ANTI-DIABETIC ACTIVITY OF *ERIOBOTRYA JAPONICA* LEAF EXTRACTS IN DIABETIC AND NON-DIABETIC RATS

BY

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DECLARATION

I, Marlven Gabaza, certify that this dissertation is my original work and has been prepared in accordance with guidelines of the Master of Clinical Pharmacology Program, University of Zimbabwe. I further attest that this work has not been submitted, in part or in full, for any other degree at any university and/or any publication.

Signature_____Date_____

I, have supervised and read this dissertation, I am satisfied that this is the original work of the author in whose name it is being presented. I confirm that the work has been completed satisfactorily for presentation in the examination.

Name of Supervisor_____

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ABSTRACT

Background and Objectives

The increased prevalence of diabetes mellitus over the years has become a major public health problem and economic burden in many countries. The limitations of the available conventional therapeutic options for diabetes mellitus, have led to a determined search for more efficacious and cost-effective alternatives. The aim of the present study was to investigate the hypoglycemic effect and mechanisms of *Eriobotrya Japonica* ethanolic leaf extracts in alloxan induced diabetic rats.

Methods

Diabetes was induced in male albino Sprague Dawley rats (250-300g) by chemical induction using alloxan monohydrate 120mg/kg body weight. *Eriobotrya Japonica* ethanolic leaf extracts were orally administered to diabetic rats at 100mg, 200mg, and 400mg/kg body weight doses for 15 days to determine the hypoglycemic activity. Blood glucose level was measured from the tail vein and the weight change was recorded.

Results

The three doses of *Eriobotrya Japonica* (100mg, 200mg, and 400mg/kg b.w) lowered the blood glucose level of alloxan induced diabetic rats significantly ($p \leq 0.01$). The reduction in blood glucose level was not dose dependent. Histo-pathological analysis did not show any protective action or change in morphology on the pancreas.

Conclusion

Eriobotrya Japonica in doses of 100mg, 200mg, and 400mg/kg b.w does not have any effect on morphology of pancreatic β cells in alloxan induced diabetic rat models. *Eriobotrya Japonica* can be tried out as a functional food for its anti-diabetic potential.

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Last but not least l would like to thank the Lord Almighty for everything, for all the guidance and protection.

DEDICATION

This project is dedicated to Christine Gabaza my lovely wife.

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ABBREVIATIONS

E J:	*Eriobotrya japonica*
b.w:	body weight
ncontrol:	normal control rats
dControl:	diabetic control rats
dmetformin:	diabetic rats on metformin
dgliben:	diabetic rats on glibenclamide

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Problem statement

The World Health Organisation (WHO) estimates that 346 million people worldwide have diabetes and it is thought that this number would double by 2030.[1,2] Diabetes Mellitus has a high prevalence worldwide and is one of the leading causes of illness, death and economic burden in many countries[1]. The World Health Organisation (WHO) estimates that 80% of the deaths from diabetes occur in developing countries.[2,3] In Zimbabwe, it is estimated that 10% of the population have diabetes[1]. Understanding and managing this crippling disorder will reduce the burden on health societies and will help reduce economic loss.

A variety of plants are used in the management of diabetes. New therapeutic agents have been discovered from plants. *Galega Officinalis* is one example of a plant that led to the discovery and synthesis of metformin[4]. There is need for scientific investigations to rationalise the use of traditional remedies. The side effect profiles and effective doses of most traditional remedies are not known, thus experimental investigations can shed more light[5,6]. Most of the available oral hypoglycemic agents have undesirable side effects.[7,8] These medicines are used in combination and rarely as monotherapy and this reduces adherence and makes diabetes mellitus management complex[8]. Therefore, there is need for medicines that are safe and that can be used as monotherapy. Natural plant sources of drugs are thought to be safe and drug discovery for a long time has had its focus on plants[4,9]. This could also represent accessible alternative medicines for the African populations to combat diabetes and its complications.

There is a huge gap in literature on the effect of medicinal plants on pancreatic cells in diabetic animals[4]. It is important to evaluate the protective or curative role and modulation on the abnormal biochemical parameters in diabetes that result from these herbal medicines. The mechanisms of action of most traditional medicines in diabetes are not known.[7,10] Most studies on the mechanisms of action of herbal plants do not investigate histo-pathology changes in the diabetic rat models.

Several reports suggest that the leaves and seeds of *Eriobotrya Japonica* have hypoglycemic effects in animal models[11-17]. However, most of these studies have been conducted in China and Japan. These studies have also shown the constituents from *Eriobotrya Japonica* leaves responsible for hypoglycemic activity. Both aqueous and ethanolic extracts have shown hypoglycemic effect. However, no experimental demonstrations have been done to show the mechanism of action. The constituents extracted from the leaves of *Eriobotrya Japonica* differ depending on the solvent used. This has shown that many compounds in the leaves are responsible for the hypoglycemic activity therefore there is a possibility of different mechanisms of action. The mechanism of *Eriobotrya Japonica* in reducing blood glucose level remains unclear despite the several pharmacological studies which were done to evaluate its hypoglycemic effect.

In another study *Eriobotrya Japonica* was found to increase blood glucose level in contrast to the many studies[12]. Amygdalin was isolated from the leaves of *Eriobotrya Japonica* and was found to both increase and decrease blood glucose level in alloxan induced diabetic rats.The aims of this present study are firstly to evaluate the hypoglycemic and insulin like effects of *Eriobotrya Japonica* and secondly to evaluate the mechanism of the glycemic control.

1.2 Research Significance

It is important to provide scientific proof in order to justify the use of the plants or their active principles. It is important to discover other alternative anti-diabetic agents to improve medicine access. This study is to confirm the hypoglycemic effect of *Eriobotrya Japonica* and to determine its mechanism of action in this regard. *Eriobotrya Japonica* easily grows in Zimbabwe, evaluating its medicinal use will give access to a medicine that is cheap and easy to access.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 Diabetes Mellitus

2.1.1 Introduction

Diabetes mellitus is a common metabolic disorder characterized by impaired pancreatic insulin secretion and insulin resistance or action in target tissues[2]. Diabetes mellitus affects the metabolism of carbohydrates, fats, proteins, and electrolytes in the body[18,19]. This leads to pathological changes in organs and subsequent micro and macro vascular complications. Acute complications include thirst, weight loss, visual blurriness and diabetic ketoacidosis. Chronic complications involve glycation of body proteins, due to persisting hyperglycaemia. This leads to kidney damage, neuropathic complications and oxidative stress[18,19].

2.2 Epidemiology and Economic burden

The prevalence of diabetes mellitus is increasing worldwide affecting both developed and developing countries[3,20]. World Health Organisation estimates that 4 % of the global population suffers from diabetes mellitus and the prevalence is expected to reach 5.4% by 2030[1,2,21]. The Asian and African population populations have been reported to have higher prevalence of diabetes compared to other ethnic groups[1]. High mortality from diabetes mellitus and undiagnosed diabetes mellitus is among the African population.[1,3] Diabetes mellitus share total of the international healthcare expenses is at least 11.6%[22].

2.3 Pathogenesis and classification

The pathogenesis of diabetes mellitus is influenced by both genetic and environmental factors. The classification of diabetes is based on aetiology and clinical presentation. Diabetes mellitus can either be type 1 or type 2. Type 1 diabetes is also known as insulin –dependent diabetes mellitus (IDDM). This is caused by destruction of pancreatic β cells by autoimmune responses, chemical toxins, viruses or many other factors. Increased oxidative stress plays a pivotal role in the aetiology and pathogenesis of diabetes mellitus.[23,24] Type 2 diabetes mellitus (non –insulin dependent diabetes (NIDDM)) is characterized by both impaired insulin secretion and insulin resistance. It is often associated with hereditary predisposition and it accounts for 90% of diabetic cases[25]. Recent studies have been done to determine whether the pathogenesis is based on the mutation and or abnormal insulin receptors[26]. The specific mechanisms of the pathogenesis of diabetes still need to be evaluated.

2.4 Signs and symptoms

The symptoms include polyuria, polydipsia, visual blurring, genital thrush, lethargy and unexplained weight loss. Many type 2 diabetes mellitus patients are asymptomatic and their disease can remain undiagnosed for many years.

2.5 Diagnosis

Screening for diabetes should be performed at an earlier age and more often in individuals with risk factors (e.g., family history of diabetes mellitus, obesity, sedentary workers). The American Diabetic Association recommends that all adults beginning at the age of 40 to be screened for diabetes every three years. [2,3] The world health organisation recommended test is the fasting plasma

glucose (FPG). The second test which can be used is the impaired fasting glucose (IFG). The third measure, though not widely recommended and used, is the oral glucose tolerance test (OGTT).

2.6 Management of Diabetes Mellitus

Management of diabetes is done pharmacologically and non-pharmacologically. Type 2 diabetes mellitus patients may be managed using diet and physical exercise alone but may progress to need oral hypoglycaemic drugs. The goals of therapy in managing diabetes mellitus include maintaining a near normal glucose level while avoiding hypoglycaemia[26]. It is important to reduce the onset and development of microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular complications[18]. The aim is also to reduce mortality and to improve the quality of life.

Diet and lifestyle changes are the key to successful treatment of type 2 diabetes[18,19]. Physical exercise improves insulin resistance and enhance sensitivity, glycaemic control and reduce cardiovascular risk factors[18]. Physical exercise also reduce cholesterol levels, lower blood pressure, augment diet, and reduce the dose requirements of hypoglycemic agents or need for insulin[19]. Physical exercise increases glucose utilization thereby reducing hepatic glucose production. Dietary modification helps in controlling lipids and ensuring moderate carbohydrate or low calories to promote loss of weight.

The available therapy for diabetes mellitus includes insulin and various oral anti-diabetic agents. This approach is reserved for patients whose disease could not be managed with diet and physical exercise. Management using drugs include laboratory assessment, self-monitoring of blood glucose, medications, physical exercise and dietary modifications. The drugs used in management are usually combined to achieve glycaemic control and monotherapy is rare. The class of drugs include

the following biguanides, sulfonylureas thiazolidinediones, insulin, alpha –glucosidase inhibitors and the meglitinide derivatives

The biguanides are considered the first choice because they rarely cause hypoglycaemia, do not cause weight gain and do not increase insulin levels[19]. Metformin is the only biguanide in clinical use. They primarily work by decreasing hepatic gluconeogenesis and by increasing insulin sensitivity in the peripheral tissues[19,27]. Metformin decreases intestinal absorption of glucose and increases peripheral glucose uptake and utilisation. Metformin is available in immediate release and extended release formulation, can be used as monotherapy or in combination with sulfonylureas. Metformin is contraindicated in patients with renal failure, heart and liver disease[27]. The adverse effects of metformin include the following lactic acidosis, anorexia, nausea, diarrhoea, vomiting, abdominal pain, taste disturbance, erythema, pruritus and urticaria[27].

Sulfonylureas increase insulin secretion, by stimulating pancreatic cells that have residual activity[27]. Equipotent doses of different sulfonylureas produce the same hypoglycemic action. This class include gliclazide, tolbutamide, chlorpropamide, glyburide and glimepiride. However, this class of drugs has many side effects and they are contraindicated in many patients for example in pregnancy. One set back is significant hypoglycaemia and this puts elderly people and those who skip meals at high risk. Other class side effects reported include; skin rash, haemolytic anemia, gastrointestinal upset, cholestasis, hyponatremia and weight gain[27].

2.7 Medicinal Plants in Diabetes

The use of traditional medicines has increased globally in the management of different conditions[28]. The use of medicinal plants in managing diabetes mellitus has also been accepted in many societies[29]. A lot of plant constituents are thought to act on a variety of targets in different mechanisms. However a lot of these claims are made with no scientific evidence. Many plants have been shown to have hypoglycemic activity but the mechanisms of action have not been explored[30]. Medicinal plants with hypoglycemic activity are preferred, due to lesser side-effects and low cost. Medicinal plants are abundant and very popular in developing countries [22,31].

