CHAPTER 3

RESULTS

The results of the study were divided into 6 parts as follows:

Part 1: Collection/Preparation of crude extracts and bioactivity screening tests

Part 2: Hypoglycemic screening of Thai/Nigerian medicinal plant extracts

Part 3: Fractionation and hypoglycemic activity of the Thai medicinal plant extracts and hypoglycemic tests of the sub-fractions obtained

Part 4: Fractionation and hypoglycemic activity of the Nigeria medicinal plant extracts and hypoglycemic tests of the sub-fractions obtained

Part 5: Extraction, isolation, purification and structure elucidation of the selected sub-fractions

Part 6: Bioactivities of the isolated compounds

Part 1: Collection of anti-hyperglycemic medicinal plants

3.1 Thai anti-hyperglycemic medicinal plants

The Thai hypoglycemic medicinal plants were selected from the Thai/Lanna medicinal plant recipe database (MANOSROI II) in the Natural Product Research and Development Center (NPRDC), Faculty of Pharmacy, Chiang Mai University, Thailand. The database has been compiled with research over a ten year period and work is still in progress. The medicinal plants were selected based on their traditional

usages, especially for diabetes mellitus. Eight (8) hypoglycemic medicinal plants were chosen after database consultation and the plants were obtained with the help of NPRDC, J. F. Maxwell and Mr. Sampan of the Faculty of Pharmacy Botanical garden, Chiang Mai University, Chiang Mai, Thailand.

3.2 Nigerian hypoglycemic medicinal plants

The selection of hypoglycemic medicinal plants from the North Eastern part of Nigeria was done after series of inquiries from within the vicinity of Mubi town and field research beginning from Mubi to Yola, Jalingo, Maiduguri and Gworza (Fig 3.1). Being the first of such field research on the traditional hypoglycemic medicinal plants of the area, the main problem encountered, is non co-operation of traditional medicine practitioners. This is because majority of those contacted are into the profession as a means of livelihood, hence their reluctance to cooperate. Though no written documents exists in the area but it is known that such knowledge are transferred orally within families or master to apprentices.

The selection of the medicinal plants was done based on their popularity and in most cases testimonies of individuals. Of the places visited similar medicinal plants were observed to be used in different towns and villages (probably because of similarities in culture and proximity to each other), hence the selection of 10 out of several medicinal plants.

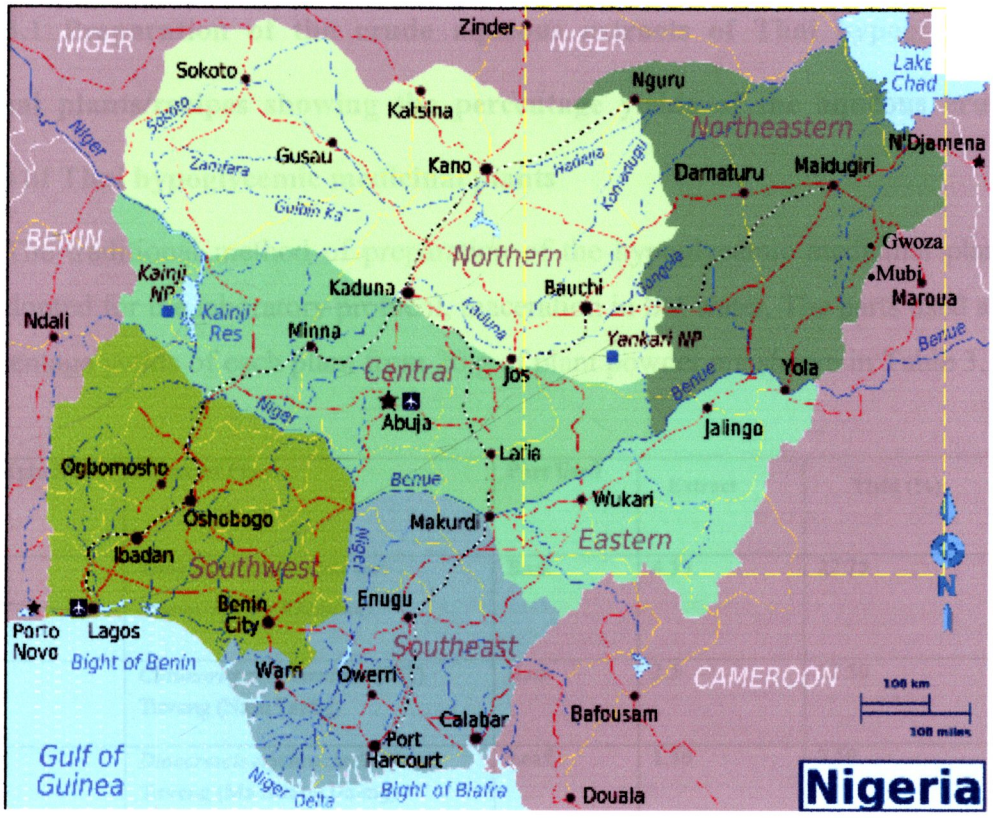


Figure 3.1 Map of Nigeria showing the North Eastern part (marked in yellow), showing the areas from which the hypoglycemic medicinal plants were obtained. Towns visited were Maiduguri, Gwoza, Mubi, Yola and Jalingo (Source: http://www.worldofmaps.net).

3.3 Preparation of the crude extract and bioactivity screening test

Table 3.1 Preparation of the crude aqueous extracts of Thai hypoglycemic medicinal plants/recipes showing the percentage yields of the aqueous crude extracts of Thai hypoglycemic medicinal plants

The traditional method of preparation of the hypoglycemic medicinal plants was adopted for the laboratory protocol, maceration in hot water. The parts used and the percentage yields of each plant from 20 g of plant powder are shown in Table 3.1.

Sample No.	Name of Plant	Part Used	Extract	Yield (%)
1.	*Anogeissus acuminata var. lanceolata (Ta Khian nu)*	Bark	4.55	22.75
2.	*Catunaregam tomentosa* (DC.) Tirveng (Nam Taeng)	Bark	2.5	12.50
3.	*Dioecrescis erythroclada* (Kurz) Tirveng (Ma Khang Daeng)	Leaf	1.50	7.50
4.	Yamed Boraped Pungchang®	Recipe	2.95	14.75
5.	Mai Tau Lusi	Recipe	2.04	10.20
6.	*Dendrophthoe pentandra* (L.) Miq (Ka Fak Ma Muang)	Leaf	4.95	24.75
7.	*Mimosa pudica Linn. var. hispida* Bren (Mai Ya Rab)	Leaf	1.83	9.15
8.	*Moringa oleifera* (Ma room)	Root	4.6	23.00
9.	*Pterocarpus macrocarpus* kurz (Pra Doo)	Bark	0.4	2.00
10.	*Rauwolfia serpentina* Benth (Rayom)	Leaf	5.1	25.50

Percentage extraction = Fraction obtained/Total fraction used x 100

Table 3.2 Preparation of the crude aqueous extracts of hypoglycemic medicinal plants/recipes showing the percentage yields of the aqueous crude extracts of Nigerian hypoglycemic medicinal plants

The traditional method of preparation of the hypoglycemic medicinal plants was adopted for the laboratory protocol, maceration in hot water. The parts used and the percentage yields of each plant from 20 g of plant powder are shown in Table 3.2.

Sample No.	Name of Plant	Part Used	Extract	Yield (%)
1.	*Anisopus mannii* N.E.Br (Kashe zaki)	Leaf	2.95	14.75
2.	*Anogeissus leiocarpus* (D.C) Guill & Perr. (Mareke)	Bark	6.60	33.00
3.	*Daniella Oliveri* (Rolfe) Hutch & Dalz. (Maje)	Leaf	5.01	25.05
4.	*Detarium macrocarpum* Harms (Taura)	Bark	3.43	17.15
5.	*Ficus Thonningii.* Blume, (chediya), *Raphia vinifera* P. Beauv, (kimba), *Leptedenia hastata* (Pers) Dec'ne (Yaa diya)	Recipe	3.26	16.30
6.	*Leptedenia hastata* (Pers) Dec'ne (Ya diya)	Leaf	4.61	23.05
7.	*Mimosa invisa* var. inermis Adelb (Idon zakara)	Leaf	2.20	11.00
8.	*Moringa oleifera* (Zogale)	Root	3.10	15.50
9.	*Pterocarpus erinaceus* (Madobiya)	Bark	2.20	11.00
10.	*Rauwolfia serpentina* (Ganyen Ghana)	Leaf	4.10	20.50

Percentage extraction = Fraction obtained/Total fraction used x 100

Table 3.3 The percentage yield of the methanol and chloroform extracts of the selected Thai and Nigerian medicinal plant

After testing all the aqueous extracts (10 Thai and 10 Nigerian hypoglycemic medicinal plants in normoglycemic and alloxan induced diabetic mice in a dose dependant trial, *Anogeissus acuminata* (Thai medicinal plant) and *Anisopus mannii* (Nigerian medicinal plant) were observed to have the highest hypoglycemic effect, hence they were chosen for fractionation after extraction with methanol and then partitioned between chloroform and methanol. The results were shown in Table 3.3.

Percentage yield of methanol and chloroform extracts of the selected extracts

Name of Plant	Part Used	Crude Methanol (%)	Sub extracts	
			Chloroform (%)	Methanol (%)
Anogeissus acuminata var. lanceolata (Ta Khian nu)	Bark	18.05	1.39	16.58
Anisopus mannii N.E.Br (Kashe zaki)	Leaf	19.89	10.47	9.35

3.4 The phytochemistry of Thai and Nigerian medicinal plants

The phytochemical analysis of the aqueous extracts of the hypoglycemic medicinal plants of Thai origin is shown on Table 3.3.1. The intensities of the phytochemicals analyzed vary from one plant to another and with constituents. Medicinal plant whose bark were used and as well as recipes showed high intensities of alkaloid except *R. serpentina* of which the leaves only was used.

Table 3.3.1 Phytochemistry of Thai medicinal plants (aqueous extracts)

Name of Plant	Part Used	Anthra quinones	Tannins	Saponins	Xanthones	Flavones	Glycosides	Alkaloids
Anogeissus acuminata var. lanceolata (Ta Khian Nu)	Bark	-	+++	++	+++	+	+++	+++
Catunaregam tomentosa (DC.) Tirveng (Nam Taeng)	Bark	-	+	+	+	+	+	+++
Dioecrescis erythroclada (Kurz) Tirveng (Ma Khang Daeng)	Leaf	-	+	+	++	+	+	++
Yamed Boraped Pungchang®	Recipe	-	+++	+++	-	-	-	++
Mai Tau Lusi	Recipe	-	+	++	++	++	+++	+++
Dendrophthoe pentandra (L.) Miq (Ka Fak Ma Muang)	Leaf	+	+++	++	+	+	++	++
Mimosa pudica Linn. var. hispida Bren (Mai Ya Rab)	Leaf	+	-	+	+	-	+	++
Moringa oleifera (Ma room)	Root	-	+	-	+	-	++	++
Pterocarpus macrocarpus kurz (Pra Doo)	Bark	-	-	-	-	-	++	++
Rauwolfia serpentina Benth (Rayom)	Leaf	++	+++	-	++	+	++	+++

Note: (+), (+ +) and (+ + +) represent intensity observed; (-) represent negative.

