ACUTE TOXICITY AND HYPOGYLCEMIC ACTIVITY OF *Plantago major* Linn. (A-kyaw-paung-tahtaung) ON EXPERIMENTAL ANIMALS

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Abstract

One of Myanmar indigenous medicinal plants, namely *Plantago major* Linn. (A-kyaw-paung-tahtaung) which is traditionally used for control of blood sugar level, has been chosen for pharmacological investigation. The acute toxicity (*in vivo* model) of dry *Plantago major* Linn. leaves has been investigated. The confidence dose of aqueous extract of the leaves of selected plant was determined by using DDY (Dutch Denken Yoken) mice. It was found that the median lethal dose (LD_{50}) was more than 10g/kg when administered orally. On the other hand, the anti-hyperglycemic activities of ethanolic and aqueous extracts were investigated in Wistar Strain rats. The animals treated with the ethanolic extract could not show significant decrease in blood glucose levels at 2 hr, 3 hr, 4 hr and 5 hr when compared with control group. Aqueous extract at dose of (1g/kg) significantly reduced blood glucose levels at 2 hr ($p<0.05$) and aqueous extract at (2g/kg) dose was observed at 2 hr ($p<0.01$) and 3 hr ($p<0.05$) after treatment of drug extracts respectively.

Introduction

Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents but also when they are used as starting materials for the synthesis of drugs or as models for pharmacologically active compounds (W.H.O, 1998). More herbal extracts have been produced from indigenous plants. Some of these medicines can now be used as substitutes for western drugs (W.H.O,1999).

Diabetes Mellitus is one of the chronic debilitating diseases that are regarded as incurable by Western medicine. A number of hypoglycemic agents (substances able to lower blood sugar level of diabetes) have been derived from plants. Many plants have long been used in different countries, usually in the form of decoctions, as folk remedies in the treatment of diabetes (Meir, P.et.al., 1985). In Myanmar, there are many medicinal plants for the treatment of diabetes (Mya, B. and Sein, G., 1967). Some of these plants have also been pharmacologically tested and shown to be valuable in diabetic treatment.

The World Health Organization says that by use of medicinal plants to treat diabetes is a topic that needs further research (Gray, A. M.,et.al., 2000). In this research, the leaves of *Plantago major* Linn. (A-kyaw-paung-tahtaung) was selected to examine acute toxicity on DDY mice and to investigate the hypoglycemic effect on adrenaline induced diabetic Albino rats. Since there is no such scientific studies conducted previous in Myanmar, this will be the first research paper on the hypoglycemic activity of *Plantago major* Linn.

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Therefore, hypoglycemic effect of ethanolic and aqueous extracts prepared from air dried leaves of *Plantago major* Linn. have been examined by using adrenaline induced hyperglycemic rat models at the Department of Medical Research (Upper Myanmar).

Acute Toxicity

Toxicology is the study of the adverse effects of chemicals on living organisms. The toxicologist is specially trained to examine the nature of these adverse effects and to access the probability of their occurrence.

Toxicity is the science of harmful effects of chemical on living organisms or is the science dealing with poison. To know the harmful effects of a new chemical, toxicity test must be evaluated first on the laboratory animals.

The first toxicity test performed on a new chemical is acute toxicity. The LD_{50} and other acute toxic effects are determined after one or more routes of administration (one route being oral or the intended route of exposure), in one or more species. The species most often used are the mouse and rat, but sometimes the rabbit and dog are employed (Klassen,C.D. and Amdur,M.O.,1995).

Acute toxicity investigations are required to supplement human experience in defining possible toxicity from short-term use.

The acute toxicity test was done to determine degree of toxicity when administration of the drug and to find out the median lethal dose (LD_{50}) of the drug. Usually the acute lethality of a compound is determined on the basis of deaths occurring in 24 hours but the survivors should be observed for at least 14 days in order to detect delayed effects (Loomis, J., 1968).