2.8 Mechanisms of Herbal medicines in Diabetes

A lot of plants have been described as efficacious in the treatment of diabetes mellitus; however many of these descriptions are unreliable accounts of traditional usage. Few of these plants or plant extracts have received thorough medical or scientific evaluation of their purported benefits[32]. New studies show that some plant leaf constituents (e.g. proanthocyanidins in grape seeds, alpha lipoic acid, fish oil) act like free radical scavengers hence protecting against oxidative stress.[7,17,24]

Other mechanisms suggest that some plant actives inhibit pancreatic α- amylase. This enzyme delays carbohydrate digestion reducing postprandial serum glucose levels[7,33]. One study indicated the protective and curative effects of *Cocos nucifera* inflorescence on alloxan-induced pancreatic cytotoxicity to be through the regulation of carbohydrate metabolic enzyme activities and islets cell repair[7]. The hypotheses made for mechanisms of hypoglycemic activity include increased utilisation of peripheral glucose and increased inhibitory effect against insulinase enzyme[32]. The other hypotheses include stimulating pancreatic β cells to produce insulin, correcting metabolic disorders of lipids and protein and increasing sensitivity of insulin receptors[34].

Table 2.1: Plant herbs with known mechanisms of action

Herbs	Constituents	Anti-diabetic mechanism
Myrcia	Flavanone glucosides (myrciacitrins) and acetophenone glucosides myrciaphenones)	Inhibit activity of aldose reductase and alpha glucosidase
Cinnamon	Cinnulin	Improve insulin sensitivity, Decrease fasting blood glucose
Scoparia	Dimethoxycoumarin	radical scavenging properties; inhibited iNOS gene expression and inhibited NFkappaB Activation.
Gymnema Sylvestre	Gymnemic acids	Controls the activities of phosphorylase, gluconeogenic enzymes and sorbitol dehydrogenase
Ipomoea batatas	Caiapo (ipomoea batatas	Decrease insulin insensitivity, increase adiponectin and decrease fibrinogen levels[35]

Adapted from: Hypoglycemic herbs and their action mechanisms, Hongxiang Hui, Tang G, Go VLW. *BioMed Central; Chinese Medicine 2009.* 2009;4(11).

2.9 *Eriobotrya Japonica* (Loquat)

2.9.1 *Eriobotrya Japonica* (Loquat)

Figure 2.1: Adapted from: http://toptropicals.com/catalog/uid/eriobotrya_japonica.htm

2.9.2 Distribution and morphology

Eriobotrya Japonica is a fruit tree that belongs to the Rosaceae family. *Eriobotrya Japonica* is native to south eastern China and southern Japan. It is also grown in other parts of the world, even in the warmest climates. The loquat trees are best described as short with evergreen leaves and they can reach 25 to 35 ft. in height. Their leaves are mostly elliptical-lanceolate to obovate-lanceolate, 12 to 30 cm long and 3 to 10 cm wide. They are dark green and glossy on the upper surface, whitish to rusty-tomentose on the lower surface. The loquats fruit are held in clusters of 4 to 30, rounded to pear-shaped, 2 to 5 cm long and weigh an average of 30 to 40 g.

2.9.3 Constituents of the Plant Leaves

The leaves of Loquat tree contain triterpene acids, sesquiterpenes, flavonoids, tannins and megastigmane glycosides.[11,17] Several studies have been done to isolate the different triterpenes in the leaves of *Eriobotrya Japonica*. Banno et al. did a study on isolation and identification of sixteen known triterpene carboxylic acids from the methanol extract of the leaves[36] . The work of Ito et al identified eighteen polyphenols one of which was procyanidin oligomer that have cytotoxicity activity against human oral tumour cell lines[37].

2.9.4 Medicinal uses of *Eriobotrya Japonica* (Loquat)

The leaves of *Eriobotrya Japonica* have been traditionally used as a medicine by the Chinese and Japanese[11,12]. Loquat has been used as an antitussive, anti-inflammatory, diuretic, expectorant, analgesic agent, and to treat women's skin diseases[12]. *Eriobotrya Japonica* has also been used to prepare oriental herbal teas for nausea and vomiting[36]. The leaves possess astringent properties and can been applied locally to wounds and ulcers[36].

It has been found that some of the constituents exhibit anti-nociceptive activity and anti – inflammatory properties[38-40]. *Eriobotrya Japonica* has been found to have antiviral, antioxidant, cytotoxic, antimutagenic, antitumor and hypoglycemic properties[41]. The triterpene 2α, 19α-hydroxy-3-oxo-urs-12-en-28-oic acid among other triterpenes have been evaluated to have anti – HIV activity[42] . This triterpene was also found to be a potent inhibitor of mouse skin cancer.[36,41,42] In a study by Kim et al. it was observed that *Eriobotrya Japonica* protects from oxidative stress and cognitive deficits induced by the Aβ peptide[43]. In a different study it was proved that *Eriobotrya Japonica* can improve immune response and production against Vibrio carchariae[44].

2.9.5 *Eriobotrya Japonica* (Loquat) in diabetes

The leaves of *Eriobotrya Japonica* have traditionally been used to treat diabetes mellitus as herbal tea[17,45]. The leaves and seeds of *Eriobotrya Japonica* contain active constituents of mainly flavonoids, ellagic acid, tannins, and amygdalin.[14,16] Amygdalin is one of the major components in the seeds and leaves of this plant and has been shown to have hypoglycemic effect in the body[12]. Other triterpenes tormentic acid, hesetormentic acid and polysaccharides have been shown to increase insulin production but the mechanisms are still not known[46,47].

Tanaka et al. studied the hypoglycemic effects of *Eriobotrya Japonica* seeds in type two diabetic Otsuka Long-Evans Tokushima fatty rats (OLETF) and KK-A mice[12]. OLETF rats are a model of spontaneous non-insulin dependent diabetes mellitus and the Long-Evans Tokushima Otsuka (LETO) are the non-diabetic control of OLETF rats. KK-A mice are type 2 diabetic animals. It was observed that the ethanolic extraction of the seeds possess hypoglycemic activity. The seed diet effectively suppressed the blood glucose concentration, the level being the same as that in the LETO group ($p \leq 0.05$). The OLETF rats fed on the seed diet showed a significantly lower level of serum insulin ($p < 0.05$) than the OLETF rats fed on the control diet. In contrast amygdalin was found to increase blood glucose in the fourth month to a level comparable to the control group ($p \leq 0.05$). This showed Eriobotrya can also increase blood glucose levels. The possible mechanism of *Eriobotrya Japonica* in reducing the blood glucose was not investigated.

A study by Chen et al. isolated a sesquiterpene glycoside (nerolidol-3-O-a-L-rhamnopyranosyl(1-4)-a-L-rhamnopyranosyl(1-2)-[a-L-rhamnopyranosyl(1-6)]-b-D-glucopyranoside) from loquat leaves and it showed remarkable hypoglycemic activity[15]. The sesquiterpene glycoside was tested for hypoglycemic activity in normal and alloxan induced diabetic mice and it lowered the blood

glucose level. The mice were induced with diabetes by intraperitoneal injection of alloxan using a dose of 200mg /kg body weight. Doses of 25mg/kg and 75mg/kg body weight of the sesquiterpene glycoside were orally given to the mice in the case groups. The two doses significantly lowered blood glucose from 29.96±5.68 to 16.05±5.51mmol/l and 14.37±6.14mmol/l ($p \leq 0.05$). The reduction in blood glucose was found to be more than the group treated with 50mg/kg body weight of gliclazide. However, this study did not make any efforts to explain the possible mechanism of action despite isolating one of the glycosides with activity.

Another study by Lu and Chen demonstrated hypoglycemic effect of the total flavonoid fraction from leaves of *Eriobotrya Japonica* [48]. Nine flavonoids were preliminarily assigned and tested for hypoglycemic effect in normal and streptozotocin-diabetic mice in graded doses. The administration of the flavonoids lowered plasma glucose concentration and glycosylated serum protein and this effect was comparable to that of gliclazide. The administration of 300mg/kg and 450mg/kg of *Eriobotrya Japonica* fraction significantly ($p \leq 0.05$) lowered plasma glucose concentration of mice in the 7th day. The hypoglycemic effect of the high dose was equivalent to 50mg/kg ($p \leq 0.01$). In this study it was seen that the glucose lowering effect was dose dependent and time dependent. The hypoglycemic effect of the third dose was significant on the 14th day ($p \leq 0.01$). The three doses significantly increased the insulin secretion to a level almost similar as normal mice ($p \leq 0.01$). The study postulated that the hypoglycemic effect on diabetic mice is by stimulating functional β-cells to secrete insulin[48].

In another almost similar study by Lu et al. an ethanolic extract of the leaves of *Eriobotrya Japonica* significantly lowered the plasma glucose level and GSP in alloxan and streptozotocin induced diabetic rats ($p \leq 0.01$)[13]. In this study gliclazide at 50mg/kg produced a significant lowering of blood glucose ($p \leq 0.05$). The results showed that a dose of 300 mg/kg of total triterpene acid

extract caused more significant ($p < 0.01$) hypoglycemic and/or hypolipidemic effects in normal, alloxan and streptozotocin-induced diabetic mice. The 300mg/kg dose of the total triterpene acid extract also significantly increased the superoxide dismutase activity (SOD) and the serum insulin level of diabetic mice ($p \leq 0.01$).

Qa'dan et al. investigated the hypoglycemic effect of the water extracts of the leaves of *Eriobotrya Japonica*[17]. The study showed that *Eriobotrya Japonica* water extract significantly increased insulin secretion from INS-1 cells and this was dose dependent. However, the results also showed that the water extracts of *Eriobotrya Japonica* when administered at 230mg/kg decreased plasma insulin for as long as 240 minutes. Cinchonain Ib enhanced insulin secretion significantly from INS-1 cells ($p \leq 0.05$) but epicatechin significantly inhibited insulin secretion from INS-1 cells ($p \leq 0.05$). 0.3 μM (micro moles) of Cinchonain Ib increased similar insulin level to two (2 μM) micro moles of glibenclamide in vitro. Cinchonain Ib when given at 108mg/kg also significantly increased plasma insulin level in rats for as long as 240 minutes. The amount of epicatechin from extraction was more than that of cinchonain Ib. This study managed to explain how extracts of *Eriobotrya Japonica* may work in diabetes, but there was no glucose challenge so the study could not test the hypoglycemic effect.

Shafi et al. also did a study on the anti-diabetic and hypolipidemic effects of *Eriobotrya Japonica* seeds in alloxan induced diabetic rats[49]. An ethanolic extract was obtained from coarsely powdered seeds *of Eriobotrya Japonica*. The extract of *Eriobotrya Japonica* was administered at three dose levels 50, 100 and 200 mg/kg daily. The results showed significant reduction in body weight after ten days ($p < 0.01$). There was also significant reduction in blood glucose levels in all groups ($p<0.01$) as compared to the diabetic control. The study also showed significant decrease in total serum cholesterol and triglyceride levels in all groups ($p<0.01$) as compared to the diabetic control.

The study by Noreen et al. investigated the effects of *Eriobotrya Japonica* on blood glucose levels of normal and alloxan-diabetic rabbits[16]. The alcoholic extract of the leaves of *Eriobotrya Japonica* was administered in doses of 100, 150, and 200 mg/kg body weight to normal and alloxan-diabetic rabbits. The blood glucose levels were estimated before and 1, 2, 3, and 4 hours after the administration of the extract. The ethanolic extract exerted a significant ($p < 0.05$) hypoglycaemic effect in normal rabbits which was, however, short-lived. In contrast to many studies the hypoglycaemic effect was not significant ($p > 0.1$) in alloxan-treated rabbits.

In another study by Li et al. ethanolic extract of leaves of *Eriobotrya Japonica* in doses of 15, 30 and 60 g (crude drug)/kg exerted a significant hypoglycemic effect on alloxan-diabetic mice in a dose dependent manner[50]. The 30 g/kg dose of *Eriobotrya Japonica* was more effective than 100 mg/kg of phenformin. The total sesquiterpenes 30 g (crude drug)/kg had significant effect on lowering blood glucose level in normal or/and alloxan-diabetic mice.

Tommasi et al. investigated the hypoglycemic effects of sesquiterpene glycosides and polyhydroxylated triterpenoids of *Eriobotrya Japonica* in genetically diabetic and normal rats[14]. The sesquiterpene glycoside 3 and the polyhydroxylated triterpenoids 5 and 6 produced a significant reduction in blood glucose levels in normoglycemic rats and alloxan induced diabetic rats.