Tannins, Saponins, Xanthones and Glycosides were observed in high intensities in *A. acuminata, C. tormentosa, D. pentandra*, Yamed Boraped

Pungchang, Mai Tau Lusi and *R. serpentina*. Anthraquinones was mildly present in *R. serpentina*.

The phytochemical analyses of the methanolic extracts of the hypoglycemic medicinal plants of Thai origin are shown in Table 3.3.2. The phytochemical intensities of the extracts are shown to be higher than in the aqueous extracts. Extracts of *D. pentandra, M. pudica, M. oleifera and P. macrocarpus* showed much improved phytochemical constituents while anthraquinones was observed in higher intensity than in the aqueous extract. The alkaloid constituents were lower than in the aqueous extract except in the *R. serpentina*.

Table 3.3.2 Phytochemistry of Thai Medicinal Plants (Methanol Extracts)

Medicinal Plant Extracts	Part Used	Anthra quinones	Flavones	Glycosides	Saponins	Xanthones	Tannins	Alkaloids
Anogeissus acuminata var. *lanceolata* (Ta Khian Nu)	Bark	-	+	+ + +	+	+ + +	+ + +	+
Catunaregam tomentosa (DC.) Tirveng (Maj Teen)	Bark	+	-	+	-	+	+	+
Dioecrescis erythroclada (kurz) Tirveng (Ma Khang Daeng)	Leaf	-	+	+	-	+	+	-
Yamed Boraped Pungchang®	Recipe	-	+ +	+ +	-	-	-	+ +
Mai Tau Lusi®	Recipe	-	+	+ +	-	+	+ +	-
Dendrophthoe pentandra (L.) Miq (Ka-Fak-Ma-Muang)	Leaf	+	-	+ +	+	+ +	+ + +	+
Mimosa pudica Linn. *Var. hispida* Bren. (Mai-Ya-Rab)	Leaf	+	-	+ +	-	+ +	+	+
Moringa oleifera (Ma-room)	Root	-	-	+ +	-	-	+	+
Pterocarpus macrocarpus Kurz (Pra-Doo)	Bark	-	+	+ +	-	+	+	-
Rauwolfia serpentina Benth (Rayom)	Leaf	+ + +	+ +	+ + +	+	-	+ +	+ + +

Note: (+), (+ +) and (+ + +) represent intensity observed; (-) represent negative.

Table 3.3.3 Phytochemistry of Nigerian medicinal plants (aqueous extracts)

The phytochemical constituents of the aqueous extracts of hypoglycemic medicinal plants of Nigerian origin were shown in Table 3.3.3. High intensities of saponin, tannins, xanthones, glycosides were observed in *A. mannii, A. leiocarpus, D. macrocarpum* and *M. invisa* as shown.

Table 3.3.3 Phytochemistry Nigerian medicinal plants (aqueous extracts)

Name of Plant	Part Used	Anthra quinones	Tannins	Saponins	Xanthones	Flavones	Glycosides	Alkaloids
Anisopus mannii N.E.Br (Kashe zaki)	Leaf	-	+	+ + +	-	-	+	+
Anogeissus leiocarpus (D.C) Guill & Perr. (Mareke)	Bark	-	+ + +	+ +	+	+	+	-
Daniella Oliveri (Rolfe) Hutch & Dalz. (Maje)	Leaf	+	+	+ +	-	+ +	+ +	+
Detarium macrocarpum Harms (Taura)	Bark	-	+ + +	+	+ + +	+ +	+	+
Ficus Thonningii. Blume, (chediya), *Raphia vinifera* P. Beauv, (kimba), *Leptedenia hastata* (Pers) Dec'ne (Yaa diya)	Recipe	-	+ +	+	+	+	+ +	-
Leptedenia hastata (Pers) Dec'ne (Ya diya)	Leaf	+	+	+	-	+	+	-
Mimosa invisa var. inermis Adelb (Idon zakara)	Leaf	-	+ +	+ +	-	+ +	+ + +	+
Moringa oleifera (Zogale)	Root	-	+	+	-	-	+ +	+
Pterocarpus erinaceus (Madobiya)	Bark	-	+ +	+ +	+	+ +	+	-
Rauwolfia serpentina (Ganyen Ghana)	Leaf	+	+	+ +	+	+	+ +	+

NB: (+), (+ +) and (+ + +) represent intensity observed; (-) represent negative

Table 3.3.4 Phytochemistry of Nigerian medicinal plants (methanolic extracts)

The phytochemical constituents of the methanolic extracts of the hypoglycemic medicinal plants of Nigerian origin were shown in Table 3.3.4. Three fold intensities observed with all the phytochemicals except anthraquinones and alkaloids. The chemical intensities were stronger than in the aqueous extracts.

Table 3.3.4 Phytochemistry of Nigerian medicinal plants (methanolic extracts)

Medicinal Plant Extracts	Part Used	Anthra quinones	Flavones	Glycosides	Saponins	Xanthones	Tannins	Alkaloids
Anisopus mannii N.E.Br (Kashe Zaki)	Leaf	-	-	+	+ + +	-	+	+
Anogeissus leiocarpus (DC.) Guill. & Perr.	Bark	-	+ + +	+	+ +	+ +	+ + +	-
Daniella oliveri (Rolfe) Hutch & Dalz.	Leaf	-	+ + +	+ +	-	-	+ +	+
Detarium macrocarpum Harms	Bark	-	+ +	+	+ +	+ + +	+ + +	+
Ficus thonningii Blume, *Raphia vinifera* P. Beauv, *Leptedenia hastata* (Pers.) Dec'ne.	Recipe	+	-	+ +	-	-	+ +	-
Leptedenia hastata (Pers.) Dec'ne (Ya'a diya).	Leaf	+	-	+	-	-	+ +	-
Mimosa invisa var. inermis Adelb (Idon zakara).	Leaf	+	+ + +	+ + +	+	-	+ + +	+
Moringa oleifera (Zogale)	Root	-	-	+ +	-	-	+	+
Pterocarpus erinaceus (Madobiya)	Bark	+	+ +	+	+	+	+ +	-
Rauwolfia serpentina (Ganye Ghana)	Leaf	+	-	+ +	-	+ +	+ +	+

NB: (+), (+ +) and (+ + +) represent intensity observed; (-) represent negative

3.5 Free radical scavenging activity (DPPH) of Thai and Nigeria medicinal plants

Table 3.4.1 SC_{50} values of the aqueous extracts of Thai hypoglycemic medicinal plants comparing with the reference compound, ascorbic acid

Spectrophotometery was used to determine the free radical scavenging activity (hydrogen donating ability) of the aqueous extracts of the Thai hypoglycemic medicinal plants. The SC_{50} of ascorbic acid was used to compare with those of the extracts and the lower SC_{50} (mean ± S.E.M) values, indicated higher activity compared to the standard (Table 3.4.1).

Table 3.4.1 SC_{50} values of the aqueous extracts of Thai medicinal plants

Medicinal Plant (Common Name)	Part Used	SC_{50} mean ± *s.e.m* (mg/ml)	Fold/Activity
Anogeissus acuminata var. lanceolata (Ta Khian Nu)	Bark	0.011 ± 0.000	5.272
Catunaregam tomentosa (DC.) Tirveng (Maj Teen)	Bark	0.292 ± 0.002	0.198
Dioecrescis erythroclada (Kurz) Tirveng (Ma Khang Daeng)	Leaves	0.063 ± 0.001	0.920
Yamed Boraped Pungchang®	Recipe	0.111 ± 0.001	0.522
Mai Tau Lusi	Recipe	0.098 ± 0.001	0.591
Dendrophthoe pentandra (L.) Miq (Ka Fak Ma Muang)	Leaves	0.059 ± 0.001	0.983
Mimosa pudica Linn. var. hispida Bren (Mai Ya Rab)	Leaves	0.136 ± 0.003	0.426
Moringa oleifera (Ma Room)	Root	0.051 ± 0.000	1.137
Pterocarpus macrocarpus kurz (Pra Doo)	Bark	0.051 ± 0.001	1.137
Rauwolfia serpentina Benth (Rayom)	Leaves	0.062 ± 0.003	0.935
Ascorbic acid	-	0.058 ± 0.003	1.0

Note: SC_{50}: Concentration of plant extract showed 50% DPPH scavenging activity, the experiments were performed in triplicate.

Table 3.4.2 SC_{50} values of the methanol extracts of Thai hypoglycemic medicinal plants comparing with the reference compound, tocopherol

Spectrophotometery was used to determine the free radical scavenging activity (hydrogen donating ability) of the aqueous extracts of the Thai hypoglycemic medicinal plants. The SC_{50} of tocopherol was used to compare with those of the extracts and the lower SC_{50} (mean ± S.E.M) values, indicated higher activity compared to the standard (Table 3.4.2).

Table 3.4.2 SC_{50} values of methanol extracts of Thai medicinal plants

Medicinal Plant (Common Name)	Part Used	SC_{50} mean ± *s.e.m*	Fold/Activity
Anogeissus acuminata var. lanceolata (Ta khian nu)	Bark	0.007 ± 0.000	7.285
Catunaregam tomentosa (DC.) Tirveng (maj teen)	Bark	0.031 ± 0.003	1.645
Dioecrescis erythroclada (Kurz) Tirveng (Ma khang daeng)	Leaves	0.066 ± 0.001	0.772
Yamed Boraped Pungchang®	Recipe	0.054 ± 0.001	0.944
Mai Tau Lusi	Recipe	0.049 ± 0.000	1.040
Dendrophthoe pentandra (L.) Miq (Ka fak ma muang)	Leaves	0.029 ± 0.003	1.758
Mimosa pudica Linn. var. hispida Bren (Mai ya rab)	Leaves	0.296 ± 0.002	0.172
Moringa pterygosperma C. F. Gaertner (Ma room)	Root	0.189 ± 0.001	0.269
Pterocarpus macrocarpus kurz (Pra doo)	Bark	0.029 ± 0.001	1.758
Rauwolfia serpentina Benth (Rayom)	Leaves	0.070 ± 0.002	0.729
Tocopherol		0.051 ± 0.001	1.0

Note: SC_{50}: Concentration of plant extract showed 50% DPPH scavenging activity. All experiments were performed in triplicate.

Table 3.4.3 SC_{50} values of the aqueous extracts of Nigerian hypoglycemic medicinal plants comparing with the reference compound, ascorbic acid

Spectrophotometery was used to determine the free radical scavenging activity (hydrogen donating ability) of the aqueous extracts of the Thai hypoglycemic medicinal plants. The SC_{50} of ascorbic acid was used to compare with those of the extracts and the lower SC_{50} (mean ± S.E.M) values, indicated higher activity compared to the standard (Table 3.4.3).