The acute toxicity give (1) a quantitative measure of acute toxicity (LD_{50}) for comparison to other substances, (2) identify the clinical manifestations of acute toxicity, and (3) give dose-ranging guidance for other studies.

Diabetes Mellitus

Diabetes is a condition in the body where the pancreas does not produce enough insulin to process glucose or the insulin receptor are not working properly. Insulin is a hormone that helps to move the glucose into the cell to produce energy. Inadequate insulin results in hyperglycemia (high blood glucose levels) and impaired glucose tolerance.

When glucose builds up in the blood instead of going into the cells, it results in damage and stress on the body. Immediately, the cells are starved for energy, which leaves the person feeling lethargic and weak and over an extendent period of time, high blood sugar levels may cause irreversible damage to eyes, kidneys, nerves and heart (Basic Diabetes Informaiton., 2002).

The symptoms of diabetes are increased blood sugar, increased thirst, unexplained weight loss, weakness, decreased blood pressure and blurred vision. Diabetes not only kills, but is also a major cause of adult blindness, kidney failure, gangrene, heart attacks and stokes.

Diabetes mellitus is one of "the six major priority diseases in Myanmar" such as malaria, tuberculosis (TB), diabetes mellitus, hypertension, diarrhoea and dysentery, and also one of the priority diseases of National Health Plan (National Health Plan, 1996-2001).

The treatment of diabetes may include a low sugar and carbohydrate diet accompanied with exercise for mild cases. For more severe and harder to control glucose levels, diabetes can be treated by the administration of insulin and or by sulfonylurea antidiabetic drugs that stimulate the production of insulin and therefore lower that blood glucose levels. Sulfonylurea antidiabetic drugs to work some cells of the pancreas and therefore are able to be stimulated to produce appropriate amount of insulin (Silverman, H., 1986).

Botanical Description

Family name	:	Plantaginaceae
Botanical name	:	*Plantago major* Linn.
Local name	:	A-kyaw-paung-tahtaung
English name	:	Greater Plantain
Parts used	:	Leaves

Medicinal Uses

Hypotensive activity (Aye Than, 1977)	_	a preliminary clinical trial was conducted by Prof. Dr. Tin May Nyunt, Consultant Physician, Thingangyun Sanpya Hospital in collaboration with Dr.Ohnmar May Tin Hlaing, Pharmacology Research Division, DMR (LM) (Paing Soe, DMR (LM), 2004).
Anti-ulcerogenic activity (Aye Than, et al., 1996)	–	a preliminary clinical trial tested in peptic ulcer patients (Thein Saw, et al., 2000)
Anti-inflammatory activity	–	tested in rat model, DMR (LM) (Aye Than, et al., 1996)
Diuretic activity	–	screening of diuretic activity *in vivo* animal model, DMR (LM) (Mu Mu Sein Myint, et al., 2000)
Anti-ascariasis activity	–	investigation on *Plantago major* Linn. plant against ascariasis, DMR (LM) (Thawka Kyin, 1976)
Other uses	–	hypoglycemic activity, dysentery, urinary disease, asthma, digestive ailments, analgesic, anti-oxidant.

Specific Objectives were

- To select and collect the indigenous medicinal plant which is traditionally used as antidiabetes herb.
- To prepare the aqueous and ethanolic extracts of *Plantago mojor* Linn.
- To examine whether the presence of acute toxicity in aqueous extract of *Plantago mojor* Linn.
- To investigate the hypoglycemic activity of aqueous and ethanolic extracts of *Plantago mojor* Linn. in albino rats.

Experimental

Sampling

The leaves of *Plantago major* Linn. (A-kyaw-paung-tahtaung) were collected from Pyin-Oo-Lwin Township, Mandalay Division. Firstly, the leaves were dried in the shade, cut into small pieces and made powder.

Acute Toxicity and Hypoglycemic Activity Tests

Preparation of Ethanolic and Aqueous Extracts of *Plantago major* Linn. Leaves

95% ethanol 0.8 L and 1 L of distilled water were used in these experiments. For testing the effect of *Plantago major* Linn. leaves on experimental animals, required amount of extract was taken and mixed with distilled water to get the required dosage of extract.

