling method was used, whereby 18 rabbits were picked from the pure-bred New Zealand rabbits and ascribed in to experimental or control group. In addition to this another set of 12 rabbits was sampled to determine safe doses of *W. ugandensis* by conducting acute oral toxicity hence making the total number of rabbits for whole study to be 30. The 18 rabbits were randomly assigned to two groups; controls and experimental groups. The control group had 12 rabbits while experimental had 6 rabbits. The process was done by assigning numbers to the sample frame and then balloting was done to choose for each category. Negative control is a group that received normal diet (150 g of rabbit chow and free access to water) while vehicle control received (50 g of high fat diet and 100 g of normal diet for 7 weeks then concurrently with DMSO for a period of 8 weeks).

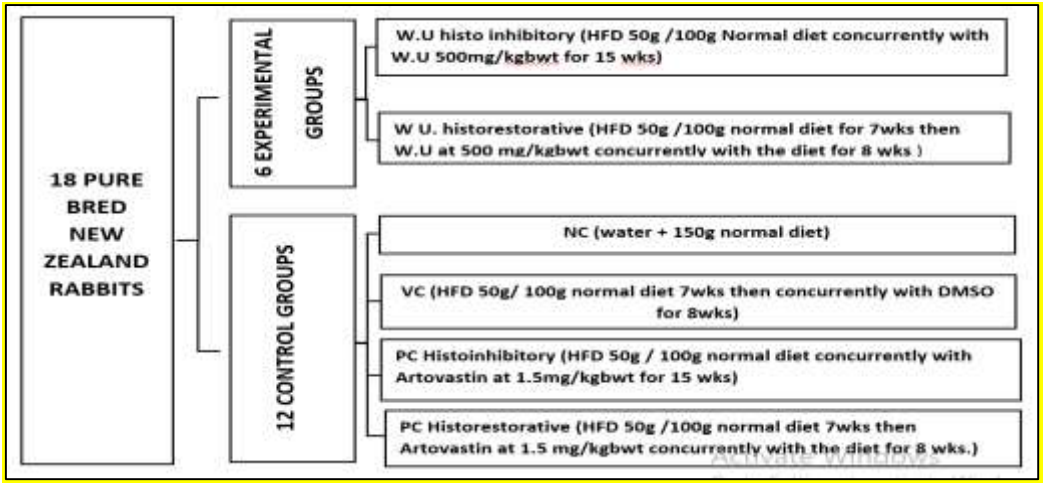

Figure 1: Grouping of animals.

Note: PC- positive control, VC-vehicle control, HFD- high fat diet and W. U.- *Warbugia ugandensis.*

Drug preparation and administration

W. ugandensis stem barks were collected randomly with assistance of a plant taxonomist from Mount Kenya forest. They were then washed, air dried and grounded into powder and stored in plastic bags until extraction. 1000 g of plant powder was soaked in room temperature for one day then filtered with Whitman no. 1 filter paper after which solvent evaporation was achieved. Phytochemical components were determined, qualitative analysis was done and acute oral toxicity conducted to determine the safe dose of extract. To induce atherosclerosis, animals were fed on 50 g of high fat diet (1.5% cholestrol) for seven weeks in positive control atorvastatin was given concurrently with high fat diet while experimental groups received *W. ugandensis*

extract concurrently with high fat diet to achieve histo-inhibitory effects.[5] During this process high hygiene standards and animal handling occupational procedures were adhered to strictly throughout the study.

Sacrificing of animals

Concentrated carbon iv oxide was used to sacrifice the animals at the end of week fifteen. A mid line incision was made from jugular notch to pubis symphysis to expose abdominal-pelvic viscera.

The heart was identified, perfusion with 10% formalin done, the heart and aorta were dissected. The aorta was then preserved in 4% formalin. Staining of atherosclerotic lesion was done using oil red O working solution. Aortic tissue fixed by Bousin`s solution for 24 hours later the slides were stained in hematoxylin eosin solution for light microscopy. Photomicrograph were obtained using Leica M125 stereomicroscope mounted with DFC450 camera at magnification of 8X.

The area fraction of aortic intima was done using image J, image analysis software. Data on aortic intima fraction was analyzed used SPSS version 26.0, one-way ANOVA was adopted to determine mean difference and later subjected to post hoc Bonferroni test. A significance level of $p<0.05$ was considered significant. The ethical approval was adopted from JKUAT (JKU/ESRC/02316/0891) and NACOSTI (NACOSTI/P/23/28152).

