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The Effect of Wild Yam *(Dioscorea Villosa)* Tincture on Memory and Learning in Mice

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Abstract

*In this work, the effect of orally administered tincture of wild yam (Dioscorea villosa) on learning and memory in young mature mice has been studied. The wild yam tincture was administered to the test animals by oral gavage and FELASA guidelines were adhered to. Twenty male swiss albino mice (n=20): ages 3 – 6 months were randomly allocated into a control and test group (n=10 each) during the experimental period of 8 months. The animals were subjected to learning and memory tests using the T-Maze apparatus. **Long term memory** was assessed using a left – right discrimination task in a T- maze. For the above test, four parameters were evaluated: Correct Response (%), Latency (sec), Distance Travelled (cm) and number of Omission Errors. In this study the effective food reward (bait) used was a breakfast cereal "Kelloggs Honey Loops". Data was analyzed using STATA version 11 software, using independent t-test and results presented as mean ± standard error of means (SEM). The significance level was $P<0.05$. **Motivation** was measured by reduction in omission errors. The test and control mice showed no significant difference in memory tests before wild yam tincture was administered. After administration of wild yam tincture the young mature test mice showed increased learning ability and improved memory. This study recommends that further studies be* *undertaken to explore the benefits of wild yam in human volunteers considering that wild yam products are widely used in Chinese and Western alternative medicine for other purposes rather than the enhancement of memory and learning.*

Key Words: *Wild Yam, Diosgenin, Tincture, Mice, T-Maze, Memory, Learning, Aging*

Introduction

Memory is central to our ability to attend and perform our daily life activities and correctly function in society. Loss in memory occurs as part of the aging process and memory deficits related to age have been significant sources of morbidity in any human population (Fjell et al., 2014). Dementia is one of the main causes of disability in older people (WHO, 2013). Memory loss is associated with the occurrence of neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease (Fjell et al., 2014). As the brain undergoes the aging process it becomes susceptible to neurodegeneration which results in memory and motor function deficits. The average weight of the normal human brain decreases steadily with age. The number of synapses decrease with aging and consequently nerve cells and neuropil is lost. This may explain decreased memory function in older individuals and declining scores in tests of memory (Anderton 2002).

Because of the great importance of memory in human affairs research studies must explore ways and means of alleviating age related cognitive and behavioral deficits. Age related changes associated with learning and memory also occur commonly in rodents (Alireza et al., 2007).

There is emerging evidence for application of phytochemicals in medicine in spite of the wide use of conventional pharmaceutical products. Medicinal herbs, plants and foods have been used for a long time to slow cognitive decline in aging and to manage age related memory deficits (Giancarlo, 2006).

Many research studies have been carried out regarding the main active ingredient in wild yam (diosgenin). However, diosgenin has been falsely marketed as a hormonal replacement therapy to alleviate menopausal or Pre-Menstrual Syndrome (PMS) symptoms. It is also said to have the ability to mimic certain hormones especially progesterone. Most of these claims however remain scientifically unproven. Studies done on humans and also in the laboratory suggest that wild yam has no hormonal effects. The steroidal saponin, diosgenin in wild yam can only be converted to the hormone progesterone in the laboratory but not in the mammalian body (Marker and Krueger, 1940). Commercially available products of wild yam have been shown to improve mood, memory and sleeping habits.

Scientific studies and reports suggest that the most important active ingredient in wild yam root (diosgenin) has potential to enhance brain function. The effect of pure diosgenin on memory has been demonstrated but the effect of wild yam on memory needs to be investigated further (Tohda et al., 2017).

The World Health Organization (WHO) estimates that up to 80% of people globally still rely on traditional remedies for their medicines resulting in the increasing demand for medicinal plants. About 85% of traditional medicines involve the use of plant extracts (Ekor, 2014). Wild yam products have been used and are still being used widely by women as a natural alternative to hormone replacement therapy for menopausal symptoms. It is estimated that at least 80% of Africans prefer traditional medicine for the treatment of common ailments (Elujoba et al., 2005).

