International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319 – 6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 8; August 2017; Page No. 5689-5694
DOI: http://dx.doi.org/10.24327/ijcar.2017.5694.0780

CASE REPORT

SYNERGISTIC EFFECTS OF *ABELMOSCHUSESCULENTUS* AND *VERNONIAAMYGDALINA* IN CONTROLLING DIABETES MELLITUS IN RAT MODELS

Vempati Poornodai[1]., Srinivasu K[2]., Manju Thomas[3] and Vulli Venkata Rao[4]

[1]Biomedical Department, College of medicine and health sciences, Dilla University, Ethiopia
[2]Department of Pharmacology, Yirgalem hospital medical college, Ethiopia
[3]Department of Pharmacology, Kampala International University of Health Sciences, Dar es salaam, Tanzania
[4]Department of Biochemistry, Kampala International University of Health Sciences, Dar es salaamTanzania

A R T I C L E I N F O

Article History:

Received 25th May, 2017
Received in revised form 13th June, 2017 Accepted 20th July, 2017
Published online 28th August, 2017

Key words:

Antidiabetic, Alloxan, Insulin

A B S T R A C T

Background: Diabetes mellitus is one of the main causes of serious maladies in the 21st century. Diabetes mellitus is defined on the basis of laboratory findings as a fasting venous plasma glucose concentration greater than 7.8mmol/l (140mg/dl) or greater than 11.1mmol/l (200mg/dl) two hours after a carbohydrate meal or two hours after an oral ingestion of the equivalent of 75g glucose, even if the fasting concentration is normal (Nwanjo, 2004).
Aim: This experiment was done to determine the synergistic antidiabetic effect of the aqueous leaf extracts of *V.amygdalina* and *A.esculentus* in alloxan induced rats
Methods: Artificial Diabetic nature was induced in rats using a diabetic inducing drug. A standard dosage of okra and bitter leaf extract was given to the rats and they were monitored for 3 weeks. Data was analyzed using SPSS Version 20. ANOVA and one sample T tests was carried out.
Results: The extracts of the two different plants worked effectively when their individual doses were increased. When combined at lower doses, (reduced blood sugar level at 3.7 mmol/l) they tend to be more effective than in high doses (reduced blood sugar level at 3.7 mmol/l) when compared to insulin (Mixtard) at 3.56mmol/l after three weeks
Conclusion: The plant extracts had antidiabetic effects individually and when combined they were more effective at lower doses

INTRODUCTION

Diabetes mellitus is one of the main causes of serious maladies in the 21st century. The world population of diabetes mellitus in year 2008 was approximately 150 million and the population of this pandemic was expected to double by year 2025 (Tan *et al*., 2008).

Diabetes mellitus is defined on the basis of laboratory findings as a fasting venous plasma glucose concentration greater than 7.8mmol/l (140mg/dl) or greater than 11.1mmol/l (200mg/dl) two hours after a carbohydrate meal or two hours after an oral ingestion of the equivalent of 75g glucose, even if the fasting concentration is normal (Nwanjo, 2004). It is a chronic metabolic disease characterized by hyperglycemia and glycosuria due to absolute or relative lack of insulin (Aguwa, 1996).

When the blood glucose elevates (for example, after eating food), insulin is released from the pancreas to normalize the glucose level.

Corresponding author:* **Vempati Poornodai
Biomedical Department, College of medicine and health sciences, Dilla University, Ethiopia

In patients with diabetes, the absence or insufficient production of insulin causes persistent hyperglycemia. (Gupta and Amartya., 2012)

Diabetes is classified into two main types: Type-1 diabetes (T1D), previously known as insulin-dependent diabetes mellitus (which is immune-mediated or idiopathic), and type-2 diabetes (T2D) formerly called non-insulin-dependent diabetes mellitus (with a complex pathophysiology that combines progressive insulin resistance and beta-cell failure and has a heritable basis). Diabetes also can be related to the gestational hormonal environment, genetic defects, other endocrinopathies, infections, and certain drugs. Patients with T1D show a state of insulin deficiency because of severe defect in islet β-cell function while T2D is characterized by a combination of resistance to action of insulin and insufficiency in insulin secretion (Ghorbani., 2013; Deshpande*et al*., 2008).