RESULTS

Comparison of mean area fraction of different historestorative groups

Vehicle control (6) verses negative control (NC) groups

The mean area fraction of vehicle control group significantly increased as compared to negative control group ($p=0.0001$) at 0.51622 mm^2 and 0.15144 mm^2 respectively.

Vehicle control vs Warbugia restorative groups

The mean area fraction of W. ugandensis restorative group significantly ($p=0.0001$) reduced when compared to vehicle control group at 0.35022 mm^2 and 0.51622 mm^2 respectively.

Vehicle control group verses atorvastatin restorative group

The mean fraction area of atorvastatin restorative group significantly reduced ($p=0.0001$) at 0.20461 mm^2 as compared vehicle group of 0.51622 mm^2 respectively.

Negative control group verses Warbugia restorative group

There was no significant difference of mean area fraction of *W. Ugandensis* restorative group when compared with negative control group ($p=1.000$).

Table 1: Comparison of mean area fraction between negative control group and vehicle control group.

Groups					
	Negative Control (water+food)	**Vehicle Control (DMSO)**	**df**	**F**	**P value**
Area fraction (mm^2)	0.1514±0.02	0.51622±0.36	5	86.493	0.0001*

Note: All values are expressed and presented as the mean±the standard error of the mean[7]; n=3. Data analyzed by one-way analysis of variance followed by post-hoc Bonferroni. Asterisks* represents significant ($p\leq0.0001$)

Table 2: Comparison of mean area fraction between vehicle control and *W. ugandensis* restorative groups.

Groups					
	Vehicle control (high fat diet-50 g/day + DMSO)	***W. ugandensis* historestorative (high fat diet-50g/day + *W ugandensis* 500mg/kg/day)**	**df**	**F**	**P value**
Area fraction (mm^2)	0.51622 ±.36	0.35022±0.017	5	86.493	0.0001*

Note: All values are expressed and presented as the mean± the standard error of the mean;[7] n=3. Data analyzed by one-way analysis of variance followed by post-hoc Bonferroni. Asterisks* represents significant ($p \leq 0.0001$

Table 3: Comparison of mean fraction area between vehicle control and atorvastatin restorative groups.

Groups					
	Vehicle control (high fat diet-50 g/day+DMSO)	**Atorvastatin historestorative (high fat diet-50 g/day + atorvastatin)**	**df**	**F**	**P value**
Area fraction (mm^2)	0.51622±0.36	0.20461±0.31	5	86.493	0.0001*

Note: All values are expressed and presented as the mean± the standard error of the mean;[7] n=3. Data analyzed by one-way analysis of variance followed by post-hoc Bonferroni. Asterisks* represents significant ($p \leq 0.0001$)

Table 4: Comparison of mean fraction area between Warbugia restorative and negative control groups.

Groups					
	Negative control (feeds + water)	***W. ugandensis* historestorative (high fat diet-50 g/day + W. ugandensis 500 mg/kg/day)**	**df**	**F**	**P value**
Area fraction (mm^2)	0.15144±0.02	0.35022±0.20	5	86.493	1.000

Note: All values are expressed and presented as the mean± the standard error of the mean;[7] n=3. Data analyzed by one-way analysis of variance followed by post-hoc Bonferroni.

Table 5: Comparison of mean area fraction between negative control and atorvastatin restorative groups.

Groups					
	Negative control (feeds + water)	**Atorvastatin historestorative (high fat diet-50 g/day + atorvastatin)**	**df**	**F**	**P value**
Area fraction (mm^2)	0.15144±.02	0.20461±0.35	5	86.493	1.000

Note: All values are expressed and presented as the mean± the standard error of the mean;[7] n=3. Data analyzed by one-way analysis of variance followed by post-hoc Bonferroni.

Table 6: Comparison of mean area fraction between *W. Ugandensis* restorative and Atorvastatin restorative.

Groups					
	Atorvastatin historestorative (high fat diet-50 g/day + atorvastatin+ $DMSO_4$)	***W. ugandensis* historestorative (high fat diet-50 g/day + *W. ugandensis* 500 mg/kg/day)**	**df**	**F**	**P value**
Area fraction (mm^2)	0.20461±.35	0.35022±.22	5	86.493	0.0001*

Note: All values are expressed and presented as the mean± the standard error of the mean;[7] n=3. Data analyzed by one-way analysis of variance followed by post-hoc Bonferroni. Asterisks* represents significant ($p \leq 0.0001$)

Negative control group verses atorvastatin restorative group

There was no significant difference of mean area fraction of negative control group when compared with Atorvastatin restorative group (p=1.000).