Based on the above studies and scientific reports, it is reasonable to hypothesize that wild yam tincture has an effect on memory and learning in mice. The present study investigated the effect and mechanisms of action of wild yam tincture on memory and learning in a young mature cohort of male mice. T – maze procedures were used to assess memory and learning ability.

Research Objective

To investigate the effect of wild yam tincture on memory and learning in male mice.

Specific Objective

To determine the effect of wild yam tincture on long term memory in young mature mice.

Research Hypothesis

H: Wild yam tincture (*Dioscorea Villosa*) has an effect on memory and learning in mice.

Materials and Methods

A. Wild Yam Tincture:

Mexican wild yam tincture (D. villosa) obtained from Healthy U, Sarit centre-Nairobi. Manufacturer: Nature's Laboratory – North Yorkshire Y022 4NH, U.K. Root Tincture: 1:3 45%.

Wild yam tincture was used because it was easily dispensed to mice using a gavage needle. Other available preparations such as root powders and creams were not found suitable for administration to mice.
The chemical analysis was done according to the following method:

1. **Extraction of *Dioscorea villosa* (wild yam) root tincture:**

1000 ml of the *D. villosa* was extracted with 1000 ml of distilled ethyl acetate to obtain the crude extract. Separation was done using a funnel to obtain the aqueous layer which was then evaporated using a rotary evaporator under reduced pressure to obtain 1 gm of the crude product.

2. **Isolation of Diosgenin:**

Crude extract of *D. villosa* (1g) was dissolved in a mixture of 1:1 CH_2Cl_2/MeOH (Dichloromethane/methanol) then transferred into a round bottomed flask containing 1g of silica gel.
Homogenization was done using rotary evaporator under reduced pressure to give a dry and uniformly adsorbed extract. The adsorbed extract was then spread on the bench to dry and then the ground powder using a mortar and pestle. The powder was carefully loaded into a small column packed with 10g of silica gel under *n*-hexane solvent.

Elution was done with increasing polarities of n-hexane/$CHCl_3$ (Chloroform) then CH_2Cl_2/MeOH (Dichloromethane/ methanol). 100 ml of the elute were collected and evaporated. Analytical Thin Layer Chromatography (TLC) plates spotting showed the compound was eluted at 100% CH_2Cl_2 (Dichloromethane) and 1% CH_2Cl_2/MeOH (Dichloromethane/ methanol)

3. **Percentage yield of Diosgenin:**

Weight of the crude extract=1g=1000mg
Weight of the diosgenin crystals=1mg
% yield= 1/1000 X 100= 0.1%

Dosage of tincture

Doses of tincture vary widely according to the strength of the preparation.
The dosage of the yam tincture used in our experiment was calculated on proportional weight basis using the manufacturer's recommended human food supplement dose of 800mg of wild yam tincture twice daily for a 70kg man.

A standard dosage of 0.5mls /day/mouse of wild yam tincture was determined and administered to the test animals by oral gavage for 30 days. The dosage was calculated on the basis of volume: weight ratio for a 70kg man (Human dosage range (40 – 50 mls/day).

B. T-Maze

T-Maze apparatus has universal application and it is used mainly to test learning and memory in rodents. Research studies using maze apparatus have been useful in investigating the effects of medicinal substances and plant extracts on learning and memory in both humans and rodents. T – maze procedures were used to test spatial learning and long-term memory in male mice.

In our study, the T-Maze Left Right Discrimination task was used to assess long term memory in the young mature mice.