Diabetes is a serious global health issue, with type 2 diabetes mellitus accounting for approximately 90 to 95% of all cases (Rodbard et *al*., 2007). The rapid increase in the prevalence of type 2 diabetes is in partly due to an ageing population but

may also be due to an increase in the number of overweight and obese people. It was estimated that approximately 6.6% of the world's population aged between 20 and 79 years had diabetes in 2010, by some of these companies or associate the higher prices to drug efficacy with this figure projected to increase to 7.8% by 2030 (Fakhoury *et al*., 2010) and prevalence of diabetes in people over 65 years of age will be 69% increase in developing countries (Wild *et al*.,2004). It is predicted that the developing countries will contribute 77.6% of the total number of diabetic patients in the world by the year 2030.

Over time, both types of diabetes lead to serious complications in the body, which include nephropathy, retinopathy, neuropathy, dyslipidemia and cardiovascular diseases (Deshpande *et al*., 2008; Ghorbani *et al*., 2010).

Diabetes treatment depends on the type and severity of the diabetes. Type 1 Diabetes is treated with insulin, exercise and a diabetic diet. Type 2 diabetes is first treated by weight reduction, a diabetic diet, and exercise. When these measures fail to control the elevated blood sugars, oral medications such as Biguanides, sulfonylureas and others are used. If oral medications are still insufficient, insulin injections are considered.

Improvement in HbA1c has become the standard surrogate outcome measure in many trial designs for a variety of therapies. In patients with diabetes, the following situations also can be considered a benefit of therapy:

1. A meaningful reduction of insulin requirements (in either type 1 or type 2 diabetes) or
2. A reduction in the number or doses of oral antidiabetic agents (in type 2 diabetes mellitus), both in the context of stable or improved HbA1c.

Although, insulin and oral hypoglycemic agents is the mainstay of treatment of diabetes, they have prominent side such as severe hypoglycemia, lactic acidosis, peripheral edema and abdominal discomfort (Lorenzati *et al*., 2010). Failure to alter the course of diabetic complications is present; moreover they are costly (Nwanjo, 2005). Non-pharmacological means like diabetic diet and exercise have also been used in the management of diabetes mellitus.

It has been estimated that a low income family with a diabetic patient devotes 25% of the income to the care of that patient and one third of the diabetic patients take alternative medications that they consider efficacious (Ryan *et al*., 2001). Garlic, Aloe vera, ginger, bitter leaf, okra and Echinacea or their herbal mixtures is commonly used alternatives (Ogbera *et al*., 2010).

The obvious limitations of these management methods stated above necessitate a search for alternatives among the arsenal of herbs available to man and have led several investigators to focus their attention on the traditional medicines. Herbal medicines have been highly esteemed source of medicine throughout the human history. They are widely used today indicating that herbs are a growing part of modern high-tech medicine (Gupta and Amartya., 2012). Hence this study aims to establish the synergistic effect of the aqueous leaf extracts of *Vernoniaamygdalina and Abelmoschusesculentus* in lowering blood glucose levels in alloxan induced rats.

MATERIALS AND METHODS

Collection and Botanical Identification of Plant Samples

The aerial parts of *Vernoniaamygdalina* and fruits of *Abelmoschusesculentus* were collected from Ishaka ('Lagos') area and a sample taken to the department of botany at Mbarara University of Science and Technology (MUST) for identification. Voucher specimens was obtained & deposited in the herbarium of the school of pharmacy KIU-WC.

Extraction method of Vernoniaamygdalina

The method of extraction was adopted and modified from (Sani *et al*., 2012). The fresh leaves of the plant *Vernoniaamygdalina*was dried in the open air in a shade for a period of about two weeks prior to the extraction process. Thereafter, the samples was grounded into fine powder and stored in air tight bottles prior to use.

The leaves were macerated with both cold and hot water. The concoction or decoction was left to settle for three days in an air tight bottle to obtain the extract.