2.9.6 Summary of Section

Most of the studies on *Eriobotrya Japonica* were to identify the leaf constituents and to prove hypoglycemic activity. A lot of research on *Eriobotrya Japonica* has managed to isolate and prove the constituents responsible for anti-diabetic activity but no study has proposed the mechanism of

action. Only one study reported *Eriobotrya Japonica* to have also increased blood glucose level when it was fed to alloxan diabetic induced rats[12].

2.9.7 Study Rationale, Objectives and Hypotheses

Study Rationale

Current pharmacological studies on the mechanism of hypoglycemic effect of *Eriobotrya Japonica* ethanolic leaf extracts in diabetic rat models are still not clear. The evidence from experimental studies that the leaves of *Eriobotrya Japonica* have hypoglycemic effect is adequate. It is important to know the mechanism of action of *Eriobotrya Japonica* in lowering blood glucose levels.

Research questions

Does the herbal plant *Eriobotrya Japonica* have hypoglycemic effect?

Does the herbal plant *Eriobotrya Japonica* help in regeneration of β pancreatic cells in alloxan induced diabetic rats?

What is the mechanism of action by which the herbal plant *Eriobotrya Japonica* reduces blood glucose in alloxan induced diabetic rats?

Aim

To determine the mechanism by which *Eriobotrya Japonica* herbal extract reduces blood glucose in alloxan-induced diabetic rat models.

Specific objectives

1 To determine the effect of *Eriobotrya Japonica* leaf extracts on the morphology of β pancreatic cells on alloxan induced diabetic rat models.

H_0 (1a) *Eriobotrya Japonica* leaf extracts will not have significant effect on the morphology of β pancreatic cells on alloxan induced diabetic rat models.

2 To determine the effects of ethanolic leaf extracts of *Eriobotrya Japonica* on blood glucose levels in non-diabetic rat models.

H_0 (2a) Blood glucose levels will not differ significantly between non diabetic rats administered *Eriobotrya Japonica* compared to control non diabetic rats.

3 To determine the effects of ethanolic leaf extracts of *Eriobotrya Japonica* on blood glucose levels in diabetic rat models.

H_0 (3a) Blood glucose levels will not differ significantly between diabetic rats administered 100mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats.

H_0 (3b) Blood glucose levels will not differ significantly between diabetic rats administered 200mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats.

H_0 (3c) Blood glucose levels will not differ significantly between diabetic rats administered 400mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats.

4 To compare the hypoglycemic effect of *Eriobotrya Japonica* herbal extract to that of glibenclamide.

H_0 (4a) Blood glucose levels will not differ significantly between diabetic rats administered 100mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats administered with 10mg/kg b.w glibenclamide.

H_0 (4b) Blood glucose levels will not differ significantly between diabetic rats administered 200mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats administered with 10mg/kg b.w glibenclamide.

H_0 (4c) Blood glucose levels will not differ significantly between diabetic rats administered 400mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats administered with 10mg/kg b.w glibenclamide.

5 To compare the hypoglycemic effect of *Eriobotrya Japonica* herbal extract to that of metformin.

H_0 (5a) Blood glucose levels will not differ significantly between diabetic rats administered 100mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats administered with 50mg/kg b.w metformin.

H_0 (5b) Blood glucose levels will not differ significantly between diabetic rats administered 200mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats administered with 50mg/kg b.w metformin.

H_0 (5c) Blood glucose levels will not differ significantly between diabetic rats administered 400mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats administered with 50mg/kg b.w metformin.

6 To determine the dose- response relationship between *Eriobotrya Japonica* and blood glucose levels in diabetic rats.

H_0 (6a) Blood glucose levels of the three doses of *Eriobotrya Japonica* (100mg, 200mg and 400mg/kg/b.w will not differ significantly between the groups in diabetic rats

7 To determine whether any pharmacodynamic interactions exist between the different doses of *Eriobotrya Japonica* and glibenclamide in the diabetic rats.

H_0 (7a) Blood glucose levels will not differ significantly between diabetic rats administered *Eriobotrya Japonica* and glibenclamide compared to diabetic rats given glibenclamide only.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Methods

3.2 Collection of plant Material

Eriobotrya Japonica fresh leaves were collected in Harare at the Pond Gardens in Marlborough. The herbal plant was authenticated by the National Herbarium of Zimbabwe (Appendix 5).

3.3 Preparation of plant extracts

Fresh leaves collected of *Eriobotrya Japonica* were air dried at room temperature and ground to fine powder using a home blending machine. The powder was sieved using a fine muslin cloth and was kept in airtight containers waiting for solvent extraction. Ethanol extract of the herbal plant was prepared. The powdered leaves (400g) were dissolved in 2L of 70 % ethanol. The mixture was kept for 48 hours with continuous stirring. The fluid obtained was then filtered through a Whattman-1 filter paper. The filtrate was evaporated using a rotary evaporator at 40ºC under vacuum. The extract was then kept in amber glass bottles until the day of the experiment.

3.4 Induction of diabetes

Sprague Dawley rats used in the experiment were obtained from the animal house at the University of Zimbabwe. The animals were housed and maintained in an animal room that allows 12 hour light/ 12 hour dark cycle with free access to pellet feed and water. The rats were fed with a high fat diet for three weeks before induction of diabetes. Alloxan monohydrate was obtained from Sigma Chemicals Germany through a local agent. Diabetes was induced in Sprague Dawley rats (150-

350g) by a single intraperitoneal injection of alloxan monohydrate dissolved in normal saline (120mg/kg b.w) after being fasted for 12 hours. The rats were given a normal pellet diet and water after induction. The animals were confirmed to have hyperglycemia four days after induction from blood tests using the code free SD check glucometer machine.

3.5 Drug preparation

Metformin generic from Cipla batch number K30279 and glibenclamide generic batch number LOT230330 were procured from PCD and Varichem pharmaceuticals respectively. The drugs were crushed into powder and suspended in distilled water at administrable concentrations. Glibenclamide was dosed at 10mg/kg/b.w in the rats and metformin was administered at 50 mg/kg/b.w. daily.

3.5.0 Experimental design

3.5.1 Evaluation of hypoglycemic effect of the ethanolic extract of *Eriobotrya Japonica* in normal and alloxan induced diabetic rats.

The animals were divided into nine groups and each group consisted of six rats. The baseline of blood glucose level was measured and recorded. The groups formed were as follows:

- Group 1 Normal control- was injected with normal saline only no diabetes induced.
- Group 2 Diabetic control- diabetic rats injected normal saline only.
- Group 3 Metformin -diabetic rat models and received metformin 50mg/kg b.w.
- Group 4 Glibenclamide -diabetic rat models and received glibenclamide 10mg/kg b.w.
- Group 5 Glibenclamide -diabetic rats treated with extract of *Eriobotrya Japonica 200mg/kg*.
- Group 6 Normal rats treated with aqueous extract of *Eriobotrya Japonica 200mg/kg b.w*.
- Group 7 Diabetic rats treated with aqueous extract of 100mg/kg *Eriobotrya Japonica.*

- Group 8 Diabetic rats treated with aqueous extract of 200mg/kg *Eriobotrya Japonica*.
- Group 9 Diabetic rats treated with aqueous extract of 400mg/kg *Eriobotrya Japonica*.

After an overnight fast the diabetic rat groups received the herbal extracts and the oral hypoglycaemics by gastric intubation and the untreated normal received distilled water only. Blood glucose level was then measured after the administration of the herbal extracts and the oral hypoglycaemics. Percentage reduction in glucose level following drug treatment was calculated.

3.5.2 The long term effect of treatment with *Eriobotrya Japonica* on glycaemic control and pancreatic function in diabetic rats.

The rats were divided into five groups and each group consisted of 6 rats

- Group 1 Normal control- the rats were injected with normal saline only no diabetes induced.
- Group 2 Diabetic control-diabetic rat models but did not receive any treatment
- Group 3 Normal rats treated with ethanolic extract of 200mg/kg/b.w of *Eriobotrya Japonica*/day
- Group 4 Diabetic rats treated with ethanolic extract of 200mg/kg of *Eriobotrya Japonica*/day
- Group 5 Diabetic rats treated with 10mg of glibenclamide/kg b.w/day

The herbal extract or glibenclamide was administered to the rats every day in the morning for 15 days by gastric intubation using oral gavage.

Blood samples were collected from the tail veins before the start of the treatment, on the 4^{th}, 7^{th} and 14^{th} days of the treatment. The fasting blood glucose levels were measured after one hour post drug administration. The body weight of all the animals was recorded prior to the treatment and the

sacrifice. On the 15th day all the animals were sacrificed, and the pancreas was collected and stored in formalin waiting for histo-pathological studies. The whole pancreas from each animal was collected in 10% formalin solution and immediately processed by the paraffin technique. Sections of 5 μm thickness were cut and stained with hematoxylin and eosin for histological examinations. Stained sections were then evaluated qualitatively using a microscope.

3.6 Statistical analysis

All the grouped data was evaluated with the Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc.). Hypothesis testing methods included Student's t-test and one way analysis of variance (ONE WAY ANOVA) followed by Tukey's and Dunnett's multiple comparisons test. $P < 0.05$ was considered statistical significant. Data was expressed as mean ± S.D in each group.

3.7 Ethical Approval

The study was approved by the The Joint Parirenyatwa Hospital and College of Health Sciences Research Ethics Committee (JREC/204/13, Appendix 2). The approval to use the animals in the experiment was granted by the University of Zimbabwe Animal House (Appendix 4). All the experiments were in accordance with the ethical guidelines and standards on animal handling from the University of Zimbabwe Animal House.

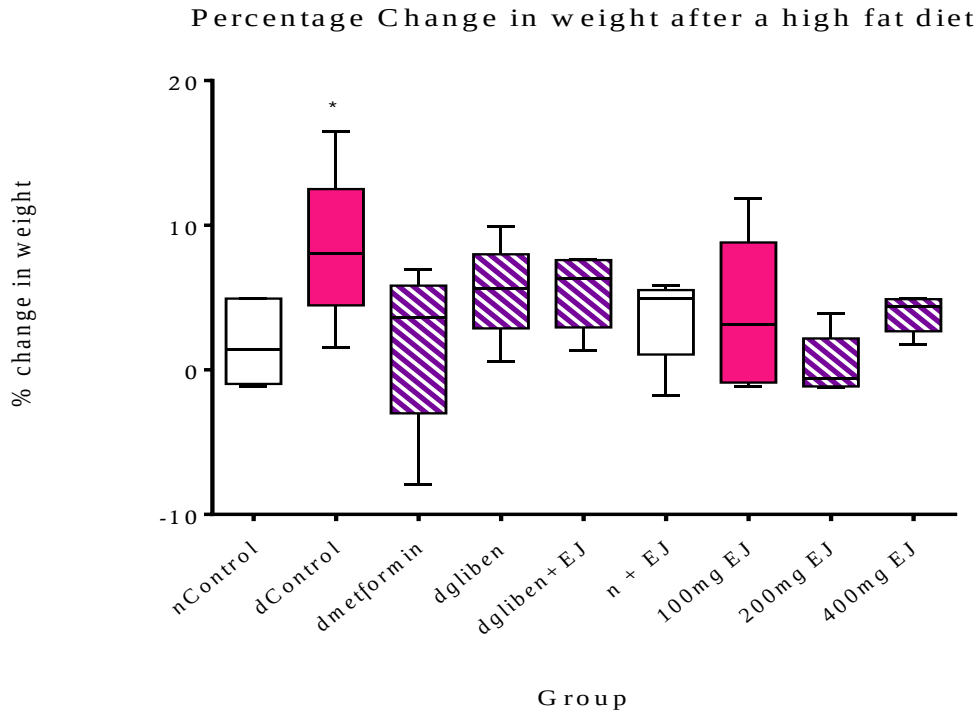
CHAPTER FOUR

4.0 RESULTS

This chapter contains a summary of the findings of the research project. The figures and graphs will show the weight, blood glucose, and the histo-pathological changes that occurred as the animal models were administered the different drugs and *Eriobotrya Japonica* leaf extract.

4.1 Induction of Obesity

Feeding of the rats with high fat diet resulted in significant weight changes. Table 4.1 and graph on Figure 4.1 shows the weight changes that occurred when the rats were fed with high fat diet before induction of diabetes.