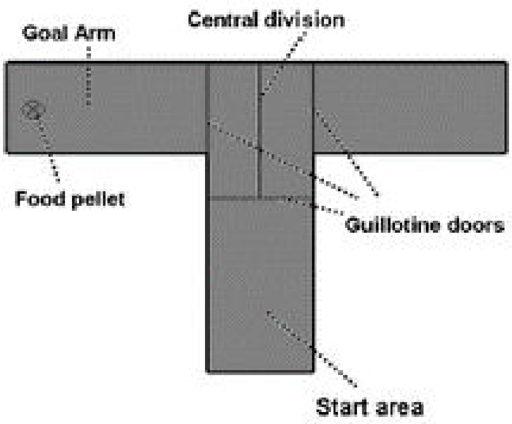

Figure 1: T - Maze

The young mature cohort of mice was subjected to the T-maze task before and after the test animals were given wild yam tincture by oral gavage. Both the test and control animals underwent training and test sessions (10 sessions per animal per day).

Experimental Animals

Twenty male Swiss albino mice obtained from Department of Public Health, Pharmacology and Toxicology, University of Nairobi were used. At the beginning of the experiment the animals ages 3-6 months divided into a control and test group (n=10) each. The sample size was determined using the G – power analysis method. The sample size is also supported by previous similar animal studies.

The animals weighed 20-30gms and were fed *ad libitum* on commercial pellets (by Unga Farm Care Africa LTD) provided water supply and kept at standard laboratory conditions in accordance with FELASA (Federation of European Laboratory Animal Science Associations) guidelines. The animals were housed in separate cages in the department of Medical Physiology, animal unit. Exclusion criteria included any animal that was clinically unwell. One-week acclimatization period was allowed for the animals before experimentation. The mouse was chosen as the experimental animal due to its close genetic make up to that of the human species.

Male mice were used because they have nearly constant hormonal levels contrary to females; and estrous cycling affects reference memory in rodents (Pompil et al., 2010). All the experiments were performed during the same time period (9.00 am to 6.00 pm) and data was collected over a period of eight months.

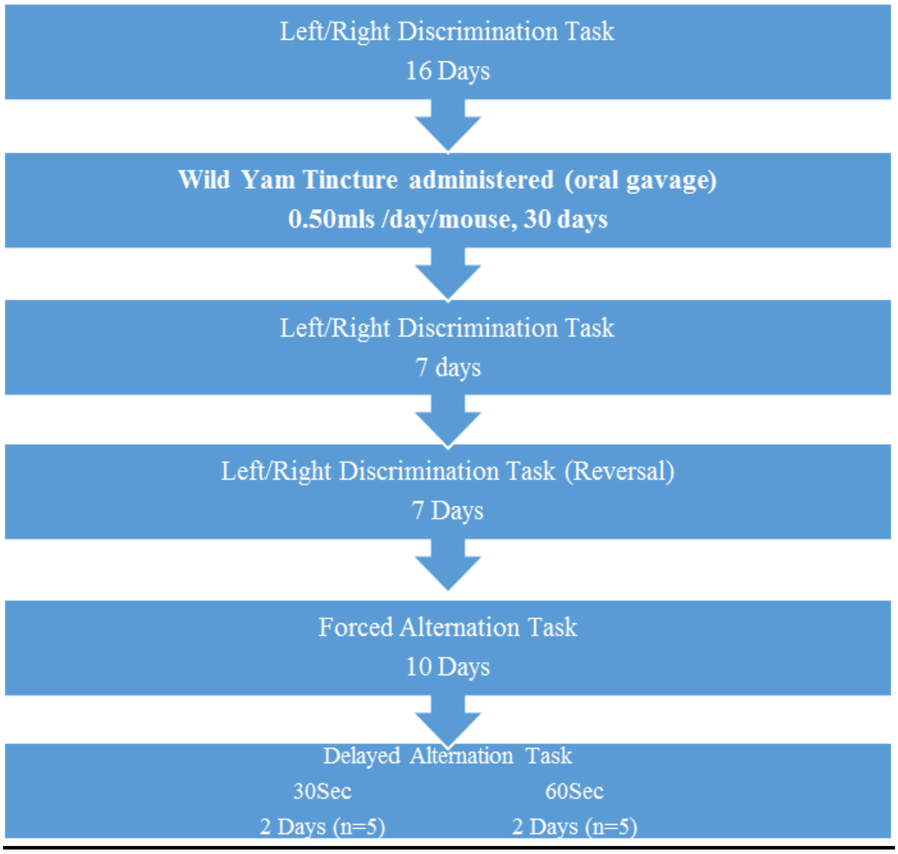

Figure 2: Experimental protocol for memory and learning tests

The experimental procedures for the memory tests/tasks were carried out as per the standard T-maze procedures described by Nature Protocols.