Table 3.4.3 SC_{50} values of the aqueous extracts of Nigerian medicinal plants

Medicinal Plant (Common Name)	Part Used	SC_{50} mean ± *s.e.m* (mg/ml)	Fold/Activity
Anisopus mannii N.E.Br (Kashe zaki)	Leaves	0.190 ± 0.002	0.31
Anogeissus leiocarpus (D.C) Guill & Perr.(Mareke)	Bark	0.028± 0.009	2.07
Daniella Oliveri (Rolfe) Hutch & Dalz.(Maje)	Leaves	0.050 ± 0.007	1.16
Detarium macrocarpum Harms (Taura)	Bark	0.027 ± 0.001	2.15
Ficus Thonningii. Blume. , *Raphia vinifera* P. Beauv, *Leptedenia hastata* (Pers) Dec'ne (Maganin Ciwon suga)	Recipe	0.066 ± 0.007	0.88
Leptedenia hastata (Pers) Dec'ne (Ya diya)	Leaves	0.118 ± 0.002	0.50
Mimosa invisa var. inermis Adelb (Idon zakara)	Leaves	0.119 ± 0.001	0.50
Moringa oleifera (Zogale)	Roots	0.322 ± 0.004	0.18
Pterocarpus erinaceus (Madobiya)	Bark	0.035 ± 0.001	1.66
Rauwolfia serpentina (Ganyen Ghana)	Leaves	0.304 ± 0.002	0.19
Ascorbic acid		0.058 ± 0.003	1.0

Note: SC_{50}: Concentration of plant extract showed 50% DPPH scavenging activity. All experiments were performed in triplicate.

Table 3.4.4 SC_{50} values of the methanol extracts of Nigerian hypoglycemic medicinal plants comparing with the reference compound, tocopherol

Spectrophotometery was used to determine the free radical scavenging activity (hydrogen donating ability) of the aqueous extracts of the Thai hypoglycemic medicinal plants. The SC_{50} of ascorbic acid was used to compare with those of the extracts and the lower SC_{50} (mean ± S.E.M) values, indicated higher activity compared to the standard (Table 3.4.4).

Table 3.4.4 SC_{50} values of the methanol extracts of Nigerian medicinal plants

Medicinal Plant (Common Name)	Part Used	SC_{50} mean ± *s.e.m* (mg/ml)	Fold/Activity
Anisopus mannii N.E.Br (Kashe zaki)	Leaves	0.356 ± 0.003	0.14
Anogeissus leiocarpus (D.C) Guill & Perr. (Mareke)	Bark	0.009 ± 0.001	5.67
Daniella Oliveri (Rolfe) Hutch & Dalz. (Maje)	Leaves	0.039 ± 0.002	1.31
Detarium macrocarpum Harms (Taura)	Bark	0.026 ± 0.001	2.00
Ficus Thonningii. Blume, *Raphia vinifera* P. Beauv,*Leptedenia hastata* (Pers) Dec'ne (Maganin suga)	Recipe	0.125 ± 0.002	0.41
Leptedenia hastata (Pers) Dec'ne (Ya diya)	Leaves	0.261 ± 0.005	0.20
Mimosa invisa var. inermis Adelb (Idon zakara)	Leaves	0.238 ± 0.005	0.21
Moringa oleifera (Zogale)	Roots	0.369 ± 0.005	0.14
Pterocarpus erinaceus (Madobiya)	Bark	0.036 ± 0.002	1.42
Rauwolfia serpentina (Ganyen Ghana)	Leaves	0.180 ± 0.003	0.28
Tocopherol		0.051 ± 0.001	1.0

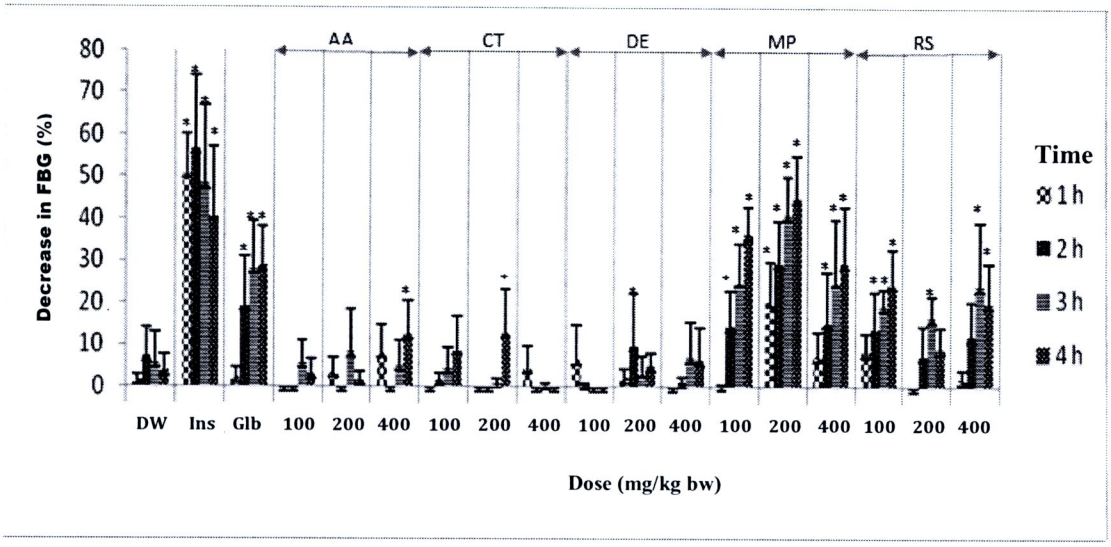
Note: SC_{50}: Concentration of plant extract showed 50% DPPH scavenging activity. All experiments were performed in triplicate.

Part 2: Hypoglycemic effects/activities of medicinal plants

3.6.1 Thai hypoglycemic medicinal plants (A)

Hypoglycemic effect of the aqueous extracts of Thai hypoglycemic medicinal plants in normoglycemic mice

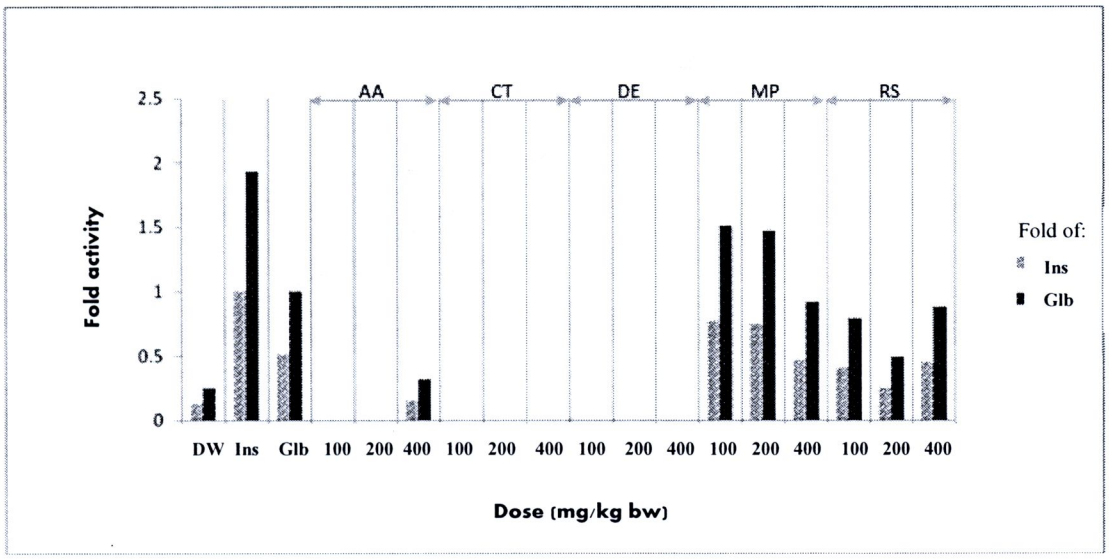
The extracts of *M. pudica* and *R. serpentina* showed significant reduction of 23.71% in FBG in normoglycemic mice (Fig 3.2). *M. pudica* extracts showed the highest reduction of 47.43% reduction with 200 mg/kg bw at the 4 h.



DW: Distilled water, Ins: Insulin, Glib: Glibenclamide, AA: *Anogeissus acuminata,* CT*: Catunaregam tormentosa,* DE: *Dioecrescis erythroclada,* MP*: Mimosa pudica,* RS*: Rauwolfia serpentina*

Fig 3.2: Hypoglycemic effects of five Thai medicinal plants in normoglycemic mice. All bars are highest percentage decrease compared with the FBG at 0 hr. Values are means ± SEM of groups of five observations (n=5).*Significant decrease ($p < 0.05$) comparing with FBG at 0 hr.

The highest hypoglycemic activity of each of the five medicinal plants was compared with the standard drugs, insulin and glibenclamide (Fig 3.2). The aqueous extracts of *M. pudica* were 0.77 and 1.51 fold compared to insulin and glibenclamide (Figures 3.3).

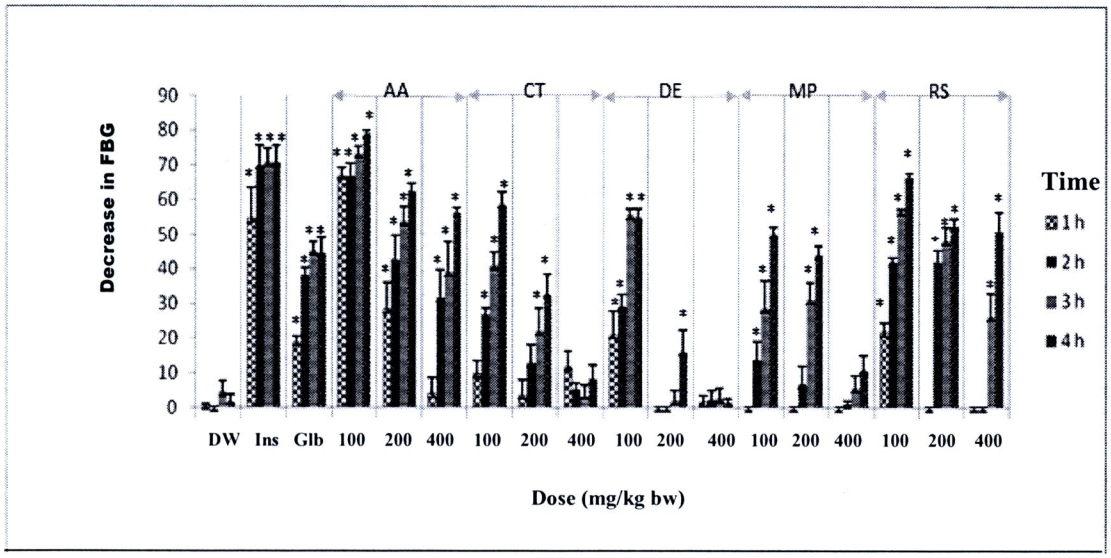


DW: Distilled water, Ins: Insulin, Glib: Glibenclamide, AA: *Anogeissus acuminata,* CT*: Catunaregam tormentosa,* DE: *Dioecrescis erythroclada,* MP*: Mimosa pudica,* RS*: Rauwolfia serpentina*