Extraction method for Abelmoschusesculentus

The mode of extraction was adopted and modified from (Saha *et al*., 2011). The fruits were air-dried in shade for three weeks and grinded to coarse powder.

About 500gm of the dried fruit powder was extracted with water by simple maceration. The maceration continued for 48 hours. The water extract was filtered and concentrated. The extract was then obtained.

This study will be experimental; involving Alloxan induced diabetic Wistar rats as well as normal Wistar rats.

Animal study

The study will be conducted on mature experimental Wistar rats. A total of 73 animals will be used, in line with (Campos et al., 2013). 28 albino rats will be used for acute toxicity tests and 45 albino rats will be used for the anti-hyperglycemic activity study. Random sampling technique will be used. Mature, healthy adult male and female rats will be used for the study and rats weighing above 100g will be used. Any pregnant or diseased rat as well as those weighing less than 100g will be excluded

Determination of Acute Toxicity

The methods of acute toxicity testing was modified from (Yeap *et al*., 2013; Ojiako and Nwanjo., 2006; Saha *et al*., 2011) respectively.

The acute toxicity of the aqueous leaf extract of *Vernoniaamygdalina* and fruit extract of *Abelmoschusesculentus* were determined by using adult male and female Wistar rats. The animals were fasted for 18 hours prior to experiment, weighed with an electronic balance and marked according to different body marks. The animals were grouped into four groups and each group (four animals) will be administered a different dose Table 1.

Dose of Extract with control **Table No 1**

Normal saline (control)	***Vernoniaamygdalina***	***Abelmoschusesculentus***	**Mixture**
1ml/kg	1000mg/kg	300mg/kg	1000+300mg/kg
Nil	2000mg/kg	500mg/kg	1500+500mg/kg

Volume to administer was gotten from:

$$\frac{\text{(Body weight x dose)}}{\text{(Concentration x 1000)}}$$

Animals were observed for signs of toxicity after 3 hours and 24 hours and 28 animals were used.

Induction of Diabetes

Rats were weighed, marked and fasted for 24 hours.

Diabetes was induced by a single intraperitoneal administration of Alloxan monohydrate (140mg/kg) with 4% saline solution (an average of 0.90mL per test animal). Before administering Alloxan, a base line glycemia was recorded from blood taken from puncture of tail vein.

After 2 days, the animals' blood glucose level was determined by testing with strips and glucometer. Rats with blood glucose above 200mg/dl (7.0mmols) were considered as diabetic and selected for the proposed study One group of rats were not administered Alloxan and served as normal control. Insulin treatment 0.03IU/kg was used as reference point.

Anti-Hyperglycemic Activity

Rats were randomized into 9 groups, each consisting of 5 rats (Ojiako and Nwanjo., 2006).

Group	Process
Group 1	Non-diabetic rats will receive Normal Saline (2ml/kg) to serve as normal control group
Group 2	Diabetic rats will receive Normal Saline (2ml/kg) and serve as diabetic control group
Group 3	Diabetic rats will receive an aqueous leaf extract of *A. esculentus* at a dose of 200mg/kg
Group 4	Diabetic rats will receive aqueous leaf extract of *A. esculentus* at a dose of 300mg/kg
Group 5	Diabetic rats will receive aqueous leaf extract of *V. amygdalina* at a dose of 50mg/kg
Group 6	Diabetic rats will receive aqueous leaf extract of *V. amygdalina*at a dose of 100mg/kg
Group 7	Diabetic rats will receive a mixture of aqueous leaf extract of *A. esculentus* :*V. amygdalina*at a dose of (300:100)mg/kg
Group 8	Diabetic rats will receive a mixture of aqueous leaf extract of *A. esculentus* :*V. amygdalina*at a dose of (200:50)mg/kg
Group 9	Diabetic rats will receive insulin at a dose of 0.03IU/kg as standard

Administration of normal saline, the aqueous leaf extract, mixture and insulin was done daily before feeding the animals.

Testing for random blood glucose and weighing of the animals was done after every seven days and the blood sample was collected from the tail and tested using a glucometer.