Data and Statistical Analysis

Data generated from the study were entered into STATA version 11 statistical software and were analyzed using independent t – test. Results were expressed as means ± standard error of means (SEM). Differences were considered to be significant if $P < 0.05$.

RESULTS

Extract yield

The weight of the crude extract of wild yam tincture (*D. Villosa*) was determined and the main active ingredient diosgenin was isolated through a solvent system of Dichloromethane/methanol (CH_2CL_2/MeOH).

T-maze left-right discrimination task:

Mice received ten trials daily for 16 days and the average percentage of correct responses for the group was determined. There were no significant differences in the percentage of correct responses between the control mice and test mice before administration of wild yam tincture **[73.6 ± 1.5% (c) Vs. 76 ± 1.1% (t) , t=1.24 p=0.2232].** A graphical representation of the results is shown in Figure 3.

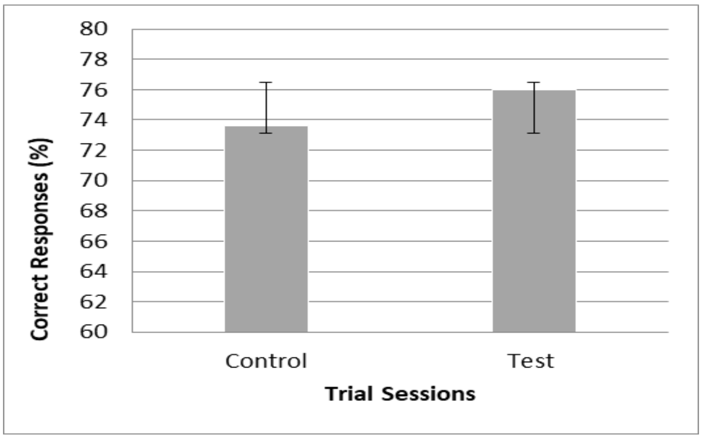

Figure 3: Bar graph showing correct responses before administration of wild yam tincture

T-maze left-right discrimination task:

Mice received ten trials daily for 16 days and the average latency for the group was determined. There were no significant differences in latency between the control mice and test mice before administration of wild yam tincture [**474.3 ± 17.5 sec (c) Vs 512.6 ± 18.8 sec (t), t=1.5 p = 0.1469**]. A graphical representation of the results is shown in Figure 4.

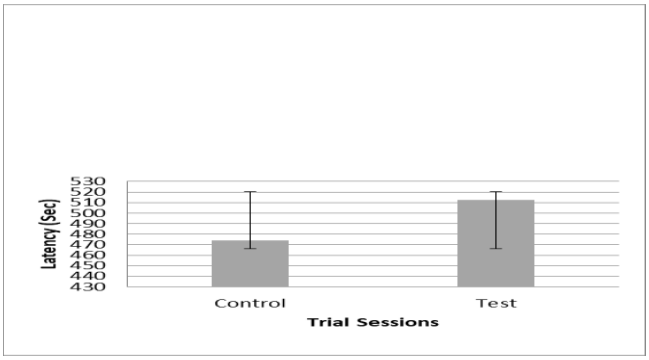

Figure 4: Bar graph showing latency before administration of wild yam tincture

T-maze left-right discrimination task

Mice received ten trials daily for 16 days and the average total distance travelled by the group was determined. There were no significant differences in the distance travelled between the control mice and test mice before administration of wild yam tincture [**3324.3 ± 13.9 cm (c) Vs 3357 ± 23.8 cm (t), t=1.20, p=0.2563**]. A graphical representation of the results is shown in Figure 5.