Key: * Groups sharing a common star had significant weight difference %- percentage

Figure 4.1: Percentage changes in weight from a high fat diet feed

Table 4.1: Change in weight after the high fat diet in grams

N control	D control	D metformin	D Gliben	D Gliben + EJ	n + 200mg EJ	100mg EJ	200mg EJ	400mg EJ
-1.13	16.52	5.45	6.80	1.36	-1.79	-1.16	-0.82	4.88
-0.93	11.17	6.96	3.63	7.58	5.393	-0.78	-1.19	1.79
1.82	10.43	-7.94	4.39	3.46	5.34	7.79	1.58	4.11
1.04	1.52	-1.37	9.91	7.62	4.50	0.21	-1.13	4.68
4.92	5.43	3.39	0.58	7.27	5.85	11.83	-0.40	4.89
4.95	5.72	3.79	7.34	5.32	2.01	5.99	3.92	2.96

Using the ANOVA test rats gained significant weight compared to the rats fed with the normal pellet feed with less protein and fat ($F=2.551$; $DFn=8$; $DFd=45$; $p=0.0220$). Dunnett's multiple comparisons test showed the diabetic control groups had significant weight change compared to the normal control group. Tukey's multiple comparisons test showed the difference in weight gain among the groups fed with high fat diet were not significant ($p \leq 0.05$). Refer to Figure 4.1 and Table 4.1

4.2 Induction of diabetes using alloxan

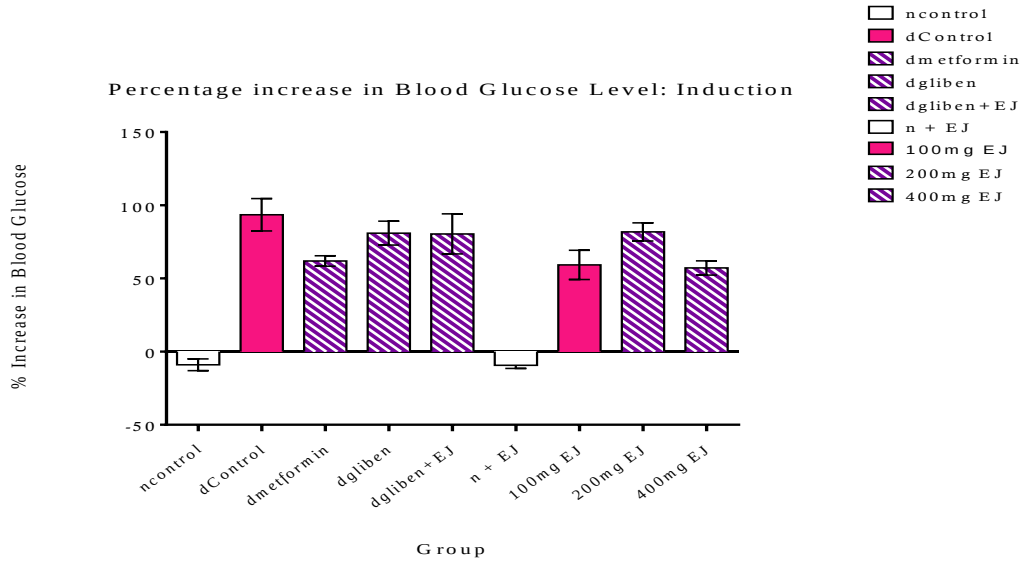


Figure 4.2: Percentage Increase in Blood Glucose after Induction

Induction of diabetes mellitus using alloxan in the rats was confirmed by the hyperglycemia in the animals. The graph on Figure 4.2 and Table 4.1 shows the significant increase in blood glucose level except in the control group following administration of alloxan. ANOVA test showed that the overall percentage increase in blood glucose level was statistically significant (F statistic=23.03; DFn=8; DFd=45; $p \leq 0.001$). Dunnett's multiple comparisons test showed all the groups had significant blood glucose increase compared to the control group ($p \leq 0.05$). Tukey's multiple comparisons test showed the differences in increase in blood glucose among the diabetic groups were not significant ($p \leq 0.05$).

Specific Objectives

Objective 1

1 To determine the effect of *Eriobotrya Japonica* leaf extracts on the morphology of β pancreatic cells on alloxan induced diabetic rat models.

H_0: (1a) *Eriobotrya Japonica* leaf extracts will not have significant effect on the morphology of β pancreatic cells of alloxan induced diabetic rat models.

Fail to reject H_0

4.3: Histo-pathological changes in the rat pancreas

The following figures show the islet cells of the pancreas after the experiment. The pictures were taken under light of low and high power intensity.

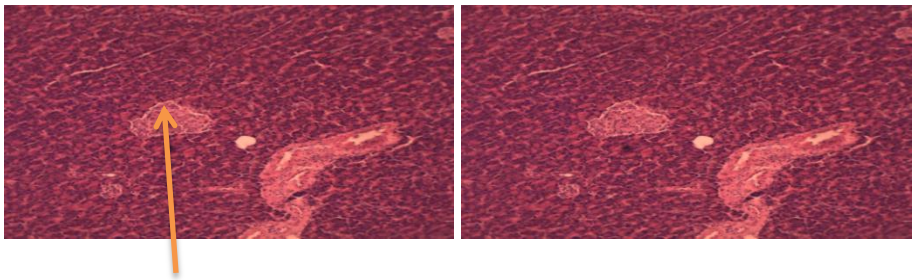
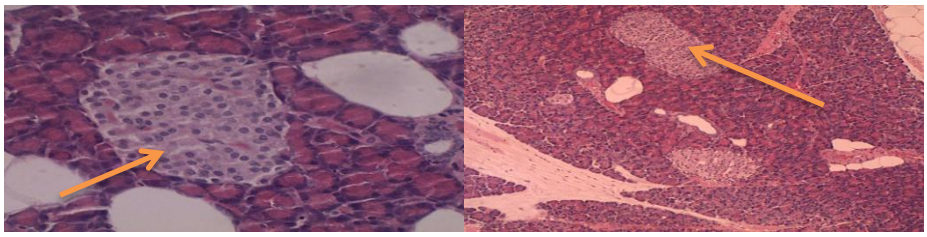


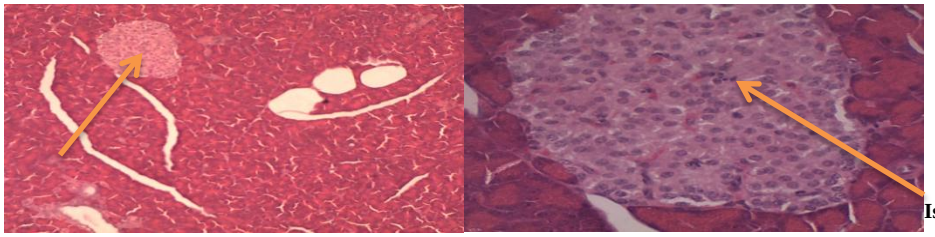
Photo micrograph of rat showing normal pancreatic islet cells

FIGURE: A Normal rats Control



High power low power

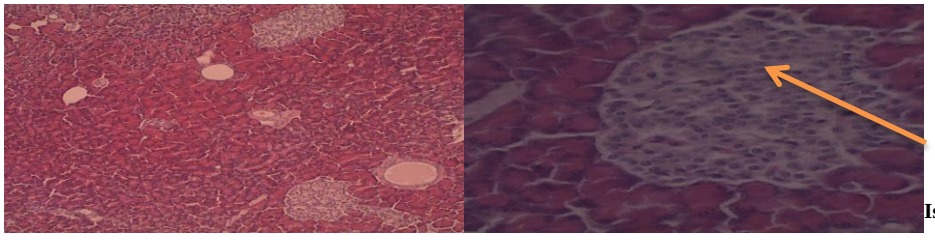
FIGURE: B Diabetic control



Islet cells showed no change

Low power high power

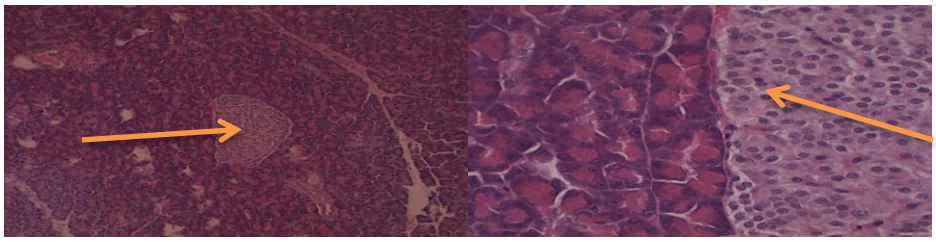
FIGURE: C Normal rats + Eriobotrya Japonica



Islet cells same normal control

Low power **High power**

FIGURE: D Diabetic rats treated with Eriobotrya Japonica



Low power **High power**

FIGURE: E Diabetic rats on glibenclamide

Figure 4.3: Histo-pathological examinations of the rat pancrease

Key: Arrows point at the islet cells

There were no changes in morphology of the pancreatic β cells in the normal and diabetic rat models administered with *Eriobotrya Japonica.* Figure 4.3 shows the histology reports of the different treatment groups and the normal controls. There was no severe necrotic damage and inflammation in the rats with diabetes. Photomicrograph of the slides showed no change in the array of the islet cells.

4.4 Effect of *Eriobotrya Japonica* on Blood Glucose level

Table 4.2 shows the changes in glucose level in the rat experiment before induction of diabetes and after administering *Eriobotrya Japonica*. The table shows the mean blood glucose level measured of the six replicates in each group and the standard error of the mean. Table 4.3 shows the changes in blood glucose levels that occurred when the animals were administered with *Eriobotrya Japonica* for four days.

Table 4.2: Effect of *Eriobotrya Japonica* on Blood Glucose level (mean ± SEM)

Groups	Fasting blood Glucose level in mg/dl			
	Baseline	induction	4th day	7th day
Normal control	100.50±3.96	90.83±2.60	91.33±3.31	94.33±1.31
Diabetic control	104.50±5.51	199.17±0.87**	196.50±1.88	188.67±4.57
Diabetic metformin	120.67±1.48	195.33±4.34**	108.00±3.61**	92.33±4.02
Diabetic glibenclamide	104.67±4.77	187.67±4.59**	102.33±1.91**	92.00±7.51
Diabetic Gliben + EJ	96.67±1.91	173.33±10.43**	111.50±2.32**	103.17±2.63
Normal + 200mg/kg EJ	97.33±3.88	88.17±3.64	93.50±3.02	93.33±4.16
100mg/kg/b.w EJ	116.50±3.42	184.17±7.56**	111.83±3.22**	117.50±3.99
200mg/kg/b.w EJ	101.83±2.14	184.83±5.91**	119.67±5.25**	118.33±3.00
400mg/kg/b.w EJ	121.67±2.84	190.83±5.06**	122.33±3.56**	103.00±3.69

To convert to mmol/l divide by 18.01 ** values not sharing a common superscript differ significantly at $p \leq 0.05$.

Table 4.3: Percentage change in blood glucose level after administration of drugs

ncontrol	dControl	Dmetformin	dgliben	dgliben+EJ	n + EJ	100mg EJ	200mg EJ	400mg EJ
3.44	-0.50	-46.73	-42.32	-24.82	17.56	-37.04	-35.91	-28.57
13.41	0.00	-46.77	-40.91	-25.71	-8.89	-35.44	-30.67	-41.29
-8.42	-3.48	-40.80	-47.59	-33.90	1.00	-44.33	-42.29	-32.95
-12.50	-0.50	-50.25	-50.75	-38.12	4.26	-35.98	-27.14	-31.03
2.04	1.52	-39.80	-39.08	-46.27	13.10	-39.80	-42.32	-40.70
8.04	-5.03	-43.68	-50.76	-39.29	12.64	-41.71	-32.39	-39.70

Key: negative sign (-) means reduction in blood glucose level

2 To determine the effects of ethanolic leaf extracts of *Eriobotrya Japonica* on blood glucose levels in non-diabetic rat models.