Acute Toxicity Study

A total of 40 DDY mice of both sexes (body weight 20-25 g) used in this study were randomly divided into four groups with 10 animals in each. Three were tested groups and one was control group. 10 albino mice in each group were fasted overnight before administration of the aqueous extract of *Plantago major* Linn. The acute toxicity study was carried out with an oral administration of aqueous extract of *Plantago major* Linn. at increasing doses of 2.5g/kg, 5g/kg and 10g/kg on the three test groups and 10ml/kg of distilled water on control group. After administering the extract orally, each group containing mice was kept in individual cage with free access to food and water and was observed toxic effects daily for two weeks. The median lethal dose (LD_{50}) was determined from the number of animals surviving at the end of two weeks period (Litchfield, J. T and Wilcoxon, F. A., 1949).

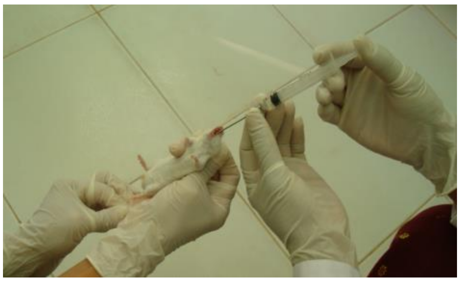

Figure 1 - Administration of Drug Solution to the Experimental Mice

Screening of Experimental Animals for Hypoglycemic Activities

This experiment was done two weeks before starting experiment. Wistar Strain albino rats of both sexes (weighing between 170 to 220 g) were obtained from Laboratory Animal Services Division, Department of Medical Research, Upper Myanmar. They were fed the standard diet and water. Then they were kept in clean and dry cages and maintained in a well-ventilated animal house. The rats were fasted overnight (18 hours) before the commencement of the experiment but water was allowed orally.

Blood Sampling

After animals were made fasting for 18 hours, tails were cut about 1 mm length. Blood was collected on the test strip by tail nipping and blood sugar level reading was done by glucometer which was expressed in mmol/L.

Preparation of Adrenaline Induced Hyperglycemic Albino Rat

Albino rats were induced by subcutaneous injection of adrenaline 0.2 ml/kg body weight and 1 hour post injection fasting blood sugar levels were measured. Fasting blood sugar levels equal and above 8 mmol/L were selected for the study and those who did not respond the induction were rejected. And then these albino rats were allowed to take rest for two weeks with standard food and water.

Test of Hypoglycemic Effect of *Plantago major* Linn. Leaves on the Albino Rats

Method

After resting of two weeks, a total of 36 fasted adrenaline induced hyperglycemic rats were randomly divided into six groups each having six animals. Rats were fasted for 18 hours prior to drug administration allowing access only to water. Before the drugs and vehicle administration, baseline fasting blood sugar levels were measured with Omnitest glucometer in all six groups.

In this experiment, the dosage of aqueous and ethanolic extracts of *Plantago major* Linn. was calculated on the body weight basis for each animal. Before giving the extract to the animals, the extract was dissolved in distilled water. After that following procedures were done.

*__Group I & II__ were given a single oral administration of ethanolic extract of leaves of *Plantago major* Linn. (suspended in distilled water) at doses of 1 g/kg and 2 g/kg body weight respectively.

***Group III & IV** were given a single oral administration of aqueous extract of leaves of *Plantago major* Linn. (suspended in distilled water) at doses of 1g/kg and 2g/kg body weight.

*__Group V__ received a single oral administration of glibenclamide at a dose of 0.5mg/kg body weight and it served as a standard.

*__Group VI__ served as an antihyperglycemic control group and received 10ml/kg of distilled water only.