The whole process was carried out in 15 days approximately.

Ethical Considerations

An approval was obtained from the School of Pharmacy Kampala International university –Western campus Uganda prior to the research and the laboratory animals were cared for appropriately. Animal experiments were conducted according to the national institute of health guide for the care and use of laboratory animal (NIH, 1996).

Production of Aqueous Extracts

The extracts of the leaves of Vernoniaamygdalina and the fruit of Abelmoschusesculentus were gotten by maceration.

Maceration is the process whereby the leaves are softened and then broken into pieces using a liquid solvent with their active ingredient dissolving in that liquid solvent at room temperature.My solvent in this case was water.

Procedure for Maceration

The leaves of Vernoniaamygdalina was air-dried for two weeks and the fruits of Abelmoschusesculentus were slit-open and air-dried for three weeks due to its high moisture content. After they had been dried, they were blended individually using a blender.

1000mls of water was used for each plant during maceration. They were macerated for 48 hours and the extracts were filtered using a filter paper to get the filtrate. The concentration for *Vernoniaamygdalina* extract was 200mg/dl and the concentration of *Abelmoschusesculentus* extract was 100mg/dl.

Acute Toxicity Testing

The rats were tested for acute toxicity testing in October 2015 using *Vernoniaamygdalina*, *Abelmoschusesculentus* and a mixture of both plants. The rats were weighed before giving the aqueous extracts

Volume for administering was adopted from (Ojiako and Nwanjo, 2003)

$$\frac{\text{(Body Weight x Dose)}}{\text{(Concentration x1000)}}$$

The formula above was used to calculate the mls for administering the rats. The rats for acute toxicity were observed for three hours and twenty-four hour.The laboratory coat was worn to reduce skin contamination of the extracts and gloves were also worn to protect the fingers.Absolute care was taken when administering the extracts to avoid them taking the oral dose into the wrong tract due to their anatomical structure and the rats were properly labeled to avoid confusion.Hands were washed before and after to avoid contamination and all rats used were sure to be weighing 100g and above.

Anti-Hyperglcaemic Activity

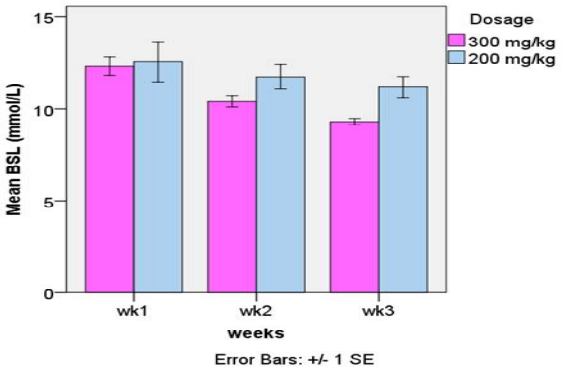
Alloxan-Toxicity Induction

Alloxan is a toxic glucose analogue which selectively destroys insulin-producing cells in their pancreas (that is the beta-cells). This causes an insulin-dependent Diabetes Mellitus called 'Alloxan-diabetes' in these animals with characteristics similar to type 1 diabetes in humans.

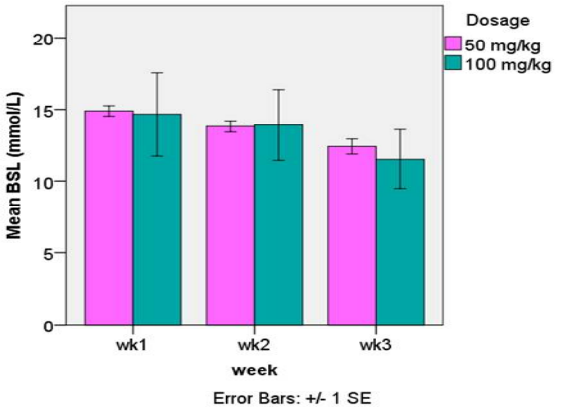
Alloxan Mono-hydrate was given to 40 animals out of 45 animals that were to be used for checking the anti-hyperglycemic activity. The remaining 5 animals were used as control. Appropriate care was taken when administering Alloxan Mono-hydrate because it can be absorbed through the skin and still be effective at a small dose.Laboratory coats were worn to reduce contact of the skin with Alloxan Mono-hydrate and gloves were doubled and worn to avoid contact with Alloxan in case of any hole in the first glove or tear (if they are eaten up by the rats when they were been given Alloxan).Appropriate care was taken during the administration since it was given intravenously and all rats were labeled correctly to reduce confusion.Hands and arms were washed before and after administration.