Figure 4.4 shows changes in blood glucose levels in non-diabetic rats negative control and normal rats which were administered 200mg/kg body weight of Eriobotrya Japonica

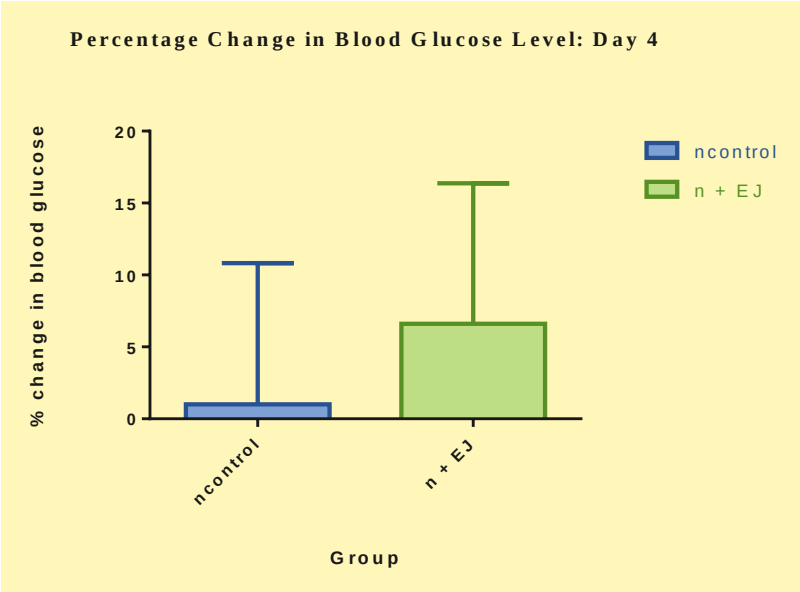


Figure 4.4: Percentage change in blood glucose in non-diabetic rats

H_0 (2a) Blood glucose levels will not differ significantly between non diabetic rats administered *Eriobotrya Japonica* compared to control non diabetic rats.

Failed to Reject H_0

T test showed that there was no significant decrease in blood glucose level between the normal rat models administered with 200mg/kg body weight *Eriobotrya Japonica* (Figure 4.4) compared to the control rats (t-statistic =0.9925; df=10; p=0.3444).

3 To determine the effects of ethanolic leaf extracts of *Eriobotrya Japonica* on blood glucose levels in diabetic rat models.

Figure 4.5 below shows the changes in blood glucose levels of the three doses of *Eriobotrya Japonica* (100mg, 200mg, and 400mg/kg b.w) against the diabetic control.

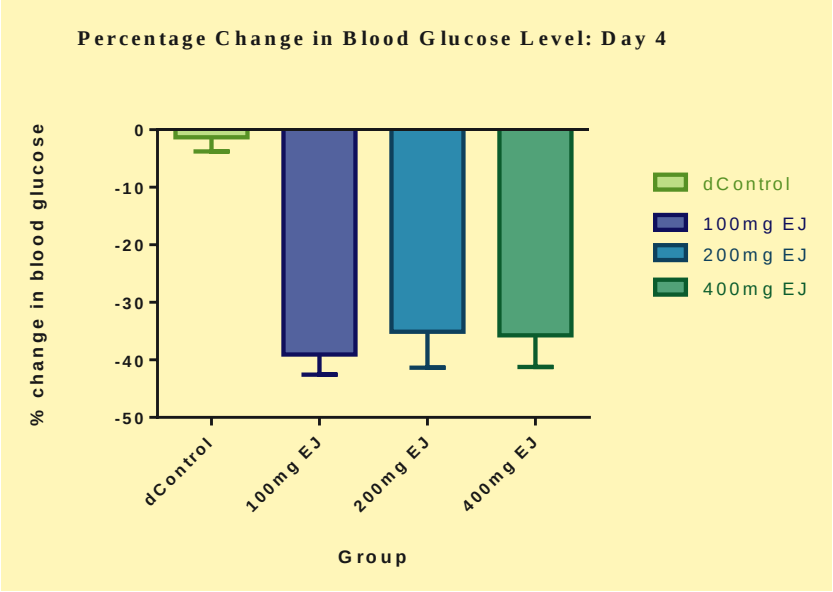


Figure 4.5: Glucose lowering effect after administering *Eriobotrya Japonica*

H_0 (3a) Blood glucose levels will not differ significantly between diabetic rats administered 100mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats.

H_0 rejected

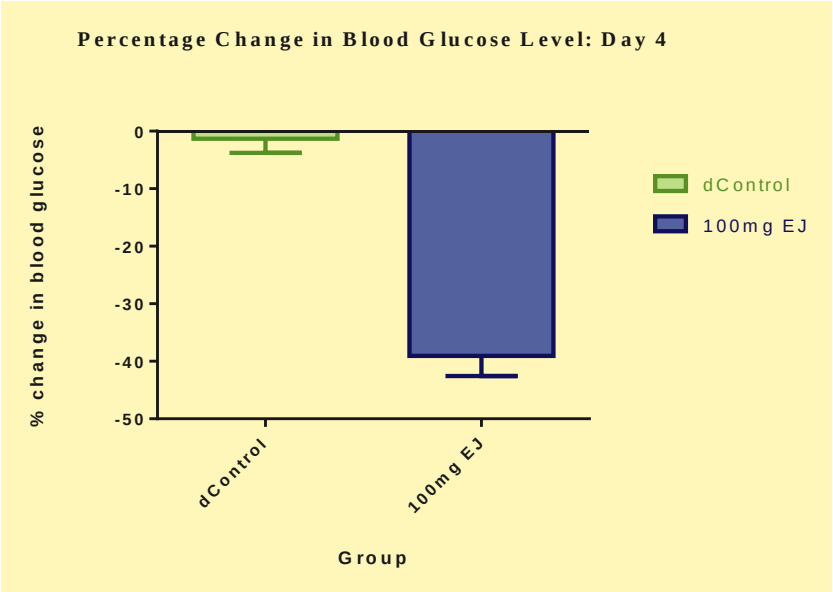


Figure 4.6: Glucose level changes with 100mg/kg b.w *Eriobotrya Japonica*

Two tailed T test showed that there was a significant decrease in blood glucose level between the diabetic rat models administered with 100mg/kg body weight *Eriobotrya Japonica* (Figure 4.6) compared to the diabetic control rats (t-statistic =21.59; df=10; p≤0.001).

H_0 (3b) Blood glucose levels will not differ significantly between diabetic rats administered 200mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats.

H_0 rejected

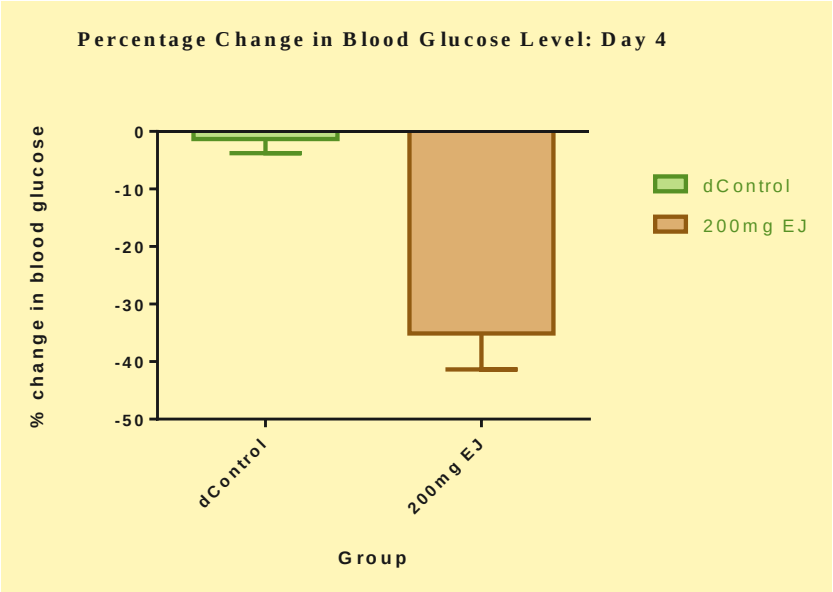


Figure 4.7: Glucose level changes with 200mg/kg b.w *Eriobotrya Japonica*

Two tailed T test showed that there was a significant decrease in blood glucose level between the diabetic rat models administered with 200mg/kg body weight *Eriobotrya Japonica* (Figure 4.7) compared to the diabetic control rats (t-statistic =12.35; df=10; p≤0.0001).

H_0 (3c) Blood glucose levels will not differ significantly between diabetic rats administered 400mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats.

H_0 rejected

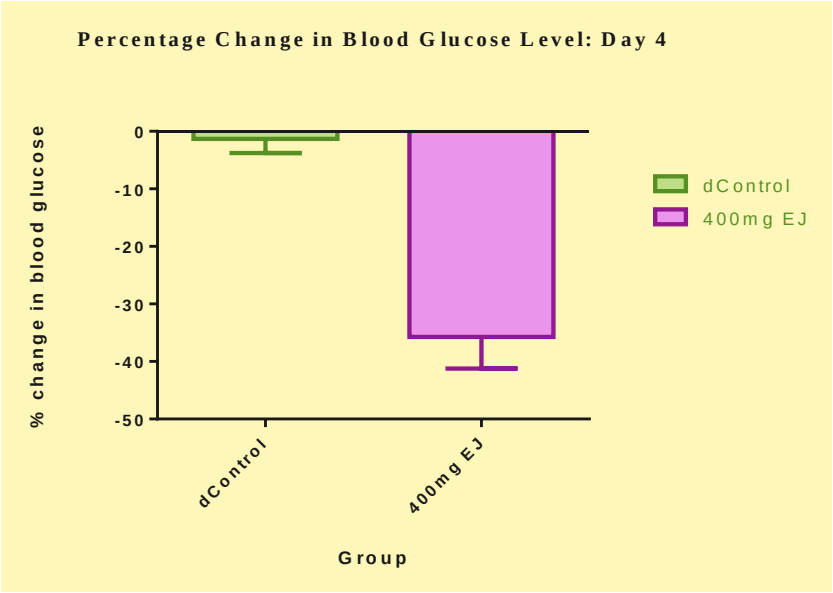


Figure 4.8: Glucose level changes with 400mg/kg b.w *Eriobotrya Japonica*

Two tailed T test showed that there was a significant decrease in blood glucose level between the diabetic rat models administered with 400mg/kg body weight *Eriobotrya Japonica* (Figure 4.8) compared to the diabetic control rats (t-statistic =13.96; df=10; $p \leq 0.0001$).

One-way analysis of variance (ANOVA) was used to compare four groups. Three doses/groups (100,200,400mg/kg/b.w) of *Eriobotrya Japonica* were compared to the diabetic control group. The reduction in blood glucose level with all the three doses was significant using one-way analysis of variance (ANOVA) test (The F statistic = 85.96; DFn=3; DFd= 20; $p \leq 0.001$). Dunnett's multiple comparisons test showed all the decreases in blood glucose level in the treatment group were significant $p \leq 0.05$ compared to the diabetic control group. Refer to Figure 4.5.

4 To compare the hypoglycemic effect of *Eriobotrya Japonica* herbal extract to that of glibenclamide.

Figure 4.9: shows the changes in blood glucose level in the three doses of *Eriobotrya Japonica* (100mg, 200mg, and 400mg/kg b.w) against the treatment group which was on 10mg/kg b.w glibenclamide.

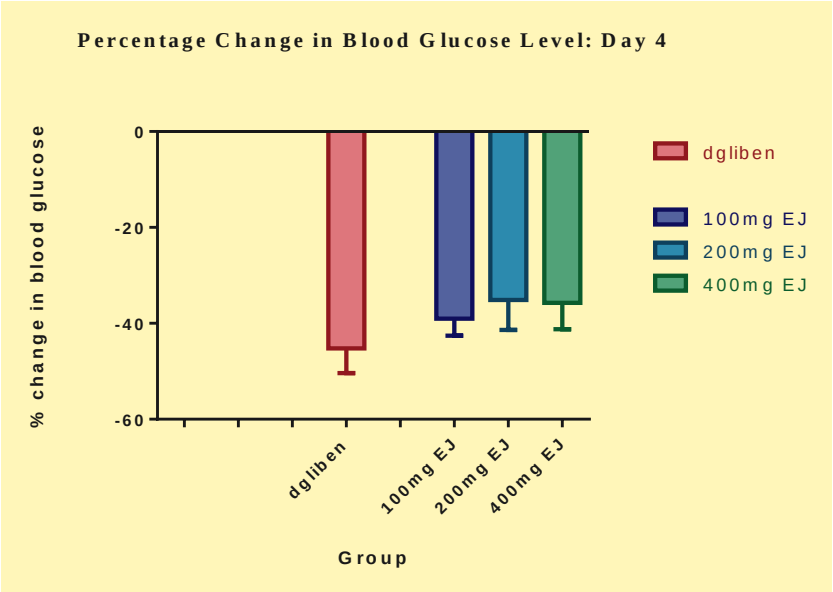


Figure 4.9: Glucose level changes of Glibenclamide and *Eriobotrya Japonica*

H_0 (4a) Blood glucose levels will not differ significantly between diabetic rats administered 100mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats fed with glibenclamide.