Adrenaline (0.2ml/kg) was injected subcutaneously to all rats at 1 hour after the administration of distilled water or standard drug (glibenclamide) or extract. Blood sampling was taken at 1 hour, 2 hours, 3 hours and 4 hours after adrenaline injection. Blood sugar estimation was done by glucometer at 2 hours, 3 hours, 4 hours and 5 hours after administration of distilled water or drug extracts and recorded the results separately and safely. Food and water were withheld during experiment (Tin Tin Thein,et al.,2008).

Statistical Analysis

The mean difference among the blood glucose levels of control and experimental rats were analyzed by unpaired "t" test. The results were expressed as Mean ± SE. Differences between groups were considered as significance when $p<0.05$.

(i) (ii) (iii) (iv)

Figure (2) - i) Wister Strain Rats of Both Sexes
ii) Administration of Drug Solution to the Experimental Rats
iii) Induction of Blood Glucose Level by Subcutaneous Injection of Adrenaline to the Experimental Rats
iv) Collection of Blood Samples at 2, 3, 4 and 5 hrs after Administration of Drug Extract for Determination of Blood Glucose Levels

Results and Discussions

Acute Toxicity of *Plantago major* Linn. Extract in Albino Mice

The mice adminstered with 2.5g/kg, 5g/kg and 10g/kg doses of aqueous extract of *Plantago major* Linn. leaves were kept under observation for two weeks. After two weeks, all the mice were alived and did not show any toxic symptoms such as body weight loss and restlessness. So, it was found that 10g/kg dose of aqueous extract of *Plantago major* Linn. showed confidence dose and considered as safe.

Therefore it was concluded that the median lethal dose (LD_{50}) was more than 10g/kg when administered orally.

Effect of Ethanolic Extract of *Plantago major* Linn. on Blood Glucose Levels in Adrenaline Induced Hyperglycemic Rats

The mean blood glucose levels of adrenaline induced hyperglycemic rats in control group, ethanolic extract treated groups and standard drug treated group are shown in Table (1).

Table (1) Hypoglycemic Effect of Ethanolic Extracts of *Plantago major* Linn. Leaves on Blood Glucose Levels (Mean ± SE) of Adrenaline Induced Hyperglycemic Rat Models (Swe Swe, et.al., 2008)

Group (n=6)	Blood glucose level (mmol/L)				
	0 hr	2 hr	3 hr	4 hr	5 hr
Distilled water (control) (10ml/kg)	5.17±0.26	12.02±1.55	11.13±1.57	8.27±0.81	5.87±0.43
Ethanolic extract (1g/kg)	6.18±0.08	11.15±1.05	10.98±0.99	9.25±0.89	7.16±0.66
Ethanolic extract (2g/kg)	5.67±0.14	10.57±0.41	12.48±1.41	9.80±0.95	7.68±0.67
Glibenclamide (standard) (0.5mg/kg)	5.93±0.19	10.95±0.79	9.28±0.42	6.25±0.84	4.58±0.49

According to this table, the animals treated with the ethanolic extracts could not show significant decrease in blood glucose levels at 2 hr, 3 hr, 4 hr and 5 hr when compared with control group.

Effect of Aqueous Extracts of *Plantago major* Linn. on Blood Glucose Levels in Adrenaline Induced Hyperglycemic Rats

The mean blood glucose levels of adrenaline induced hyperglycemic rats in control group, aqueous extract treated groups and standard drug treated group are shown in Table (2).

Table (2) Hypoglycemic Effect of Aqueous Extracts of *Plantago major* Linn. Leaves on Blood Glucose Levels (Mean ± SE) of Adrenaline Induced Hyperglycemic Rat Models

Group (n=6)	Blood glucose level (mmol/L)				
	0 hr	2 hr	3 hr	4 hr	5 hr
Distilled water (control) (10ml/kg)	5.17±0.26	12.02±1.55	11.13±1.57	8.27±0.81	5.87±0.43
Aqueous extract (1g/kg)	4.95±0.19	8.11±0.78*	8.75±1.02	7.83±0.77	6.33±0.61
Aqueous extract (2g/kg)	5.05±0.18	7.00±0.68**	7.05±0.83*	6.26±0.44	5.57±0.27
Glibenclamide (standard) (0.5mg/kg)	5.93±0.19	10.95±0.79	9.28±0.42	6.25±0.84	4.58±0.49

* denotes $p < 0.05$ ** denotes $p < 0.01$

According to this table, the animals treated with the aqueous extracts showed decrease in blood glucose levels at 2 hr, 3 hr, 4 hr and 5 hr when compared with control group.