Fig 3.3 Hypoglycemic effects of five Thai medicinal plants in normoglycemic mice in fold comparing with the standard drugs, insulin and glibenclamide. All bars are highest percentage decrease compared with the FBG at 0 hr. Values are means ± SEM of groups of five observations.*Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

In the alloxan induced diabetic group, the aqueous extract of *A. acuminata* showed the highest significant ($p<0.05$) FBG reduction of 78.97% with the 100 mg/kg bw at the 4 h (Fig 3.4). *C. tormentosa* extract showed significant ($p<0.05$) decrease in

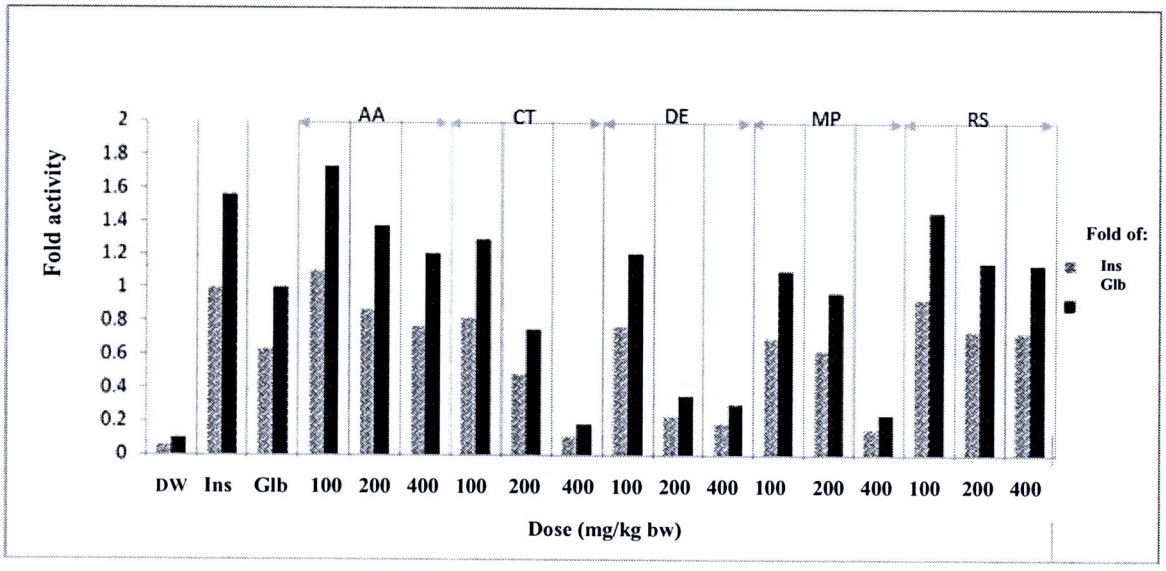
FBG with 100 mg/kg bw at the 4 h, *D. erythroclada* showed 55.51% with 400 mg/kg bw at the 3 h while *M. pudica* and *R. serpentina* showed 50.35 and 66.60% with 100 mg/kg bw at the 4 h, respectively.



DW: Distilled water, Ins: Insulin, Glib: Glibenclamide, AA: *Anogeissus acuminata,* CT*: Catunaregam tormentosa,* DE: *Dioecrescis erythroclada,* MP*: Mimosa pudica,* RS*: Rauwolfia serpentina*

Fig 3.4 Hypoglycemic effects of five Thai medicinal plants in alloxan induced diabetic mice. All bars are highest percentage decrease compared with the FBG at 0 hr. Values are means ± SEM of groups of five observations.*Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

The aqueous extract of *A. acuminata* which showed the highest significant ($p<0.05$) FBG reduction in the group at the 4 h was 1.10 fold of insulin and 1.76 of glibenclamide while *C. tormentosa aqueous* 0.8 and 1.26 fold compared to insulin and glibenclamide.

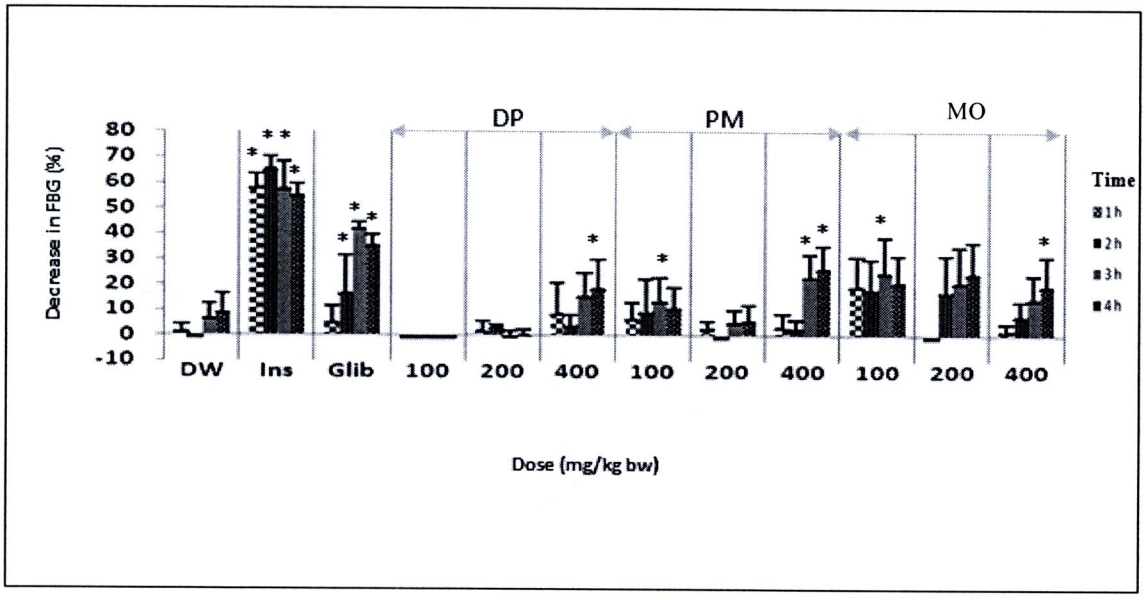


DW: Distilled water, Ins: Insulin, Glib: Glibenclamide, AA: *Anogeissus acuminata,* CT*: Catunaregam tormentosa,* DE: *Dioecrescis erythroclada,* MP*: Mimosa pudica,* RS*: Rauwolfia serpentina*

Fig 3.5 Hypoglycemic effects of five Thai medicinal plants in alloxan induced diabetic mice in fold comparing with the standard drugs. All bars are highest percentage decreases compared with the FBG at 0 h. Values are means ± SEM of groups of five observations. *Significant (p<0.05) decrease comparing with FBG at 0 h.

3.6.2 Thai hypoglycemic medicinal plants (B)

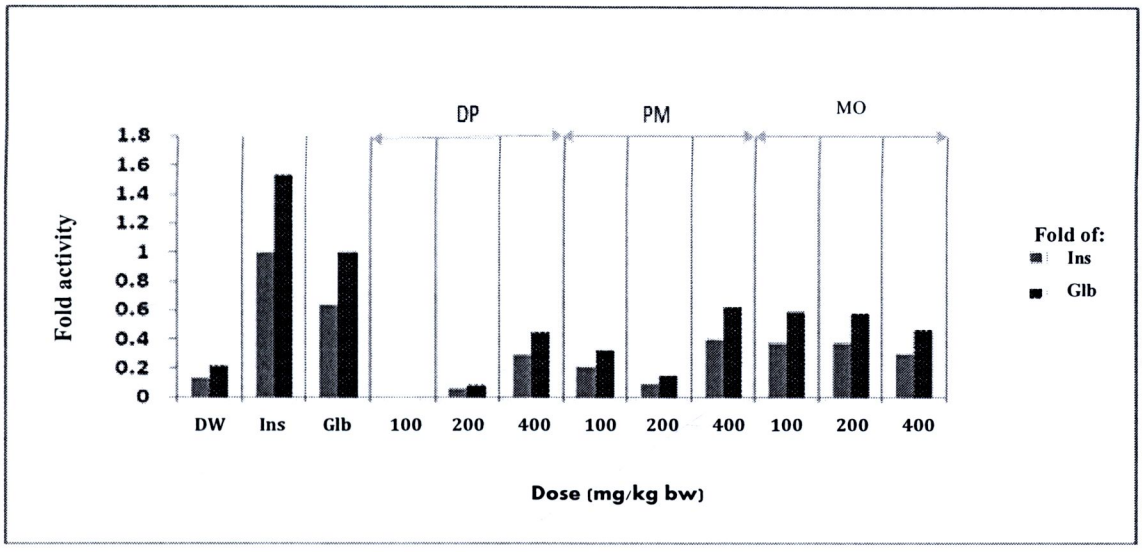
The aqueous extracts of *P. macrocarpus* showed the highest significant (p<0.05) FBG decrease of 26.71% at the 4 h with 400 mg/kg bw dose in normoglycemic mice (Fig 3.6), it was followed by *M. oleifera* and *D. pentandra* with 26.46 and 19.10% FBG reduction at the 4 h with 200 mg/kg bw, respectively.