H_0 rejected

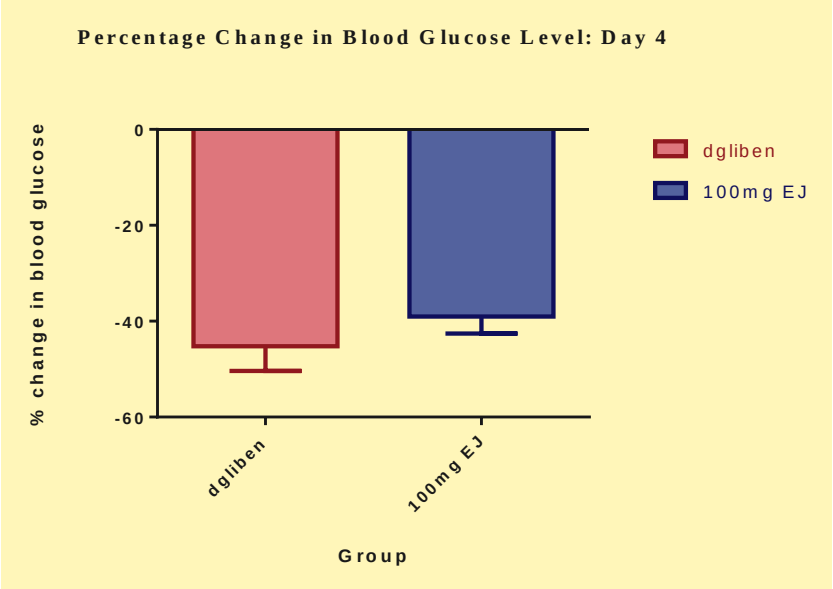


Figure 4.10: Glucose level changes with glibenclamide + 100mg *Eriobotrya Japonica*

T test showed that there was significant difference in decrease in blood glucose level between the diabetic rat models administered with 100mg/kg body weight *Eriobotrya Japonica* (Figure 4.10) compared to the diabetic rats administered with 10mg/kg b.w glibenclamide. (T-statistic =2.436; DF=10; p=0.035).

H_0 (4b) Blood glucose levels will not differ significantly between diabetic rats administered 200mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats fed with glibenclamide.

H_0 rejected

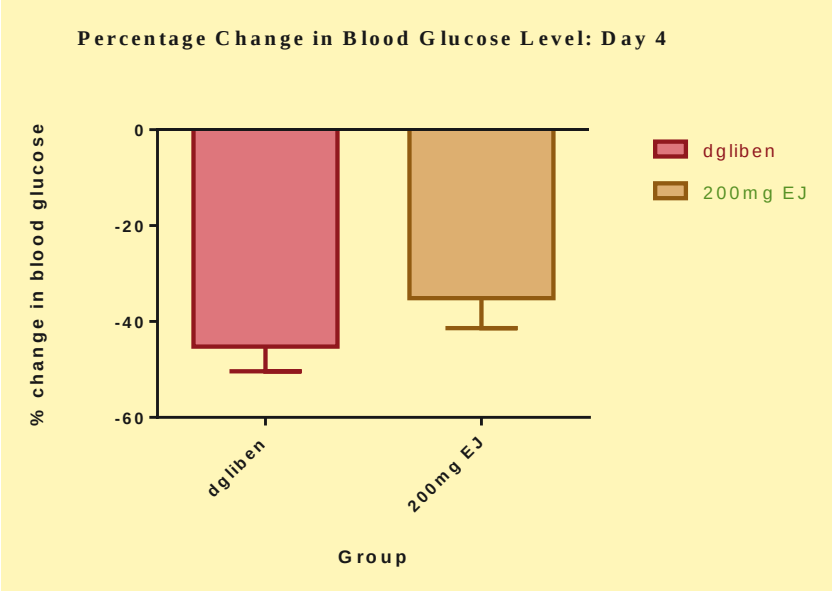


Figure 4.11: Glucose level changes with glibenclamide + 200mg *Eriobotrya Japonica*

T test showed that there was significant difference in decrease in blood glucose level between the diabetic rat models administered with 200mg/kg body weight *Eriobotrya Japonica* (Figure 4.11) compared to the diabetic rats administered with 10mg/kg b.w glibenclamide. (T-statistic =3.066; DF=10; p=0.0119).

H_0 (4c) Blood glucose levels will not differ significantly between diabetic rats administered 400mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats fed with glibenclamide.

H_0 rejected

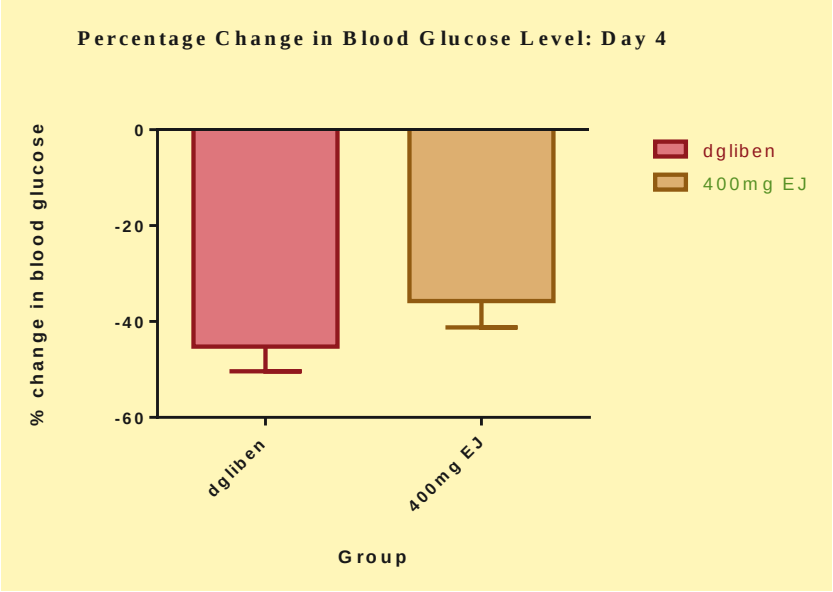


Figure 4.12: Glucose level changes with glibenclamide + 400mg *Eriobotrya Japonica*

T test showed that there was significant difference in decrease in blood glucose level between the diabetic rat models administered with 400mg/kg body weight *Eriobotrya Japonica* (Figure 4.12) compared to the diabetic rats administered with 10mg/kg b.w glibenclamide. (T-statistic =3.097; DF=10; p=0.0113).

5 To compare the hypoglycemic effect of *Eriobotrya Japonica* herbal extract to that of metformin.

H_0 (5a) Blood glucose levels will not differ significantly between diabetic rats administered 100mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats fed with metformin.

H_0 rejected

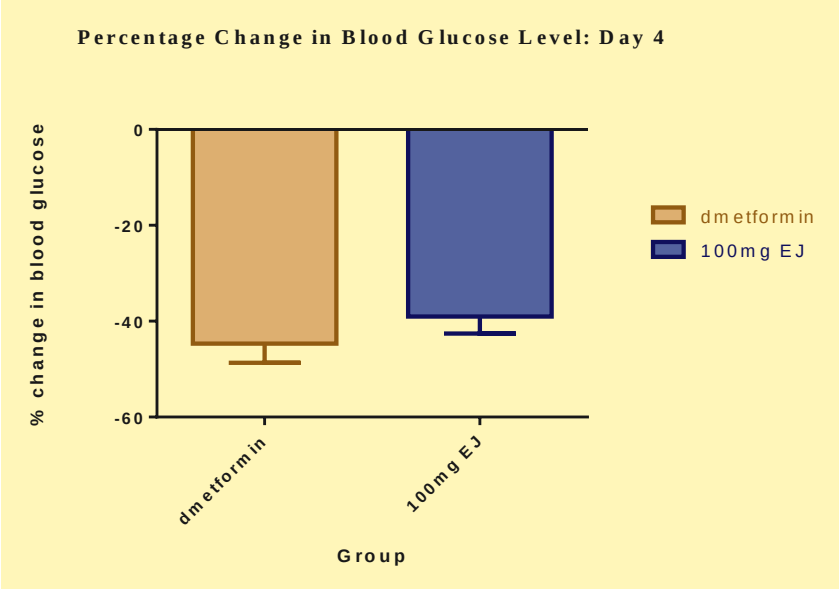


Figure 4.13: Glucose level changes with metformin + 100mg *Eriobotrya Japonica*

T test showed that there was significant difference in decrease in blood glucose level between the diabetic rat models administered with 100mg/kg body weight *Eriobotrya Japonica* (Figure 4.13) compared to the diabetic rats administered with 50mg/kg b.w metformin. (T-statistic =2.588; DF=10; p=0.027).

H_0 (5b) Blood glucose levels will not differ significantly between diabetic rats administered 200mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats fed with metformin.

H_0 rejected

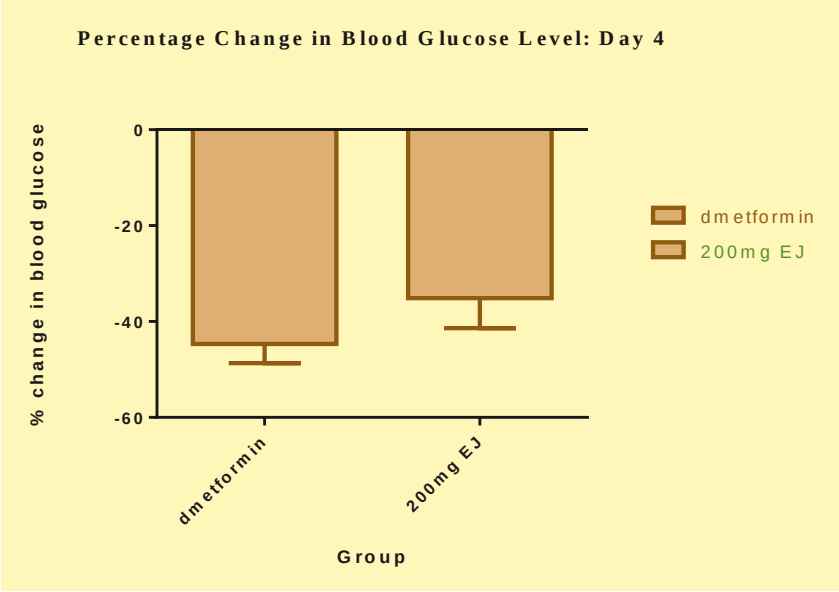


Figure 4.14: Glucose level changes with metformin + 200mg *Eriobotrya Japonica*

T test showed that there was significant difference in decrease in blood glucose level between the diabetic rat models administered with 200mg/kg body weight *Eriobotrya Japonica* (Figure 4.14) compared to the diabetic rats administered with 50mg/kg b.w metformin. (T-statistic =3.157; DF=10; p=0.0102).

H_0 (5c) Blood glucose levels will not differ significantly between diabetic rats administered 400mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats fed with metformin.