Aqueous extract at (1g/kg) dose provided significantly decrease in blood glucose levels at 2 hr ($p<0.05$) and aqueous extract at (2g/kg) dose provided prominantly decrease in blood glucose level at 2 hr ($p<0.01$) and 3 hr ($p<0.05$) after administration of drug extract.

The significantly decrease in blood glucose levels of aqueous extract (1g/kg) was observed at 2 hr ($p<0.05$) and aqueous extract (2g/kg) was observed at 2 hr ($p<0.005$) and 3 hr ($p<0.05$) when compared with glibenclamide.

The hypoglycemic effect of the aqueous extracts (1g/kg and 2g/kg), are shown in Figure (3) and (4). For comparison purpose, the blood glucose level of control group and glibenclamide treated group are also presented.

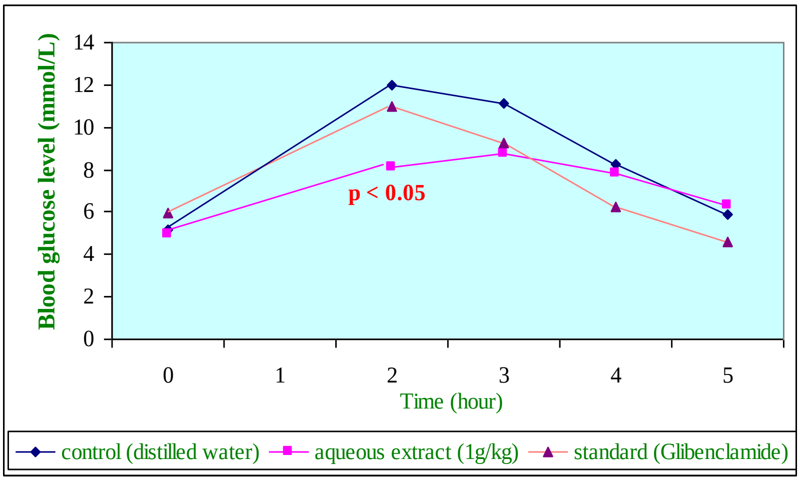

Figure (3) Hypoglycemic Effect of Aqueous Extract (1 g/kg) of *Plantago major* Linn., Standard and Control Group

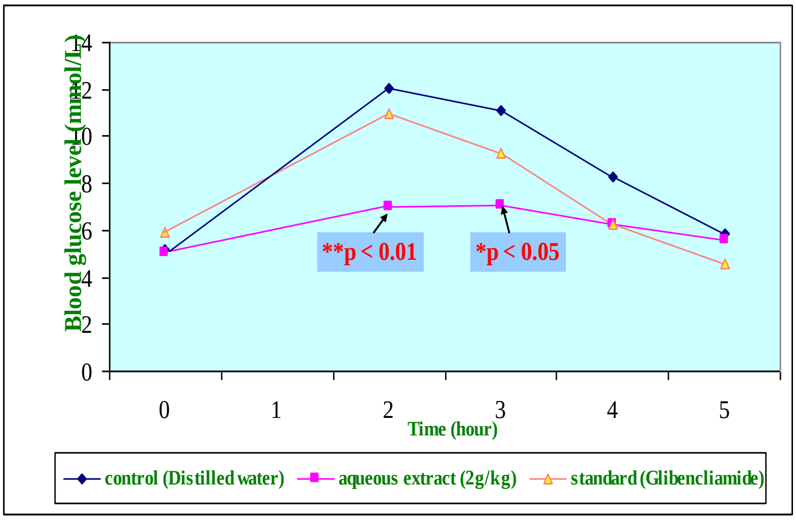

Figure (4) Hypoglycemic Effect of Aqueous Extract (2 g/kg) of *Plantago major* Linn., Standard and Control Group