DW: Distilled Water, Ins: Insulin, Glib: Glibenclamide, DP: *Dendrophthoe pentandra*, PM: *Pterocarpus macrocarpus*, MO: *Moringa oleifera*

Fig 3.6 Hypoglycemic effects of three Thai medicinal plants in normoglycemic mice. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five (n=5) observations.*Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

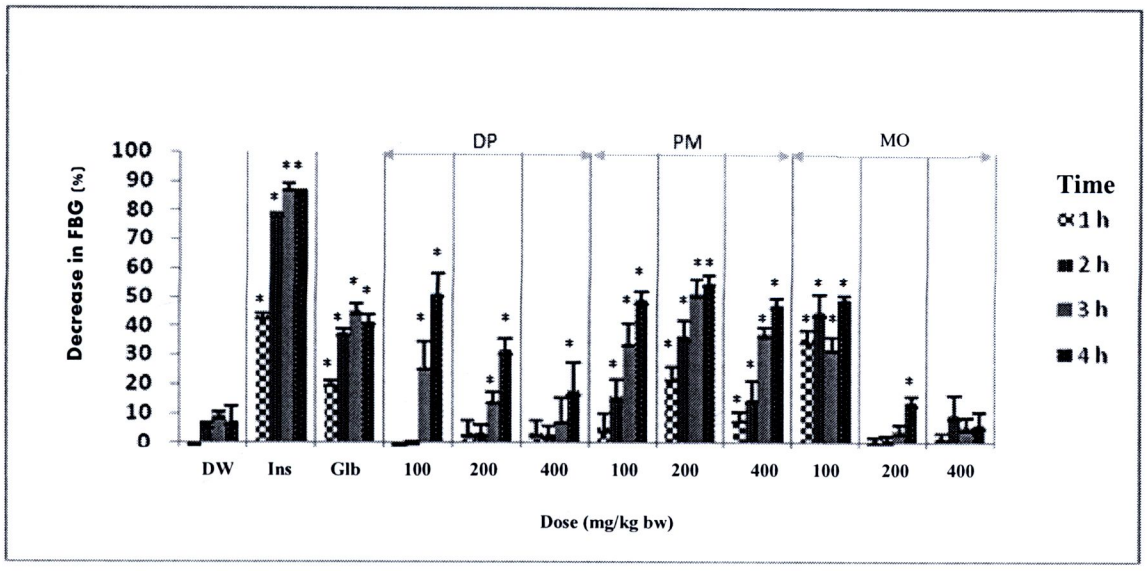
The aqueous extracts of *P. macrocarpus* showed the highest significant ($p<0.05$) FBG decrease, which was 0.40 and 0.62 folds of insulin and glibenclamide and it was followed by *M. oleifera* and *D. pentandra* with 0.38 and 0.59, and 0.29 and 0.45 folds of insulin and glibenclamide, respectively (Fig 3.7).



DW: Distilled Water, Ins: Insulin, Glib: Glibenclamide, DP: *Dendrophthoe pentandra*, PM: *Pterocarpus macrocarpus*, MO: *Moringa oleifera*

Fig 3.7 Hypoglycemic effects of Thai medicinal plants in normoglycemic mice in fold comparing with the standard drugs. All bars are highest percentage decreases compared with the FBG at 0 hr. Values are means ± s.e.m of groups of five observations. *Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

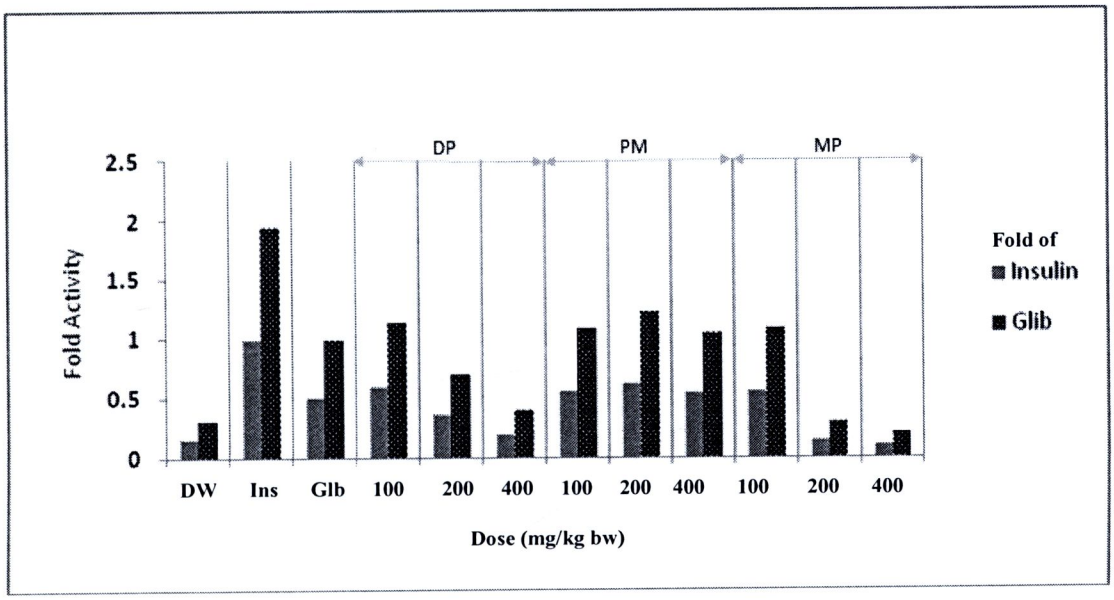
In the alloxan induced diabetic group, *P. macrocarpum* showed the highest FBG reduction of 54.63% at the 4 h with 200 mg/kg bw dose. It was followed by *D. pentandra* and *M. oleifera* with 50.81 and 49% at the 4 h with 100 mg/kg bw doses, respectively.



DW: Distilled Water, Ins: Insulin, Glib: Glibenclamide, DP: *Dendrophthoe pentandra*, PM: *Pterocarpus macrocarpus*, MO: *Moringa oleifera*

Fig 3.8 Hypoglycemic effects of three Thai medicinal plants in alloxan induced diabetic mice. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five (n=5) observations.* Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

In the alloxan induced diabetic group, *P. macrocarpum* which showed the highest FBG reduction at the 4 h was 0.62 and 1.22 fold of insulin and glibenclamide (Fig 3.9). It was followed by *D. pentandra* and *M. oleifera* with 0.56 and 1.09, and 0.58 and 1.13 folds of insulin and glibenclamide, respectively.

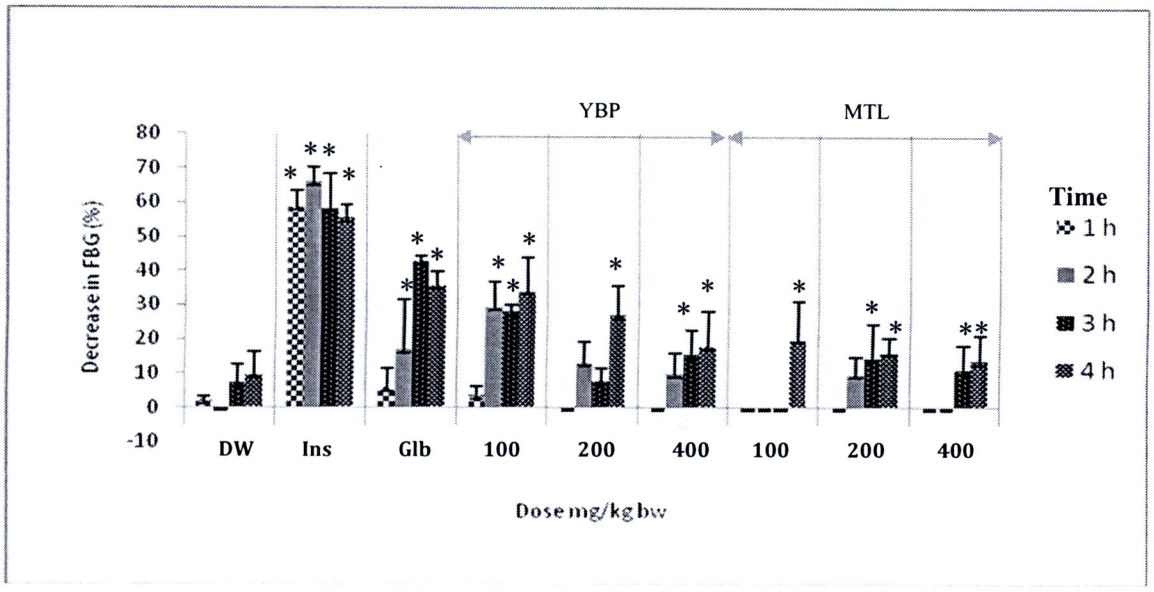


DW: Distilled Water, Ins: Insulin, Glib: Glibenclamide, DP: *Dendrophthoe pentandra*, PM: *Pterocarpus macrocarpus*, MO: *Moringa oleifera*

Fig 3.9 Hypoglycemic effects of three Thai medicinal plants in normoglycemic mice in fold comparing with the standard drugs. All bars are highest percentage decreases compared with the FBG at 0 h. Values are means ± SEM of groups of five observations. *Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

3.6.3 Thai hypoglycemic medicinal recipes (C)

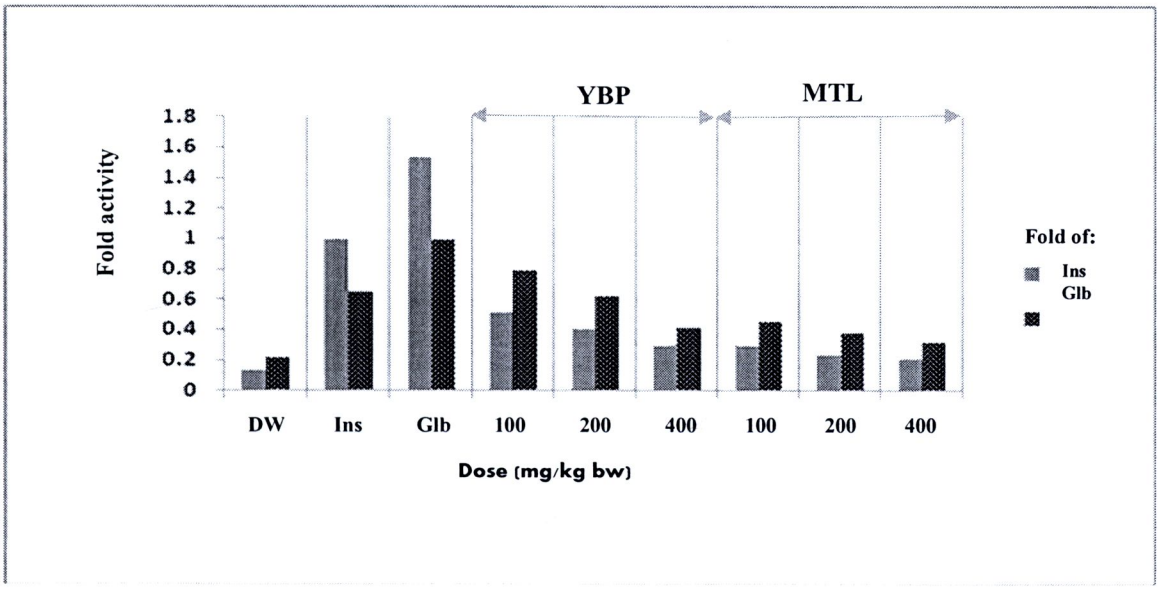
The Thai hypoglycemic recipes in normoglycemic mice showed significant hypoglycemic effects (Fig 3.10). YBP showed the highest FBG reduction of 34% at the 4 h with 100 mg/kg *bw* dose, while MTL was 19% at the 4 h with 100 mg/kg *bw,* respectively.