H_0 rejected

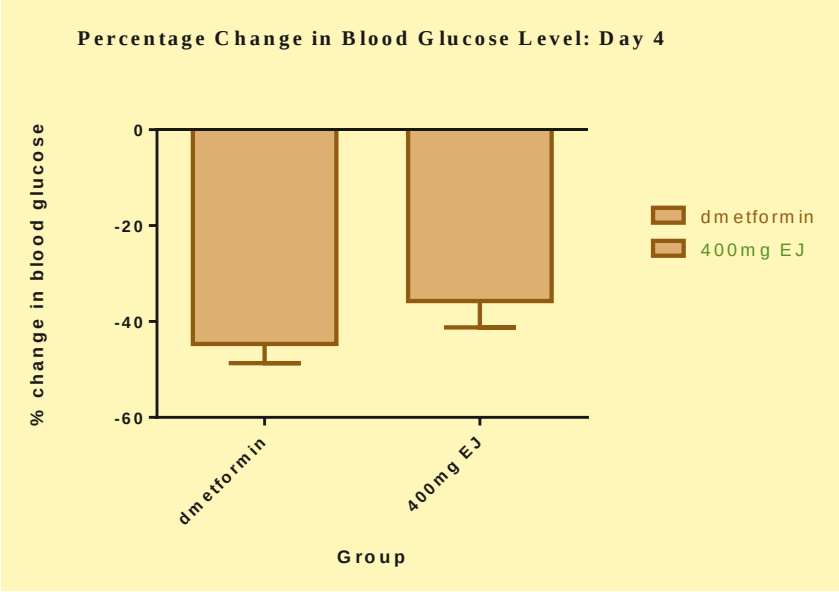


Figure 4.15: Glucose level changes with metformin + 400mg *Eriobotrya Japonica*

T test showed that there was significant difference in decrease in blood glucose level between the diabetic rat models administered with 400mg/kg body weight *Eriobotrya Japonica* (Figure 4.15) compared to the diabetic rats administered with 50mg/kg b.w metformin. (T-statistic =3.222; DF=10; p=0.0091).

6 To determine the dose- response relationship between *Eriobotrya Japonica* and blood glucose levels in diabetic rats.

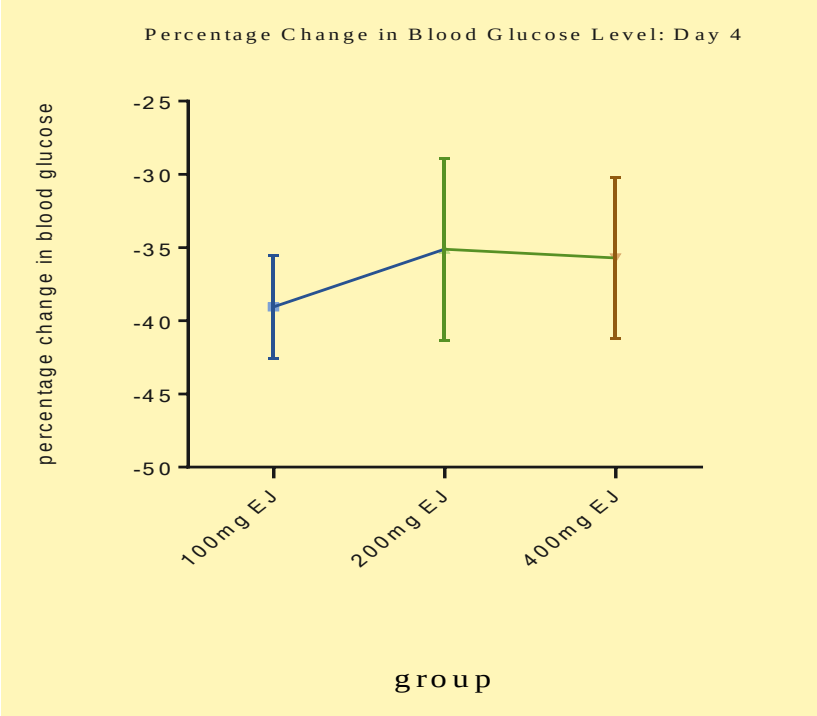


Figure 4.16: Dose – Response Changes after administering *Eriobotrya Japonica*

H_0 (6a) Blood glucose levels of the three doses of *Eriobotrya Japonica* (100mg, 200mg and 400mg/kg/b.w will not differ significantly between the groups in diabetic rats.

Fail to reject H_0

The ANOVA test showed there was no significant difference in the reduction blood of blood glucose level between all the treatment groups on *Eriobotrya Japonica.* (F=3.441; DFn=2; DFd=15; p = 0.059). Refer to Figure 4.16.

7 To determine whether any pharmacodynamic interactions exist between the different doses of *Eriobotrya Japonica* and glibenclamide in the diabetic rats.

H_0 (7a) Blood glucose levels will not differ significantly between diabetic rats administered 200mg/kg b.w *Eriobotrya Japonica* + 10mg/kg b.w glibenclamide compared to diabetic rats given 10mg/kg b.w glibenclamide only.

H_0 rejected

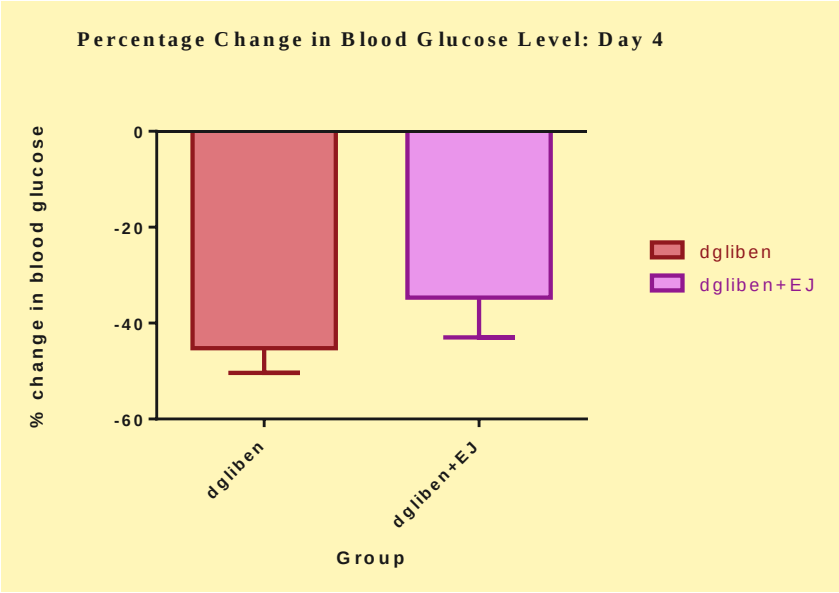


Figure 4.17: Glucose lowering effect of glibenclamide and *Eriobotrya Japonica*

T test showed that there was no significant difference in decrease in blood glucose level between the diabetic rats models administered with 200mg/kg body weight *Eriobotrya Japonica* + 10mg/kg b.w glibenclamide compared to the diabetic rats administered with 10mg/kg b.w glibenclamide only. (T-statistic = 0.2762; DF=10; p=0.7880). Refer to Figure 4.17.

Table 4.4: Hypotheses Testing Summary

OBJECTIVES	HYPOTHESIS	RESULT
Main Objective 1	*Eriobotrya Japonica* leaf extracts will not have significant effect on the morphology of β pancreatic cells of alloxan induced diabetic rat models.	**Fail to reject H0**
2	**(2a)** Blood glucose levels will not differ significantly between non diabetic rats administered *Eriobotrya Japonica* compared to control non diabetic rats.	**Fail to reject H0**
3	**(3a)** Blood glucose levels will not differ significantly between diabetic rats administered 100mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats.	**H0 rejected**
	(3b) Blood glucose levels will not differ significantly between diabetic rats administered 200mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats.	**H0 rejected**
	(3c) Blood glucose levels will not differ significantly between diabetic rats administered 400mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats.	**H0 rejected**
4	**(4a)** Blood glucose levels will not differ significantly between diabetic rats administered 100mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats fed with glibenclamide.	**H0 rejected**
	(4b) Blood glucose levels will not differ significantly between diabetic rats administered 200mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats fed with glibenclamide.	**H0 rejected**
	(4c) Blood glucose levels will not differ significantly between diabetic rats administered 400mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats fed with glibenclamide.	**H0 rejected**
5	**(5a**) Blood glucose levels will not differ significantly between diabetic rats administered 100mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats fed with metformin.	**H0 rejected**
	(5b) Blood glucose levels will not differ significantly between diabetic rats administered 200mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats fed with metformin.	**H0 rejected**
	(5c) Blood glucose levels will not differ significantly between diabetic rats administered 400mg/kg/b.w of *Eriobotrya Japonica* compared to control diabetic rats fed with metformin.	**H0 rejected**
6	**(6a**) Blood glucose levels of the three doses of *Eriobotrya Japonica* (100mg, 200mg and 400mg/kg/b.w will not differ significantly between the groups in diabetic rats	**Fail to reject H0**
7	**(7a**) Blood glucose levels will not differ significantly between diabetic rats administered *Eriobotrya Japonica* and glibenclamide compared to diabetic rats given glibenclamide only.	**Fail to reject H0**

CHAPTER FIVE

5.0 Discussion and Conclusion

The purpose of the present study was to evaluate the mechanism through which *Eriobotrya Japonica* ethanolic leaf extracts reduce blood glucose level in alloxan induced diabetic Sprague Dawley rats. The objective was to investigate whether the plant has an effect on the morphology of β pancreatic cells in alloxan induced diabetic rats. The study was also to confirm the hypoglycemic effect of *Eriobotrya Japonica* ethanolic leaf extracts in alloxan induced diabetic rat models.

The results showed that the ethanolic extracts of the leaves of *Eriobotrya Japonica* had a significant hypoglycemic effect on alloxan induced diabetic rats. The administration of all the three doses of *Eriobotrya Japonica* total ethanolic extraction in diabetic rats had a significant glucose lowering effect ($p \leq 0.05$). There was no significant reduction of blood glucose levels in normal rats administered with 200mg/kg body weight of *Eriobotrya Japonica.* The response was almost similar in all the three doses of *Eriobotrya Japonica* tested, doses below 100mg/kg/b.w could also lower increased blood glucose level in the rat models.

The glucose lowering effect of *Eriobotrya Japonica* was short lived. The reduction in blood glucose level on the 7^{th} day of administering the plant was not significant in all the doses given. This showed *Eriobotrya Japonica* cannot maintain normal glucose levels in the rat models. The reason could be the presence of other actives in the leaf extracts that increase blood glucose level or simply because the hypoglycemic effects are short lived. The glucose lowering effect was not accumulative in the group that was given glibenclamide and *Eriobotrya Japonica* suggesting no synergistic effect. Metformin and glibenclamide were found to be more potent hypoglycemic agents compared *to Eriobotrya Japonica*. In another study *Eriobotrya Japonica* was reported to be comparable to 50mg/kg body weight of gliclazide[15].

In two other separate studies the hypoglycemic effect of *Eriobotrya Japonica* was reported to be dose dependent[13,48]. However, in these studies hypoglycemic effect was not investigated in total ethanolic extraction of the leaves but isolated triterpenes. The total ethanolic extraction of the leaves of *Eriobotrya Japonica* could contain other active components which may increase blood glucose level. In contrast to other studies, *Eriobotrya Japonica* in the present study did not decrease blood glucose level in normal rats.

Several other studies have been carried out to investigate the protective effect of herbal plants on morphology or architecture of β pancreatic cells[29,33,51]. There was no change in morphology of the β pancreatic cells in the treatment groups which were given the plant *Eriobotrya Japonica.* This could be because the plant does not have the ability to help regeneration of the insulin producing cells. The lack of morphological changes might have been because of the type of stain used and the sample collection method. The other reason for this result could be because *Eriobotrya Japonica* was administered to the rats for a very short time. It is difficult to measure the size of the β pancreatic cells in order to determine the change in morphology. The islets also contain four different types of cells hence the change in size of these cells is not attributed to the change in size of β cells only.

Conventional drugs manage diabetes by improving insulin sensitivity, increasing insulin production and decreasing the amount of glucose in the blood[35]. There was no evidence of change of morphology of the islet cells suggesting no increased insulin secretion or pancreatic regeneration. This, therefore suggest *Eriobotrya Japonica* works through mechanisms that decrease the amount of glucose in the blood. Two mechanisms can be postulated by which *Eriobotrya Japonica* exert its hypoglycemic effect, one which will be by increasing peripheral glucose uptake into tissues. It can also be postulated that it decreases absorption of glucose from the small intestines.