Comparison of Percent Inhibition Effect of the Glibenclamide and Aqueous Extract of *Plantago major* Linn. Leaves

Percent inhibition was calculated by the following formula.

$$\text{Percent inhibition of tested drug} = \frac{\text{Blood glucose level of (control-tested drug)}}{\text{Blood glucose level of control}} \times 100\,\%$$

Percent inhibition of glibenclamide, high and low dose of aqueous extract of *Plantago major* Linn. leaves were calculated and shown in Table (3).

Table (3) Percent Inhibition of Glibenclamide and Aqueous Extract (1g/kg and 2g/kg)

Type of drug	2 hour (%)	3 hour (%)	4 hour (%)
Glibenclamide(0.5 mg/kg)	8.9	16.6	24.4
Aqueous extract (1g/kg)	32.5	21.4	5.3
Aqueous extract (2g/kg)	41.8	36.7	24.3

According to this table, the percent inhibition of aqueous extract (2g/kg) dose was higher than that of glibenclamide and aqueous extract (1g/kg) dose at 2 hr and 3 hr respectively. Maximum inhibition was found at 2 hr for aqueous extract (2g/kg) dose as shown in Figure (5).

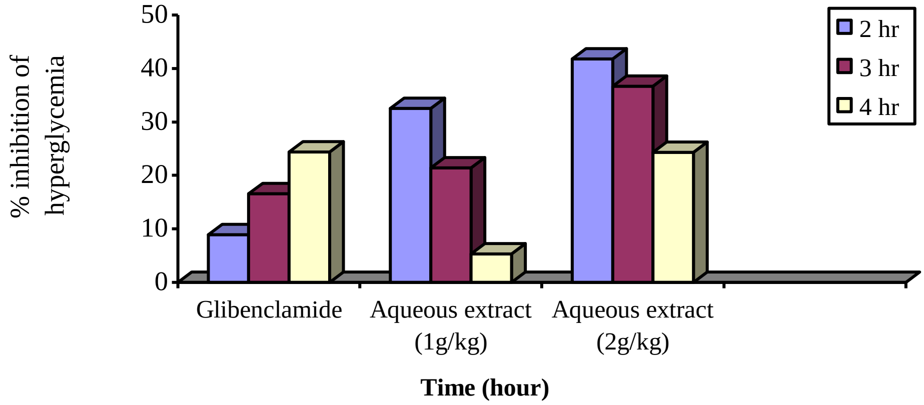

Figure (5) Times Course of the Percent Inhibition Effect of Glibenclamide, Aqueous Extract (1g/kg) and Aqueous Extract (2g/kg) on Adrenaline Induced Hyperglycemic Rat Models.

Conclusion

In this research work, the leaves of *Plantago major* Linn. was selected for acute toxicity and hypoglycemic activities. Acute toxicity study of 10 g/kg of aqueous extract of *Plantago major* Linn. leaves on albino mice did not cause any death within 24 hours and for two weeks. So, it was found that 10g/kg dose of aqueous extract of *Plantago major* Linn. showed confidence dose and considered as safe. Hypoglycemic activities of ethanolic and aqueous extracts of *Plantago major* Linn. leaves were investigated on experimental animals. The animals treated with ethanolic extracts dose at (1g/kg and 2g/kg) could not show significant decrease in blood glucose levels but aqueous extracts dose at (1g/kg and 2g/kg) showed significant decrease in blood glucose levels. In addition, results of aqueous extracts with $p < 0.05$ were statistically significant. Therefore, aqueous extract of leaves of *Plantago major* Linn. can be used in the treatment of diabetes.