Treatment of Diabetic Rat with V. Amygdalina, a. Esculentus, Mixtures, Insulin and Normal Saline

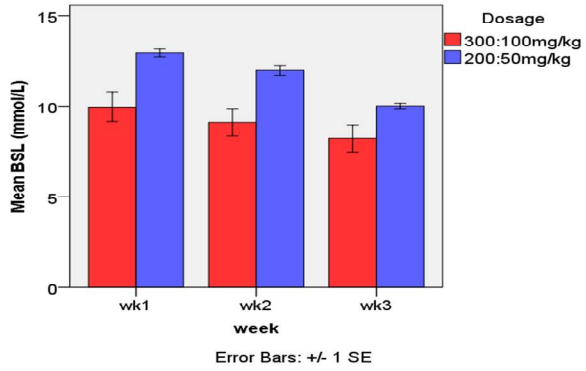
The treatment started on the 22nd of october 2015 after the animals were all tested to be diabetic.



The graph above is for the administration for belmoschusesculentus 200mg/kg and 300mg/kg

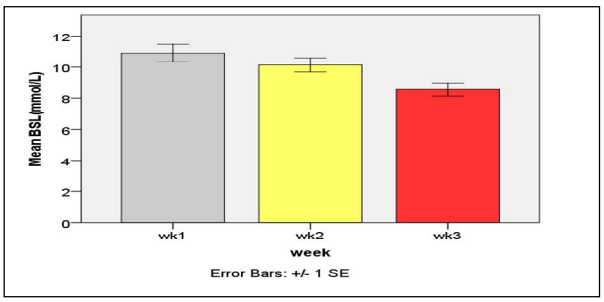


The graph above is for the administration of Vernoniaamygdalina 50mg/kg and 100mg/kg



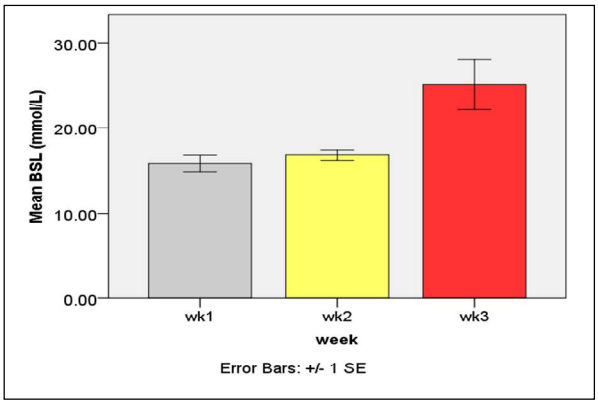
The graph above is for the mixture of A.esculentus and V.amygdalina at the ratio-300:100mg/kg amd 200:50mg/kg

Insulin



The graph above is for the administration of insulin 0.03IU/Kg (Mixtard)

Normal saline



The graph above is for the administration of control using normal saline 2ml/kg

Using a calculator the below results were gotten for the average Blood Sugar Level mmol/l

Description	Initial BSL-Final BSL	BSL mmol/l
BK RA	15.6-11.2	4.4
TL LL RL	16.2-11.5	4.7
RA	10.1-9.6	0.5
LL RL	16.4-12.4	4.0
TL	11.5-10.2	1.3
Total BSL mmol/l 14.9 / 5= 2.98		

The above table shows the average blood sugar level that was reduced by A. *esculentus*200mg/kg