Alloxan a diabetogenic agent induces diabetes in animal models by selective cytotoxic action on pancreatic β cells through reactive oxygen species[52,53]. Alloxan induces partial pancreatic damage thereby causing insulin deficiency[52,54]. In a study by Mir, the damage of alloxan was investigated and the hematoxylin and eosin stained sections of the pancrease showed severe necrotic damage, congestion and degenerative changes in acini[54]. Alloxan does not cause insulin resistance in the diabetic animals hence it produces type 1 like diabetes mellitus[55]. The disadvantage of using alloxan is that it brings variability of the results on development of hyperglycaemia[56,57]. This is attributed by the difference in extent of damage of the pancrease in the animal models. This drawback of alloxan leaving residual function of the β cells that continue to produce insulin means pancreatic function has to be assessed from time to time. The results of the treatment under study can be due to normal β cells that would have regained functionality without any assistance from the plant.

Diabetes mellitus is a chronic metabolic disorder characterised by high blood glucose caused by insulin deficiency and insulin resistance[27]. Effective management of diabetes mellitus is essential to prevent complications and to improve the patient's quality of life[4,53] .There is no medication available to combat all the diabetic complications hence there is still need for other therapeutic alternatives. Phytotherapy is still important in developing populations because of cultural reasons, perceived safety and affordability reasons[29,51]. The use of herbal plants is argued to be safe compared to the conventional treatment options that have a lot of side effects. The use of herbal plants such as *Eriobotrya Japonica* can help solve some of the problems of diabetes mellitus and improve medicine access. It can also be the birth of a drug that can be used as monotherapy in diabetes mellitus.

Conclusion

This study demonstrated that *Eriobotrya Japonica* exhibit hypoglycemic activity in alloxan induced diabetic models. Hypoglycemic activity of *Eriobotrya Japonica* was not dose dependent for doses up to 400mg/kg body weight in the rat models. Glibenclamide and metformin alone proved to be more efficacious than *Eriobotrya Japonica* leaf ethanolic extract alone. There was no synergism observed when glibenclamide was used with *Eriobotrya Japonica*.

Recommendations

Further research is needed to clarify the mechanisms of action of this plant in reducing blood glucose level. Further studies are needed in humans to assess its suitable use as an effective functional food with therapeutic benefits. A repeat of the study is needed whilst increasing the time of administering *Eriobotrya Japonica* maybe there will be significant changes. Confirmatory tests are also need to measure insulin secretion and sensitivity to assess the change in pancreatic function.

Limitations of the Study

1) The time of feeding the rat was short, long exposure of the animals to *Eriobotrya Japonica* might have resulted in significant changes.
2) It was difficult to make the high fat diet into pellets which are easy to feed the rat models.
3) It was difficult to measure blood glucose levels from other body parts except the tail vein of the rats which may not reflect the actual plasma glucose level.
4) It was not feasible to measure the size change of the β pancreatic cells.

5) It was not possible to perform histological examinations with another stain besides the hematoxylin and eosin basic stain for comparison purposes.
6) There were no resources to measure insulin levels/secretion and also assessing insulin sensitivity.

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APPENDIX

LIST OF APPEDICES

EXPERIMENTAL TESTING PROTOCOL

STANDARD OPERATING PROCEDURE FOR THE RAT TREATMENT

1. PURPOSE

- Alloxan will be used to induce diabetes mellitus.
- A high fat diet will be used to induce obesity and type 2 diabetes mellitus.
- *Eriobotrya Japonica* will be fed to the animals to treat diabetes mellitus.
- Histological examinations will be done on the rat pancreas to see change in morphology.

2. SCOPE OF THE STANDARD OPERATING PROCEDURE

This procedure will apply to all individuals who will be conducting the testing procedure and the protocol should be strictly observed. The following procedures were extrapolated from the Clinical pharmacology department laboratory guidelines, rules and regulations.

3. RESPONSIBILITIES

The following will be the responsibilities of the individuals conducting the study:

i. To ensure that bedding of the test animals is changed at least three times a week.

ii. To ensure that the animals have sufficient food and water throughout the experimental study.

iii. To ensure that water supplied to the test animals are changed every other day.

iv. To ensure that noise is kept at minimum levels in the experimental rooms.

v. To ensure that humidity is maintained at 55±5%, temperature at 22+/- 2^{o}C and light is maintained using the natural cycle(0600-1800)

4. SAFETY REQUIREMENTS

i. Drinking and eating in the experimental rooms is strictly prohibited

ii. Protective clothing, lab coats and gloves should be worn at all times during the experimental procedures

iii. Good laboratory practices should be done at all times and safety of the test animal should be monitored throughout the study as well as that of the investigator

iv. No jewelry or cosmetics should be worn at any time during the experimental work

5. INGREDIENTS AND MATERIALS

ITEM	QUANTITY	SUPPLIER/ MANUFACTURER	COST
Male Wistar rats	90	UZ animal house	270
Glucometer	1	Retail Pharmacy	55
Equivalent glucose test strips	600	Retail Pharmacy	240
Lancets	600	Retail Pharmacy	30
High-fat diet	50kg	National Foods Zimbabwe	30
Standard diet	10kg	UZ animal house	0
Glibenclamide	3g	Retail Pharmacy	21
Metformin	120g	Retail Pharmacy	10
Mass balances	1	Pharmacology Department	0
Eriobotrya Japonica leaves	400g	The Pond Gardens Marlborough	0
1ml and 5ml graduated syringes	30	Pharmacology Department	0
10ml graduated syringes	10	Retail Pharmacy	1
Alloxan Monohydrate	10g	SIGMA Germany	86

standard polypropylene cages	10	Pharmacology Department	0
1L 0.9% w/v normal saline	2	Retail Pharmacy	10
1L 5% Dextrose vacolitre	8	Retail Pharmacy	40
500g cotton swabs	1	Pharmacology Department	0
100mL volumetric flask	1	Pharmacology Department	0
10mL volumetric flask	1	Pharmacology Department	0
100mL beakers	2	Pharmacology Department	0
Gastric lavage pipes	1	Pharmacology Department	0
95% v/v ethanol	5000mL	Pharmacology Department	0
Wood shavings	4*50kg	Halsted Timbers	4
Tissue papers and towels		Pharmacology Department	0

6. PREPARATION OF THE PLANT EXTRACT AND DRUGS

6.1 Induction of diabetes

- Use the high fat diet to induce obesity and diabetes which is typical of type 2 diabetes.
- Use alloxan monohydrate 120mg/kg b.w in normal saline to chemically induce diabetes.

6.2 Preparation of *Eriobotrya Japonica*

- Mill/crush the dried leaves of Eriobotrya Japonica using a mortar and pestle or a home blender.
- Dissolve the powdered leaves in 2000ML 70 % ethanol for extraction at room temperature for 48hours.
- Pass the soaked material through a muslin cloth to remove the vegetative material.
- Vacuum filter the obtained fluid through a Whattman-1 filter paper and then evaporate the filtrate using a rotary evaporator (Rota vapor, EL130) at 40ºC
- Freeze dry the sample paste in temperatures ranging from -50 ºC to -55 ºC using the Freeze drier Heto FD3, Belgium.
- Keep the extract in a glass vacuum desiccator until the day of the experiment.
- Give the rats the respective doses of Eriobotrya japonica depending on the group in which they are.

6.3 Preparation of Glibenclamide

- Dissolve seven 5mg tablet of glibenclamide in 100ml of 0.9% normal saline solution
- Give the respective doses to the respective rat in each of the groups. The dose for each rat would be 10mg/kg body weight (weight of the rat/weight of the largest rat)*1ml

6.4 Preparation of Metformin

- Dissolve 1g of metformin in 100mL of 0.9% normal saline solution

- Give the respective doses to the respective rat in each of the groups. The dose for each rat would be 50mg/kg body weight (weight of the rat/weight of the largest rat)*1ml

7. ANIMAL HUSBANDRY AND HANDLING OF MICE

7.1 Rat strain

- Use eight weeks old male Wistar rats acquired from the animal house.

7.2 Housing

i. Put six rats per cage to avoid fighting and ensure free ventilation and adequate space

ii. Use wood shavings as the bedding

iii. Standard diet and high fat diet was obtained from the animal housing department and National Foods Zimbabwe respectively

7.3 Handling

- Pick the mice by the proximal part of the tail, put in a rat holder and clamp to avoid any movements
- The rats should not be picked up by the distal half of the tail as this might cause discomfort
- If the rats are used to handling they can be picked up by clamping the body in the hands then putting in a rat holder
- Take blood samples from the distal part of the tail and measure the sugar levels

- For oral gavage, the rats are clamped by the back with hands allowing minimum movements of any of the body parts of the rat and held in that position until they have been dosed

7.4 Marking the rats for identification

- Mark the tails of the rats with indelible ink
- Perform regular checks to renew these markings

8. TESTING PHASE: THE PROCEDURE

i. Record the initial masses of each and every rat using mass balances.

ii. Feed the rats for two-four weeks with the respective type of feed according to the group's feed requirements i.e. either standard diet or high fat diet

iii. Measure and record the changes in masses after every two weeks

iv. Fast the rats being fed on a high fat diet overnight, a day before chemical treatment with alloxan injection

v. Weigh the rats on the day of induction (before the induction) using mass balances

vi. By tail tipping, take blood samples from each and every rat and measure the blood glucose levels using the glucometer

vii. Dissolve the alloxan monohydrate in 0.9% normal saline solution

viii. Calculate individual doses for each rat of alloxan monohydrate injection 120mg/kg b.w in normal saline and perform an i.p injection on each of the rats.

ix. Give 5% dextrose orally to each of the alloxan dosed rats for 3days to prevent the early phase hypoglycemia

x. After three days of alloxan dosing, take blood samples and measure the blood glucose levels of each rat using the glucometer

xi. Rats with blood glucose levels of >140mg/dl are then selected for further study.

Experimental group one

i. Give the metformin solution at 50mg/kg b.w by a 14g gastric gavage tube to the respective rats in the respective groups
ii. Give *Eriobotrya Japonica* suspension by a 14g gastric gavage tube to the respective rats in the respective groups
iii. Give the Glibenclamide suspension 10mg /kg b.w using a 14g gastric gavage tube to the respective rats in the respective groups
iv. Give the respective doses of normal saline to the control groups through gastric gavage
v. Wait for 2 hours and then take blood samples from each of the rats and measure their blood glucose levels
vi. Record all the blood glucose levels measured

Experimental group two

i. Gavage with normal saline, metformin, glibenclamide and *Eriobotrya Japonica* same time every day for 4 weeks.
ii. Take blood samples and measure the blood glucose levels before and after the gastric gavage procedure after every ten days
iii. Sacrifice the rats after the four weeks and collect the pancreas from each rat in 10% formalin solution.
iv. Immediately process the pancreatic tissues using the paraffin technique.
v. Cut sections of 5 μm thickness and stain with hematoxylin and eosin for histological examinations.

vi. Qualitatively evaluate the stained sections using an electron microscope.

9. DOSAGE ADMINISRATION

The following treatments will be administered for the different groups:

i. Normal saline 10ml/kg – negative control

ii. Alloxan – 120mg/kg b.w in normal saline

iii. *Eriobotrya Japonica* – 100mg/kg/day,200mg/kg/day,400mg/kg/day

iv. *Eriobotrya Japonica* + Glibenclamide 10mg/kg/day

v. Eriobotrya Japonica +Metformin

vi. Glibenclamide – 10mg/kg/day

vii. Metformin – 50mg/kg/day

10. METHOD OF DOSING BY GASTRIC GAVAGE

Method Adopted from the Animal House University of Zimbabwe.

- Gavage the rats using a 14 gauge gavage tube
- Immobilize the rat by holding it by its back with the left hand palm and making sure that the tail is also held so that there will not be any movement
- Hold the base of the tail against the palm with the middle finger making sure that the rat is comfortable but immobile
- Hold the head of the rats as far back as possible so that the esophagus through the throat and oral cavity is in a straight line
- Introduce the tip of the dosing tube into the mouth running it slightly off-center down the tongue into the esophagus

- With gentle movements gently ease the tube down the throat and inject the syringe contents into the mouth. Regurgitation only occurs if the tube is not far enough down past the cardiac sphincter of the esophagus. At no point should force be used, the tube should slide down easily with minimum pressure if it is placed correctly.