DW: Distilled water, ins: Insulin, Glb: Glibenclamide, YBP: Yamed Boraped Pungchang, MTL: Mai Tau Lusi

Fig 3.10 Hypoglycemic effects of two Thai medicinal recipes in normoglycemic mice. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

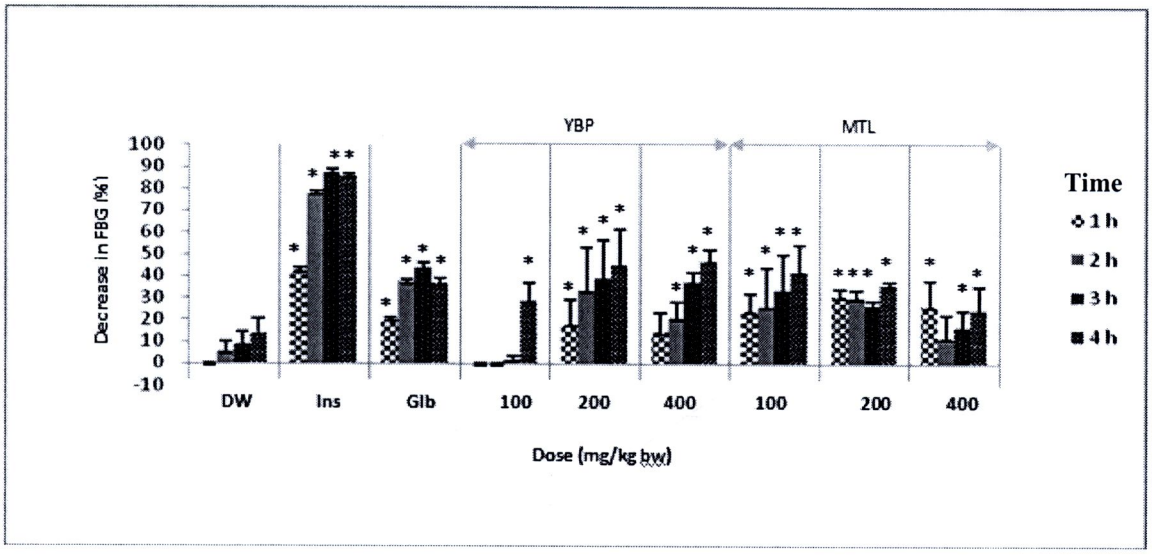
Yamed Boraped Pungchang (YBP) which showed the highest significant ($p<0.05$) FBG decrease was 0.52 and 0.8 fold of insulin and glibenclamide. Mai Tau Lusi (MTL) was 0.41 and 0.63 fold of insulin and glibenclamide (Fig 3.11).



DW: Distilled water, ins: Insulin, Glb: Glibenclamide, YBP: Yamed Boraped Pungchang, MTL: Mai Tau Lusi

Fig 3.11 Hypoglycemic effects of two Thai medicinal recipes in normoglycemic mice in fold comparing with the standard drugs. All bars are highest percentage decreases compared with the FBG at 0 h. Values are mean ± SEM of groups of five observations. *Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

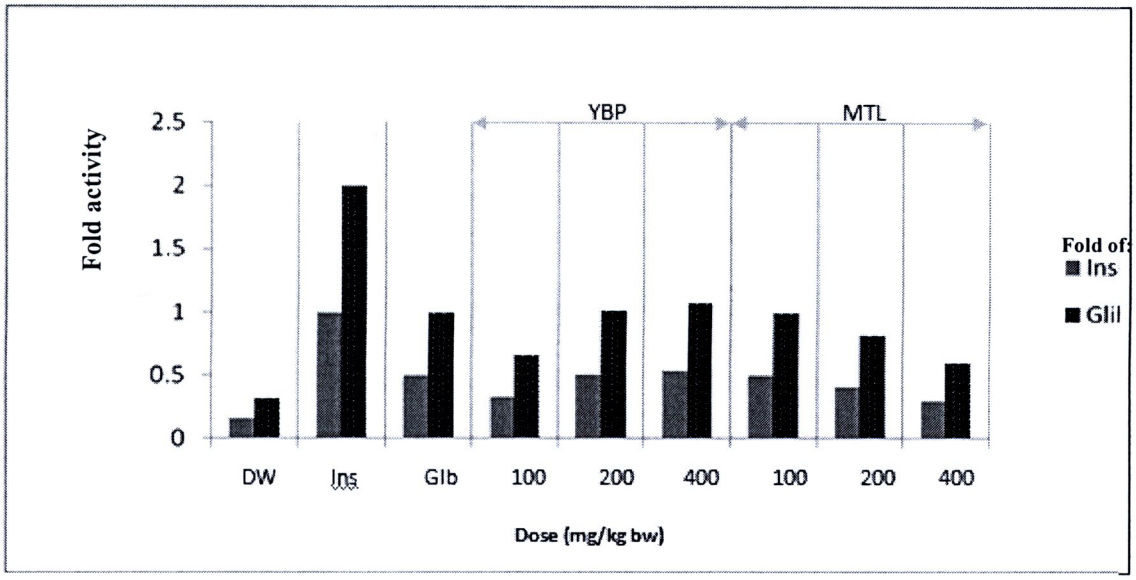
In the alloxan induced diabetic mice, the two polyherbal medicinal recipes showed significant ($p<0.05$) hypoglycemic effects at various time intervals, the highest being YBP which showed 45% FBG reduction at the 4 h, while MTL showed peak values 36% at the 4 h with 200 mg/kg bw, respectively (Fig 3. 12).



DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, YBP: Yamed Boraped Pungchang, MTL: Mai Tau Lusi

Fig 3.12 Hypoglycemic effects of Thai medicinal recipes in alloxan induced diabetic mice. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.* Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

The two polyherbal medicinal recipes showed significant ($p<0.05$) hypoglycemic effects at various time intervals, the highest being YBP showed the highest FBG reduction at the 4 h was 0.54 and 1.07 fold of insulin and glibenclamide while MTL was 0.50 and 1.00 fold of the standard drugs, respectively (Fig 3.13).



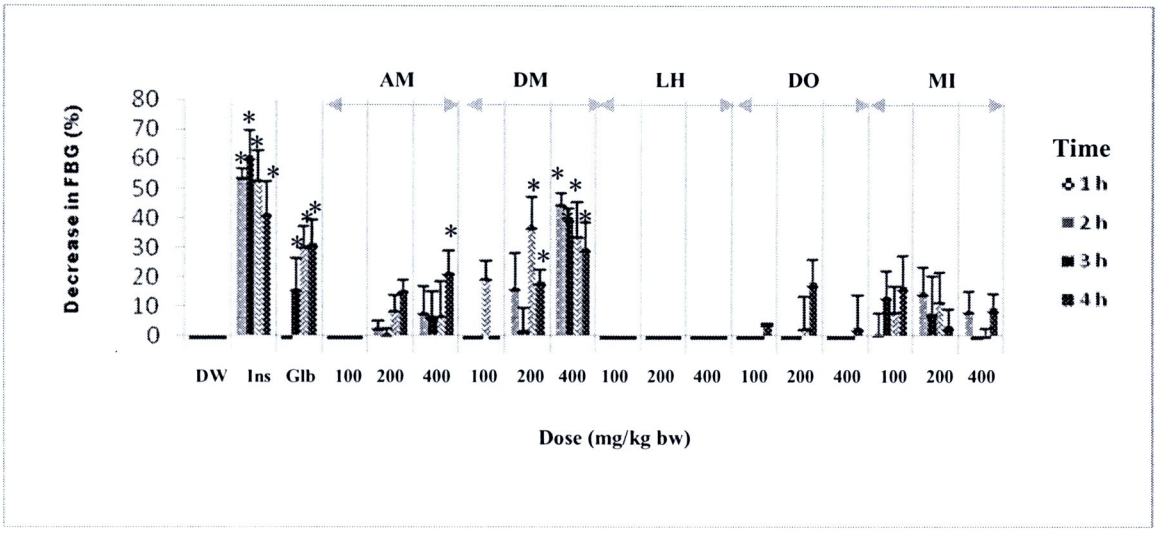
DW: Distilled water, ins: Insulin, Glb: Glibenclamide, YBP: Yamed Boraped Pungchang, MTL: Mai Tau Lusi

Fig 3.13 Hypoglycemic effects of Thai medicinal recipes in normoglycemic mice in fold comparing with the standard drugs, insulin and glibenclamide. All bars are highest percentage decreases compared with the FBG at 0 h. Values are means ± SEM of groups of five observations. *Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

3.6.4 Hypoglycemic effects of Nigerian medicinal plants (A)

Hypoglycemic effect of five medicinal plants in normoglycemic mice

The results of the Nigerian hypoglycemic medicinal plant aqueous extracts showed that *A. mannii* and *D. macrocarpum* significantly ($p<0.05$) reduced the FBG in normoglycemic mice, with the highest reduction of 44.26% shown by *D. macrocarpum* at the 1 h while *A. mannii* showed a reduction of 21.63% at a dose of 400 mg/kg bw, respectively (Fig 3.14).



DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, AM: *Anisopus mannii*, DO: *Daniella olivieri*, DM: *Detarium macrocarpum*, LH: *Leptedania hastata*, MI: *Mimosa invisa*

Fig 3.14 Hypoglycemic effects of five Nigerian medicinal plants in normoglycemic mice. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*: Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