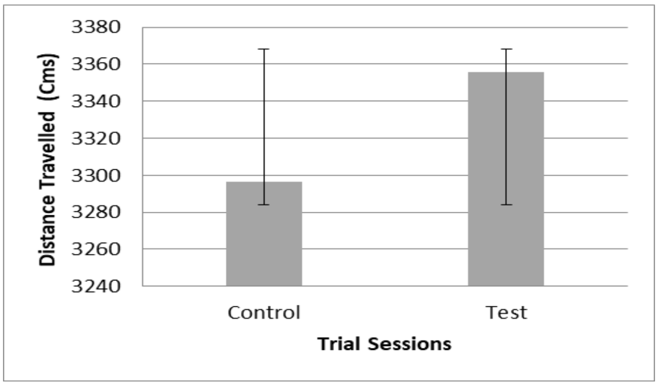

Figure 5: Bar graph showing distance travelled before administration of wild yam tincture

T-maze left-right discrimination task

Mice received ten trials daily for 16 days and the average number of omission errors for the group was determined. There were no significant differences in the number of omission errors between the control mice and test mice before administration of wild yam tincture [**4.7 ± 1.0 (c) Vs 3.9 ± 0.7 (t), t= 0.7039, p=0.4950]**. A graphical representation of the results is shown in Figure 6.

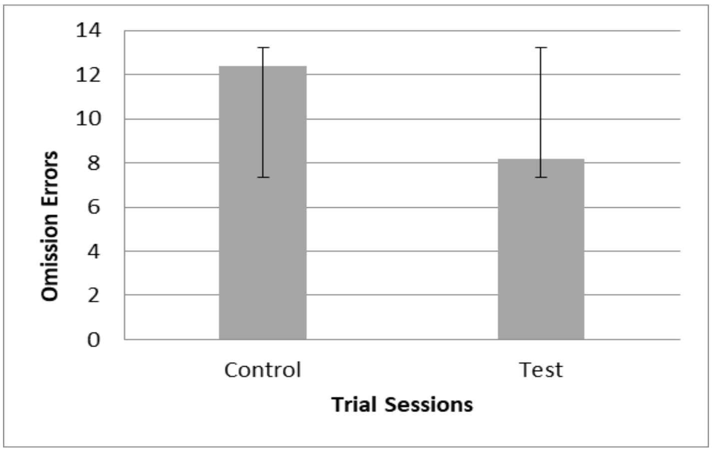

Figure 6: Bar graph showing omission errors before administration of wild yam tincture

T-maze left-right discrimination task

Mice received ten trials daily for 7 days and the average percentage of correct responses for the group was determined. There were no significant differences in the percentage of correct responses between the control mice and test mice after administration of wild yam tincture [**83.2 ± 2.4 % (c) Vs. 89.4 ± 2.1 % (t), t= 1.94, p=0.0757**]. A graphical representation of the results is shown in figure 7.