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References

Aye Than (1977). "Pharmacological investigations of *Plantago major* extracts." M.Sc Thesis (Zoology) Rangoon Arts and Sciences University, 173-178.

Aye Than, Mu Mu Sein Myint, Win Myint, & Su Su Hlaing . (1996). " The anti-ulcerogenic activity of *Plantago major* Linn." *The Myanmar Health Sciences Research Journal*, 8(2): 74-77.

Aye Than, Mu Mu Sein Myint, Tin Myint and Win Myint. (1996). " Anti-oedema activity of *Nyctanthes arbortritis* L., *Curcuma longa* L. and *Plantago major* L.", *The Myanmar Health Sciences Research Journal*; 8(2); 36-40.

Basic Diabetes Information. (2002) "American Diabetes Association." May 3, www. Diabetes org.

Gray, Alison M., Y.H.A.Ab del. Watab, and P.R Flatt. (2000). " The traditional plant treatment, *Sambucus Nigra* (elder), exhibits insulin-like and insulin-releasing actions *in vitro*," *Journal of Nutrition*, 130: 15-20.

Klassen,C.D,Amdur,M.O.(1995). "Casarett and Doull's Toxicity." The basic Science of Poisons, edited by John Doull.

Litchfield, J. T & Wilcoxon, F.A (1949). "Simplified method of evaluating dose-effect experiments." *Journal of Pharmacology and Experimental Therapeutics*; 96:99-113.

Loomis Ja (1968). "Essentials of Toxicology Lea and Fehiger, Philadelphia." pp. 144-146.

Meir, P and Yaniv, 2- (1985). "An *in Vitro* Study on the Effect of *Momardica charantia* on Glucose Uptake and Glucose Metabolism in Rats," *Planta Med*, Vol 46, 12-16.

Mu Mu Sein Myint, Aye Than, Aye Aye Thein, Win Win Maw, Kyi Kyi Myint & San San Myint. (2000). "Screening of diuretic activity of medicinal plant extracts on *in vivo* animal model." Myanmar Health Research Congress, Programme and abstracts, p-44.

Mya Bwint and Sein Gwan (1967). "Plants with reputed hypoglycemic action, In: Burmese Indigenous Medicinal plants," Special report series No.4, Burma Medical Research Institute, 48-51.

National Health Plan (1996-2001). "Planning Document Series 3", Forum on Health Sector Development, Ministry of Health, Department of Planning and Statistics.

Paing Soe, DMR (LM). (2004). "The New Light of Myanmar, Tuesday, 14 December."

Silverman, Harold (1986). "The Pill Book, 9th Edition", Pill Book Company, New York.

Swe Swe, Ei Ei Htway, Khin May Thi, Zaw Win Tun, Tin Moe Khaing, Aye Aye Phyu, Than Than Maw, Ni Ni Aung & Tin Tin Thein (DMR, UM). (2008). "Acute Toxicity and Hypoglycemic Effect of *Ocimum sanctum* Linn."

Thawka Kyin (1976). "Investigation on some Burmese indigenous plants against ascariasis." M.Sc Thesis. Rangoon Arts and Science University, 66-69.

Thein Saw, Myo Khin, Win Myint, Than Than Swe, Aye Than & Kyaw Swe (2000). " The potential usefulness of *Plantago major* Linn. in management of peptic ulcer." Myanmar Health Research Congress Programme and abstracts, p-96.

Tin Tin Thien, Maung Maung, Khin Phyu Phyu, Hein Myo Htet, Swe Swe, Myint Myint Khaing & Mee Mee Lwin (DMR,UM) (2008). "Experimental study on hypoglycemic activity of ethanolic extract of *Andrographis paniculata* Nees. On rats." *Myanmar Health Sciences Research Journal*, Vol.20, No.3

W.H.O (1998) "Regulatory Situduation of Herbal Medicines", 1-4.

W.H.O (1999) "W.H.O Monographs on selected Medicinal Plants" Geneva, 1.