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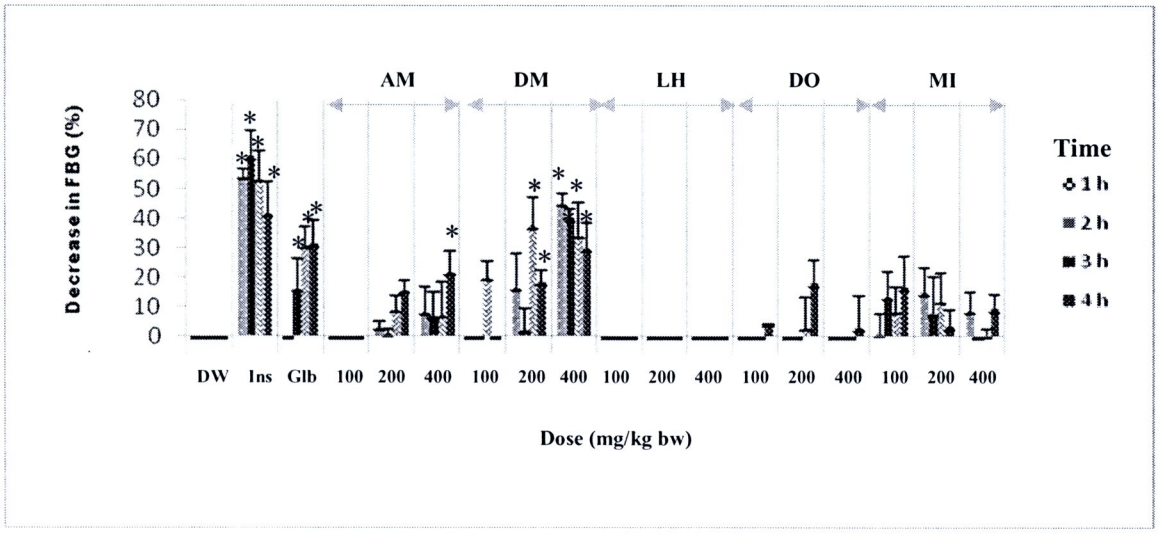
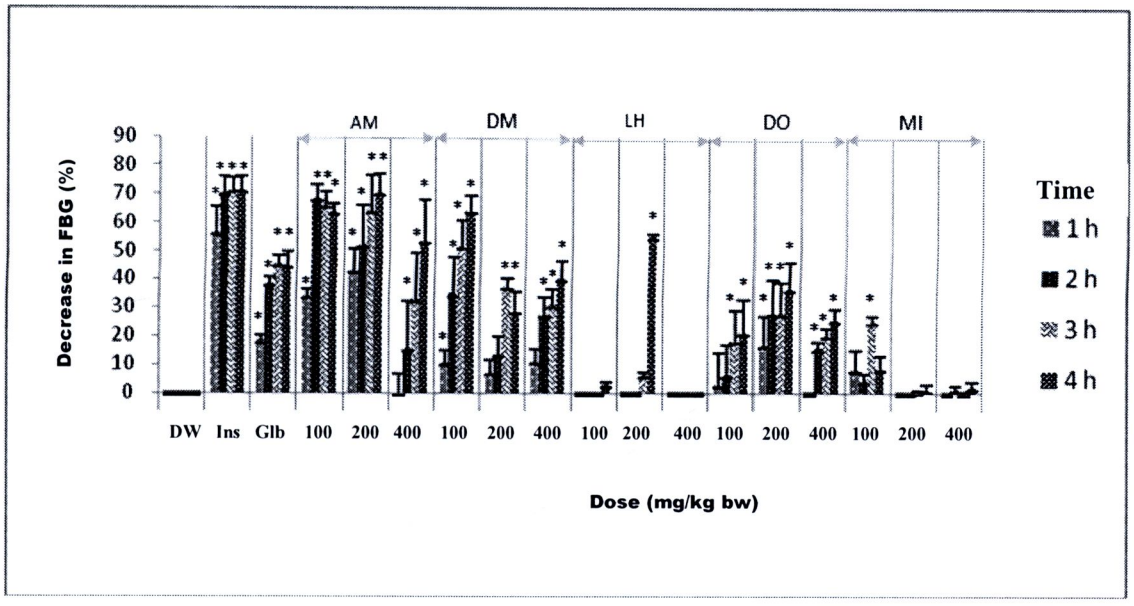


DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, AM: *Anisopus mannii*, DO: *Daniella olivieri*, DM: *Detarium macrocarpum*, LH: *Leptedania hastata*, MI: *Mimosa invisa*

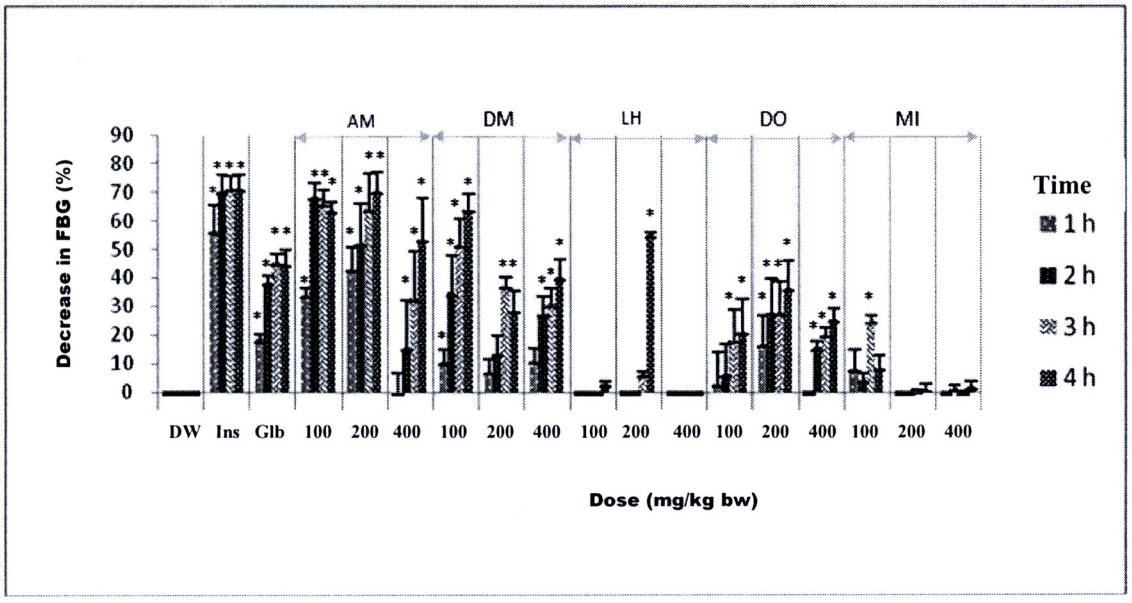
Fig 3.14 Hypoglycemic effects of five Nigerian medicinal plants in normoglycemic mice. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*: Significant decrease ($p < 0.05$) comparing with FBG at 0 h.



DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, AM: *Anisopus mannii*, DO: *Daniella olivieri*, DM: *Detarium macrocarpum*, LH: *Leptedania hastata*, MI: *Mimosa invisa*

Fig 3.16 Hypoglycemic effects of five Nigerian medicinal plants in alloxan induced diabetic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*Significant decrease ($p < 0.05$) comparing with FBG at 0 hr.

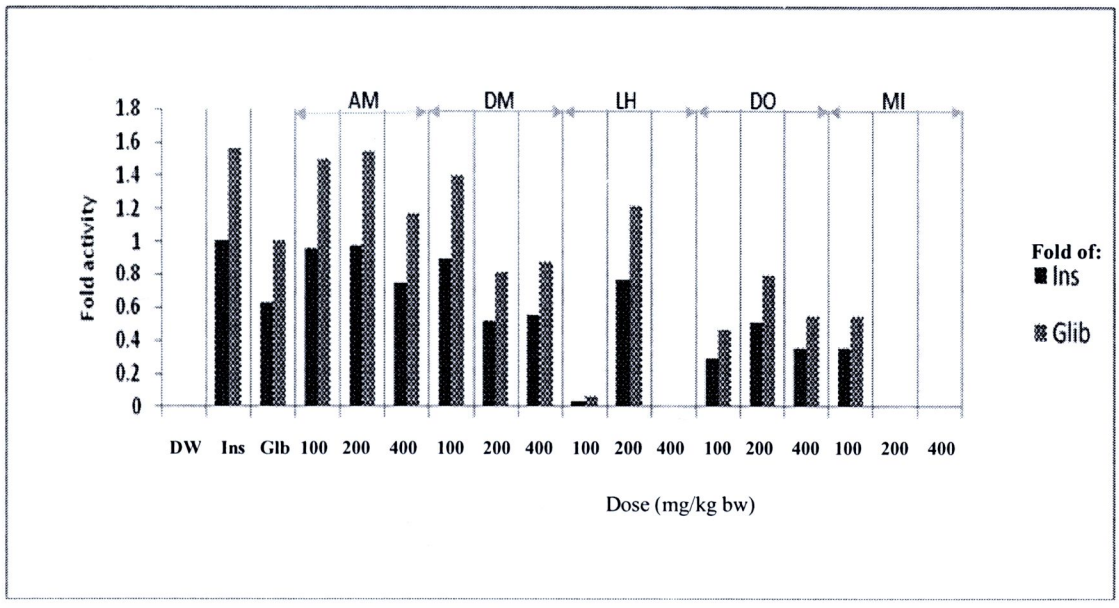
The *A. mannii* and *D. macrocarpum* showed 0.98 and 1.54, and 0.89 and 1.40 folds of insulin and glibenclamide, respectively (Fig 3.17).



DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, AM: *Anisopus mannii*, DO: *Daniella olivieri*, DM: *Detarium macrocarpum*, LH: *Leptedania hastata*, MI: *Mimosa invisa*

Fig 3.16 Hypoglycemic effects of five Nigerian medicinal plants in alloxan induced diabetic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*Significant decrease ($p < 0.05$) comparing with FBG at 0 hr.

The *A. mannii* and *D. macrocarpum* showed 0.98 and 1.54, and 0.89 and 1.40 folds of insulin and glibenclamide, respectively (Fig 3.17).

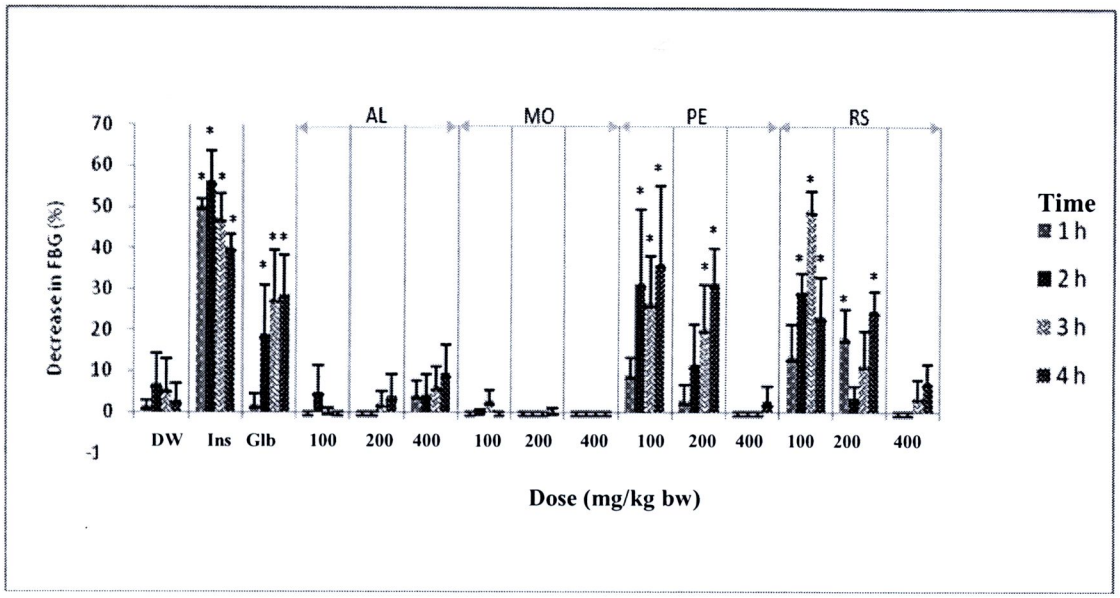


Abbreviation: DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, AM: *Anisopus mannii*, DO: *Daniella olivieri*, DM: *Detarium macrocarpum*, LH: *Leptedania hastata*, MI: *Mimosa invisa*

Fig 3.17 Hypoglycemic effects of five Nigerian medicinal plants in alloxan induced diabetic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