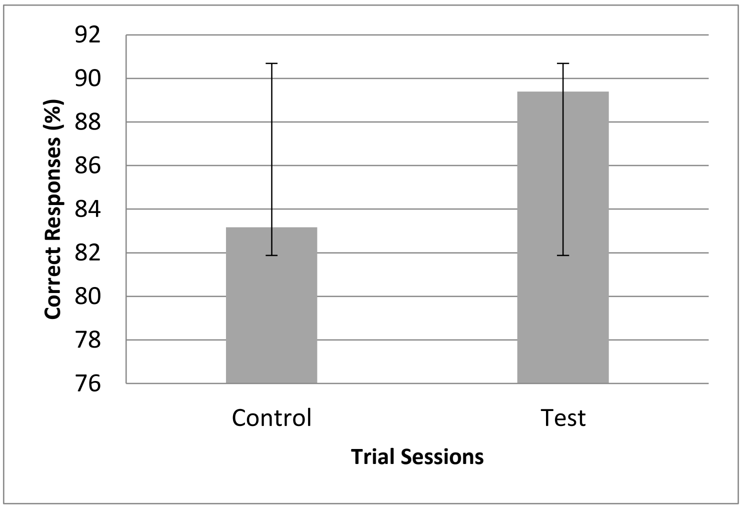

Figure 7: Effect of wild yam tincture on correct response

T-maze left-right discrimination task

Mice received ten trials daily for 7 days and the average latency for the group was determined. There were significant differences in latency between the control mice and test mice after administration of wild yam tincture [**610.6 ± 3.6 sec (c) Vs. 549 ± 17.8 sec (t) , t=-7.1197, p<0.0001**]. A graphical representation of the results is shown in Figure 8.

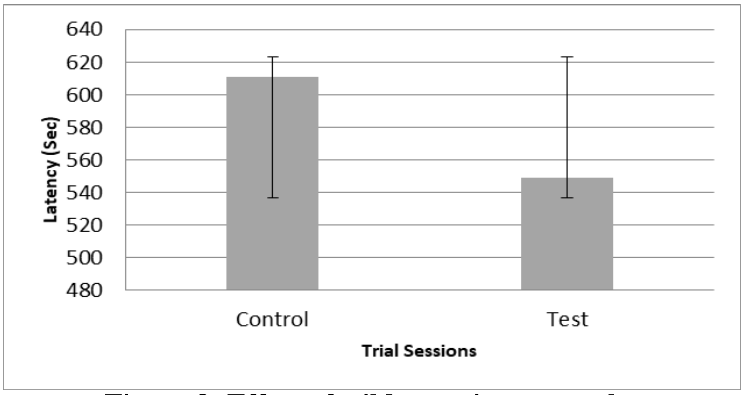

Figure 8: Effect of wild yam tincture on latency

T-maze left-right discrimination task

Mice received ten trials daily for 7 days and the average total distance travelled for the group was determined. There were no significant differences in the distance travelled between the control mice and test mice after administration of wild yam tincture [**3324.3 ± 13.9 cm (c) Vs. 3357 ± 23.8 cm (t), t=1.20, p=0.2563**]. A graphical representation of the results is shown in figure 9.

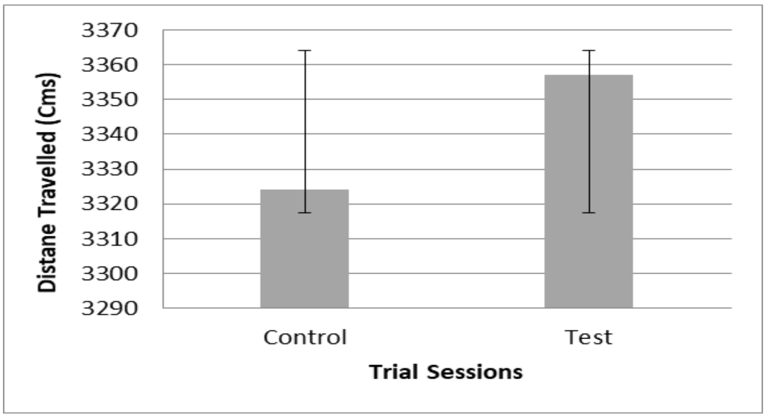

Figure 9: Effect of wild yam tincture on distance travelled

T-maze left-right discrimination task

Mice received ten trials daily for 7 days and the average number of omission errors travelled for the group was determined. There were significant differences in the number of omission errors between the control mice and test mice after administration of wild yam tincture [**12.4 ± 1.34 (c) Vs. 8.2 ± 1.4 (t), t=2.2, p=0.0136**]. A graphical representation of the results is shown in Figure 10.