Warbugia ugandensis restorative Verses atorvastatin restorative

There was a significant increase in the area fraction in *W. Ugandensis* restorative group (high fat diet- 50 g/day and Wu- 500 mg/kg/day) compared to atorvastatin restorative group (high fat diet- 50 g/day and atorvastatin) which recorded a mean of 0.35022 mm and 0.20461 mm respectively. A statistically significant difference ($p<0.005$) was observed between *W. Ugandensis* restorative group compared to atorvastatin restorative group.

Histological comparison between control and historestorative groups

W. ugandensis historestorative group had smaller lesion as compared to vehicle control but not smaller than Atorvastatin restorative group. The lipid core was smaller in size with a large fibrous cap around it. Atorvastatin historestorative had a small lesion with endothelial cells covering the lesion as opposed to vehicle control and *Warbugia ugandensis* historestorative group.

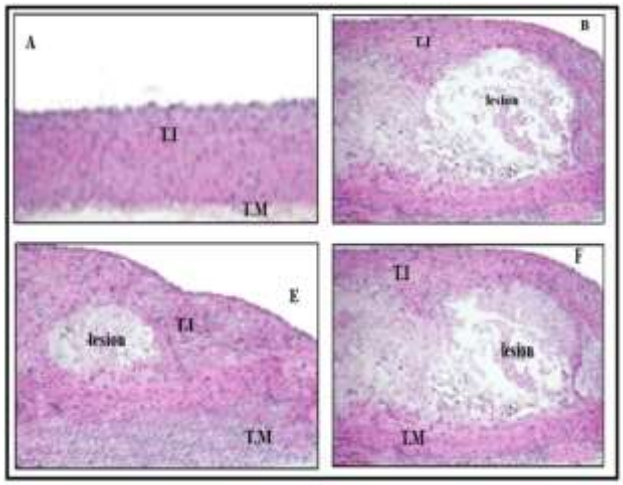

Figure 1 (A-D): (A) Negative control group; (B) vehicle control group; (C) atorvastatin restorative group; and (D) *W. ugandensis* restorative group.
Note: T. I= tunica intima and T.M= tunica media.

DISCUSSION

Historestoration is the ability of a tissue or cell to return to its normal status after a pathological process has occurred. It is evaluated through many parameters

however; in present study it was evaluated through measuring the area fraction of lesion within the tunica intima and correlating with the different histological findings. Any deviation from the normal findings as evidenced by area fraction of lesion in tunica intima and histological observations of different group denoted restoration.

It was observed that there was significant reduction in area fraction of W. ugandensis historestorative group as compared to vehicle control group. This significant reduction in area fraction of lesion might have been due to high phyto-steroids in methanolic extract of *W. ugandensis* which concurs with the findings of in which the researchers clearly state that phyto-steroids defend inflammation by pulling down the pro-inflammatory mediators.[8] According to Tiwari and colleagues, Phyto-steroids have similar structure to glucosteroids therefore are of high value in disease healing.[9] They also play a role in gene expression as they might inhibit gene expression which plays a role in treatment of malignancies.[10] On the same note the restorative effects is also attributed to presence of flavonoids which has both anti-inflammatory and antioxidant properties which contributes to slowing down the development of atherosclerosis hence cardio protective.[11]

On historestorative effects, it was observed that lipid core was smaller as compared to vehicle control with numerous cells, a small area of lipid spicules and thick fibrous cap. This is synonymous to reducing the progression of atherosclerotic lesion. On atorvastatin restorative group, the lesion was even smaller, with no lipid spicules and thicker fibrous cap because of the effects of antioxidant and anti-inflammatory properties of atorvastatin. The same was replicated in *W. ugandensis* historestorative however; it was not as much as compared to atorvastatin hence making the drug more effective compared to *W. ugandensis*. The histological changes were attributed to presence of high amounts of flavonoids, phytosteroids, tannins and saponins in the present study which gives both physiological and histological picture of the use of *W. ugandensis* in historestoration and histo-inhibition of tissue especially when rabbits were subjected to high fat diet. This therefore improves normal cardiac function.[12] Demystifies that saponins play an important role in cardio protection due to its structural characteristics and pharmacological effects. Some of its properties are Ca2+ ion regulation, antiapoptotic, antiatherosclerotic, antihyperlipidemic and vasodilatory while pharmacologically its high permeability through cell membrane makes it a better cardio protective agent.

Limitations

Some of the animals did not acquire weight at prescribed time and therefore were fed differently so as to achieve the weight before being included into the study.

CONCLUSION

Therefore, it can be concluded that *W. ugandensis* has historestorative benefits portrayed by reduction of atherosclerotic lesion with a lipid core covered by a large fibrous cap.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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