Description	Initial BSL-Final BSL	BSL mmol/l
HD	12.6-9.7	2.9
LA	13.1-8.8	4.3
BK	14.0-9.1	4.9
RL	11.4-9.2	2.2
RL RA	12.0-9.7	2.3
Total BSL mmol/l 16.6 / 5= 3.32		

The above table shows the average blood sugar level that was reduced by A. *esculentus*300mg/kg

Description	Initial BSL-Final BSL	BSL mmol/l
US RL	14.7-13.0	1.7
RL LL LA RA	15.1-14.5	0.6
HD LA	16.0-16.0	0
RA LL	15.6-12.9	2.7
HD RL LL	14.7-11.4	3.3
Total BSL mmol/l 8.3 / 5=1.6		

The above table shows the average blood sugar level that was reduced by V. *amygdalina*50mg kg

Description	Initial BSL-Final BSL	BSL mmol/l
HD TL	15.6-11.5	4.1
HD LL	16.8-10.0	6.8
US RA	12.5-7.5	5
BK LA	28-17.2	10.8
BK LL	8.9-10.4	-1.5
Total BSL mmol/l 25.2 / 5 =5.04		

The above table shows the average blood sugar level that was reduced by V. *amygdalina*100mg/kg

Description	Initial BSL-Final BSL	BSL mmol/l
TL LA	10.6-7.6	3.0
LL	14.4-11.1	3.3
US LA	9.9-7.4	2.5
LA LL	10.8-7.0	3.8
TL RL	11.6-8.1	3.5
Total BSL mmol/l 16.1 / 5 =3.22		

The above table shows the average blood sugar level that was reduced by A. *esculentus*and V.*amygdalina*at the ratio 300:100

Description	Initial BSL-Final BSL	BSL mmol/l
US LL	14.6 – 10.4	4.2
HD TL US	13.2 - 10.1	3.1
HD BK TL	13.0 – 10.0	3.0
US RL LL	14.1 - 9.5	4.6
LA RL	13.7 – 10.1	3.6
Total BSL mmol/l	18.5 / 5= 3.7	

The above table shows the average blood sugar level that was reduced by A. *esculentus*and V.*amygdalina*at the ratio 200:50

Description	Initial BSL-Final BSL	BSL mmol/l
RA LA	13.0 – 8.0	5.0
BK RL	12.7 – 8.5	4.2
US	12.4 – 8.3	4.1
TL LA RA	10.9 – 10.2	0.7
TL LL	11.6 – 7.8	3.8
Total BSL mmol/l	17.8 / 5 =3.56	

The above table shows the average blood sugar level that was reduced by Insulin 0.03IU/Kg (Mixtard)

Description	Initial BSL-Final BSL	BSL mmol/l
HD US	16.4-28	-11.6
TL RA	11.3-19.3	-8
TL US	13.2-13.2	0
HD RL	14.0-28	-14
TL	15.1-15.1	0
Total BSL mmol/l	-33.6 / 5= -6.72	

The above table shows the average blood sugar level that was reduced by Normal Saline 2ml/kg

Abberevation

T1D - Type 1 Diabetes
T2D - Type 2 Diabetes
HbA1c - Glycated hemoglobin
KIU-WC - Kampala International University-Western Campus
NIH - National Institute of Health
TL - Tail
HD US - Head Underside
TL RA - Tail Right arm
TL US - Tail Underside
HD RL - Head Right leg
RA LA - Right arm Left arm
BK RL - Back Right leg
US - Underside
TL LA RA - Tail Left leg Right arm
TL LL - Tail Left leg
BSL - Blood Sugar Level
US LL - Underside Left leg
HD TL US - Head Tail Underside
HD BK TL - Head Back Tail
US RL LL - Underside Right le Left leg
LA RL - Left arm Right leg
TL LA - Tail Left arm
LL - Left leg
US LA - Underside Left arm
LA LL - Left arm Left leg
TL RL - Tail Right leg
HD TL - Head Tail
HD LL - Head Left leg
US RA - Underside Right arm
BK LA - Back Left arm
BK LL - Back Left leg
US RL - Underside Right leg
RL LL LA RA - Right leg Left leg Left arm Right arm
HD LA - Head Left arm
RA LL - Right arm Left leg
HD RL LL - Head Right leg Left leg
HD - Head
LA - Left arm
BK - Back
RL - Right leg
RL RA - Right leg Right arm
BK RA - Back Right arm
TL LL RL - Tail Left leg Right leg
RA - Right arm
LL RL - Left leg Right leg