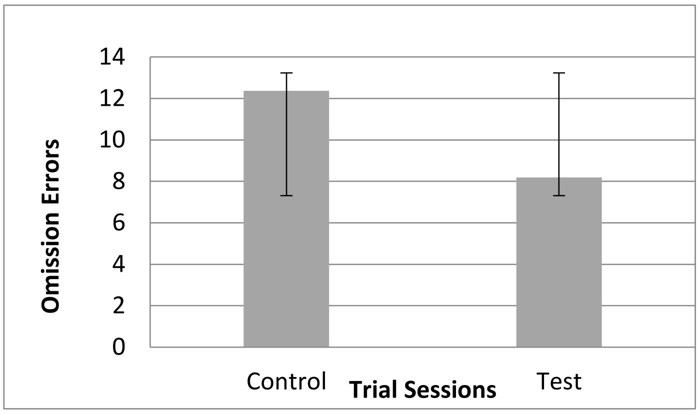

Figure 10: Effect of wild yam tincture on omission errors

DISCUSSION

In our study the results suggest that wild yam tincture had a s positive effect on learning and memory in young mature male mice. These findings support our research hypothesis regarding the effect of wild yam tincture on long term memory in mice.

In the left-right discrimination task after the wild yam tincture was administered, the young, mature adult test mice showed a shorter latency (**p<0.0001**) and made few omission errors (**p=0.0136**) than the control mice (figure 8 and 10). These results indicate a greater spatial learning ability and improved memory by the test animals. The test mice also showed a higher percentage of correct responses (**p=0.0757**) than the control mice (figure 9). Consideration of a high percentage of correct responses, short latency and significantly few omission errors indicate greater learning ability and improved performance in the T – maze. These findings indicate that wild yam tincture enhances long term memory (reference memory) in the young mature adult mice.

These results are supported by previous studies on rodents fed on fruits and vegetable extracts (Joseph et al., 2005). The age phase of young mature adult mice of 3-6 months represents mice that are mature and have not undergone senescence. This age group can be considered the reference for any significant age change. The above mice age corresponds to the human age equivalent of 20-30 years (Flurkey K, Currer JM, Harrison DE. 2007). The benefits of wild yam in enhancing learning and memory may be explored in the young human brain as well as in the aging brain with the possibility of alleviating memory loss and associated deficits occasioned by old age.

Our findings are supported by previous animal studies which indicate that Mexican wild yam (*D. Villlosa*) supplements provide benefits for brain function in rats and mice (Alireza et al., 2007). Wild yam products are commonly available as dietary supplements taken for the antioxidant and anti-inflammatory properties attributed mainly to their diosgenin and polyphenolic content. Diosgenin is a major constituent of wild yam occurring as a steroidal saponin and is found abundantly in legumes and yams (Dioscorea Sp.). The memory enhancing effect of diosgenin maybe mediated by endogenous anti-oxidant enzymes (Chiu et al., 2011).

Conclusion

This study has demonstrated that wild yam tincture enhances spatial learning and memory in male mice and hence has potential benefits in alleviating memory deficits in the aging human brain. The findings of this study are also in-keeping with previously documented research studies in rodents. The results of this study recommend that further studies be undertaken to explore the benefits of wild yam in learning and memory in human volunteers considering the fact that wild yam products are widely used in traditional and modern therapy.

Limitations of the Study

The current study had several limitations which include; inability to control precisely the external cues that determine the learning process and clarity required on what would constitute a sufficient amount of extra maze cue(s), performance may be influenced by many factors and animal characteristics such as species (strain), nutritional status, infection and stress, Content of active ingredients in the wild yam can range significantly due to the growth and quality of processing conditions. Possible sources of error include; inadequate habituation, intertrial handling of the animals, environmental cues (e.g. noise, external cues etc.), emotional and health status of the animals. An automated T-maze apparatus has been developed and that would minimize occurrence of human error. However, this apparatus is not available in Kenya yet.

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