RESULT AND DISCUSSION

A.*esculentus* and V.*amygdalina* brought about their anti-diabetic activity by acting as an anti-oxidants where by they captured oxidants that destroyed the beta cells of the pancreas which is responsible for insulin production. Anti-oxidants can be found in anthraquinones. The phytochemical studies showed that they had lots of anthraquinones present in them. The research study showed that*V.amygdalina* is more potent than A. esculentus when given at a dose of 100mg/kg in agreement with a previous study (Khatun *et al*., 2011).

Increasing the dose of the two individual extracts showed an increase in the hypoglycemic effect on the rats and significant synergistic hypoglycemic effect was observed at 200: 50 mg/kg dosage probably due to high activity of *Vernoniaamygdalina* (Anastasia, 2010)

Combination of the two extracts at lower doses showed to be more effective than insulin (Mixtard) especially at week 3.

Mixtard insulin had significant effects which is in agreement with the general knowledge (Wilcox, 2005). Normal saline had no effects.

CONCLUSION

Aqueous extracts of *V. amygdalina and A. esculentus* leaves have higher synergistic hypoglycemic effect at lower doses. Combination of both extracts at lower doses was more efficacious than insulin after three weeks.

Reference

1. Leon lachman, herbert a. Liberman, the theory and practice of industrial pharmacy: p.293-302.
2. Robinson jr, lee v.h.l, controlled drug delivery: fundamentals and applications, 2nd edn. Marcel dekker, new york: (1978) p.24-36.
3. Brahmankard.m, jaiswals.b, biopharmaceutics and pharmacokinetics a treatise, 1st ed. Vallabhprakashan; newdelhi:(1995)p.64-70.
4. Cheiny.w, novel drug delivery systems, 2nd ed.: marcel dekker; new york: (1992) p.4-56.
5. Ansel, pharmaceutical dosage form and drug delivery system, lipincott, 7th edition: p. 553.
6. Gennaror.a.Remington,the science and practice of pharmacy., 20th ed. New york :lippincottwilliams: (2000) p.1045. Banker g.s, rhodes c.t, modern pharmaceutics. 3rd ed. Marcel dekker, new york: (1996) p.678-721.
7. Vyass.p, kharr.k, controlled drug delivery: concepts and advances, 1st ed. Vallabhprakashan, new delhi: (2002) p.345-376.

8. Shwetaarora, floating drug delivery: a review, aapspharmscitech., (2005): 47(11); p.268-272.
9. Libo yang, a new intragastric delivery system for the treatment of h.pylori associated with gastric ulcers, elsevier j. Of controlled release.,apr(1999): 34 (5); p. 215-222.
10. Ross and wilson, anatomy physiology and health education. 9th ed. Churchillivingston, p. 295-311.
11. Wilson k.r.w, waugh a. Anatomy and physiology in health and illness, 9th ed. Churchill livingstone: london: (1996). P. 342-345.
12. Garimachawla- a means to address regional variability in intestinal drug absorption: pharmtech., (2003) p.234-238.
13. Chawla g, gupta p, koradia v, bansal a, gastroretention: a means to address regional variability in intestinal drug absorption, pharm. Tech., (2003); p.50-68.
14. Desai s, bolton s. A floating controlled release system: in-vitro and in-vivo evaluation, Journal of Pharma Research., (1993): 10; p.1321-1325

How to cite this article:

Vempati Poornodai *et al* (2017) 'Synergistic Effects of Abelmoschusesculentus And Vernoniaamygdalina in Controlling Diabetes Mellitus in Rat Models', *International Journal of Current Advanced Research*, 06(08), pp. 5689-5694.
DOI: http://dx.doi.org/10.24327/ijcar.2017.5694.0780

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