3.6.5 Hypoglycemic effects of Nigerian medicinal plants (B)

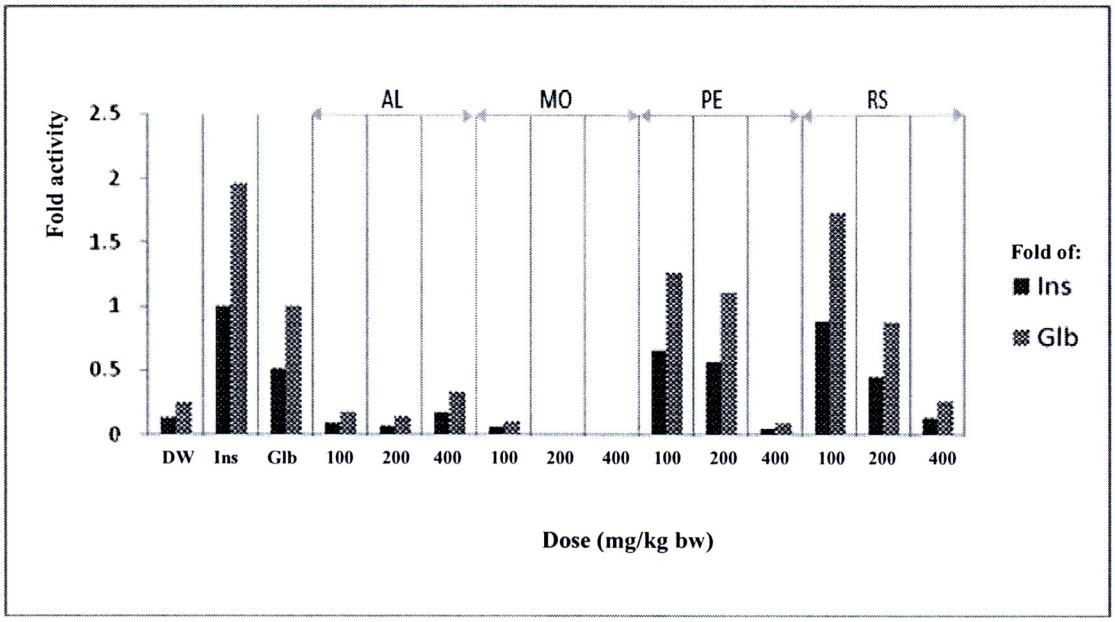
The aqueous extracts of *Rauwolfia serpentina* and *Pterocarpus erinaceous* showed significant FBG reduction with 100 mg/kg bw at the 3 and 4 h with 49.75 and 31.84% reduction, respectively (Fig 3.18).



DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, AL: *Anogeissus leiocarpus*, MO: *Moringa oleifera*, PM: *Pterocarpus erinaceous,* RS: *Rauwolfia serpentina*

Fig 3.18 Hypoglycemic effects of four Nigerian medicinal plants in normoglycemic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± s.e.m of groups of five observations.*: Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

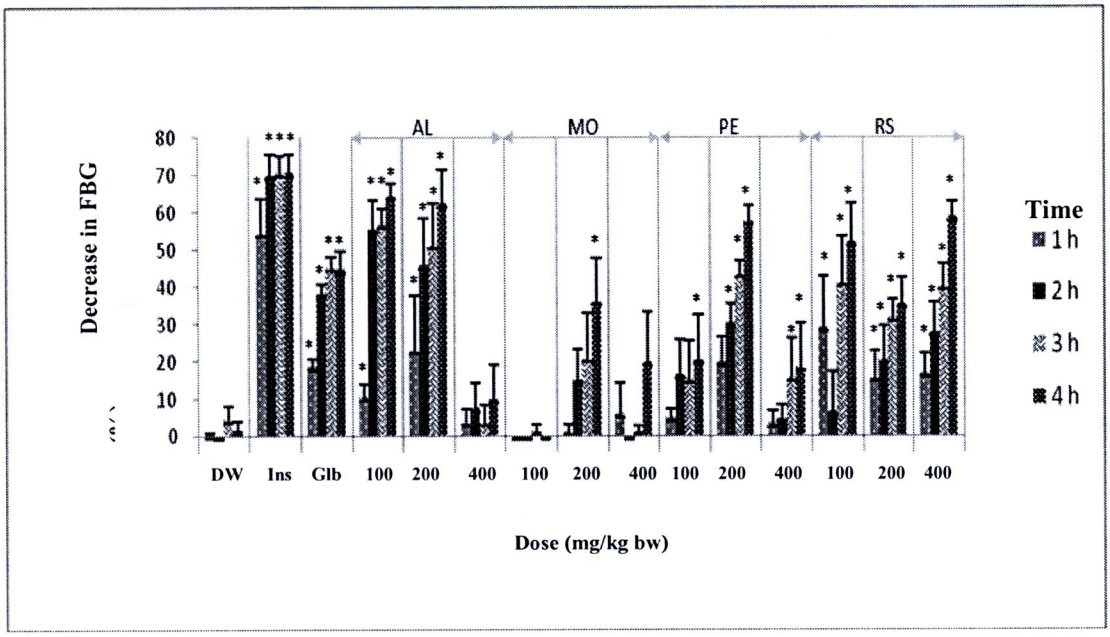
The *Rauwolfia serpentina* and *Pterocarpus erinaceous* which showed significant FBG reduction, represents a 0.88 and 1.73, and 0.64 and 1.26 fold of insulin and glibenclamide, respectively (Fig 3.19).



DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, AL: *Anogeissus leiocarpus*, MO: *Moringa oleifera*, PM: *Pterocarpus erinaceous*, RS: *Rauwolfia serpentina*

Fig 3.19 Hypoglycemic effects of four Nigerian medicinal plants in normoglycemic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

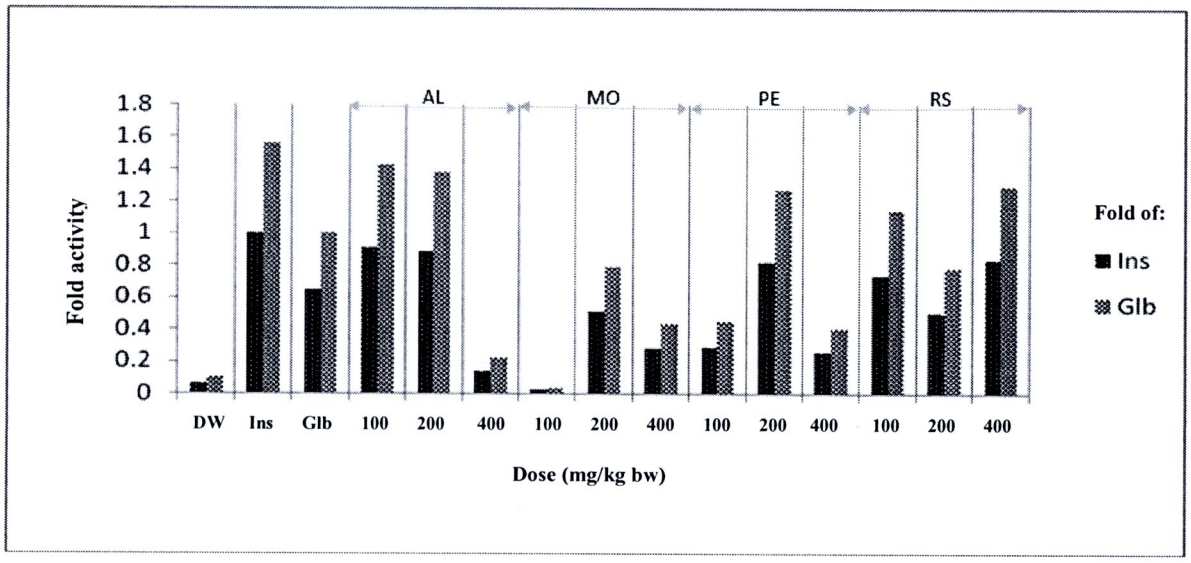
In the alloxan induced diabetic mice (Fig 3.20), the aqueous extract of all the hypoglycemic medicinal plants showed all showed significant decrease in FBG at different time intervals. *A. leiocarpus* showed the highest FBG reduction with 100 mg/kg bw at the 4 h it was followed by *P. erinaceous* with 57.83% FBG reduction. *R. serpentina* showed 51.99% and *M. oleifera* showed 35.95%.



DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, AL: *Anogeissus leiocarpus*, MO: *Moringa oleifera*, PM: *Pterocarpus erinaceous,* RS:*Rauwolfia serpentina*

Fig 3.20 Hypoglycemic effects of Nigerian medicinal plants in alloxan induced diabetic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations. *Significant decrease ($p < 0.05$) comparing with FBG at 0 hr.

A. *leiocarpus* and *P. erinaceous* which showed the highest FBG reduction showed 0.91 and 1.42 fold of insulin and glibenclamide while *R. serpentina* showed 0.73 and 1.14 fold, respectively (Fig 3.21).

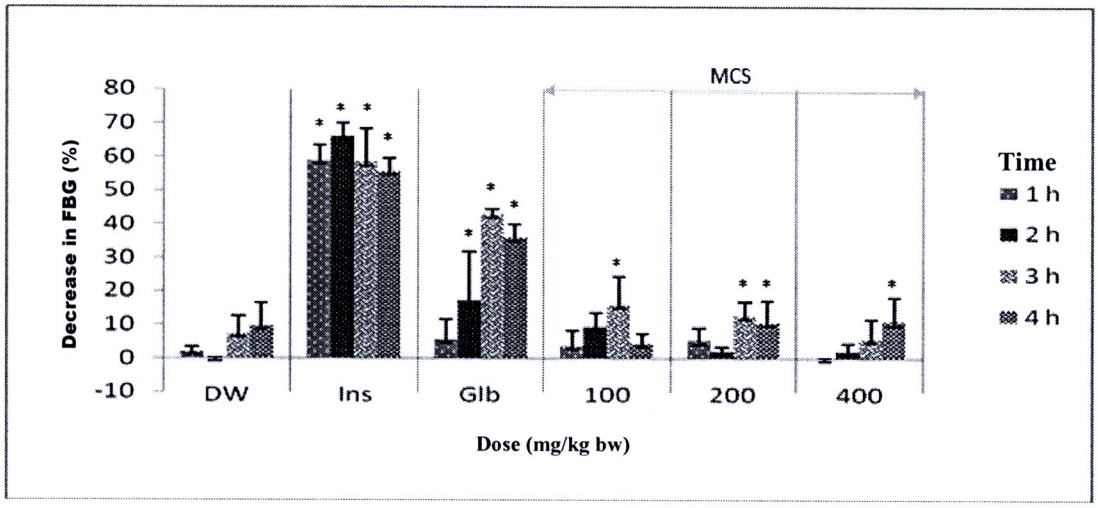


DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, AL: *Anogeissus leiocarpus*, MO: *Moringa oleifera*, PM: *Pterocarpus erinaceous,* RS:*Rauwolfia serpentina*

Fig 3.21 Hypoglycemic effects of four Nigerian medicinal plants in alloxan induced diabetic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*: Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

3.6.6 Hypoglycemic effects of Nigerian medicinal recipe (C)

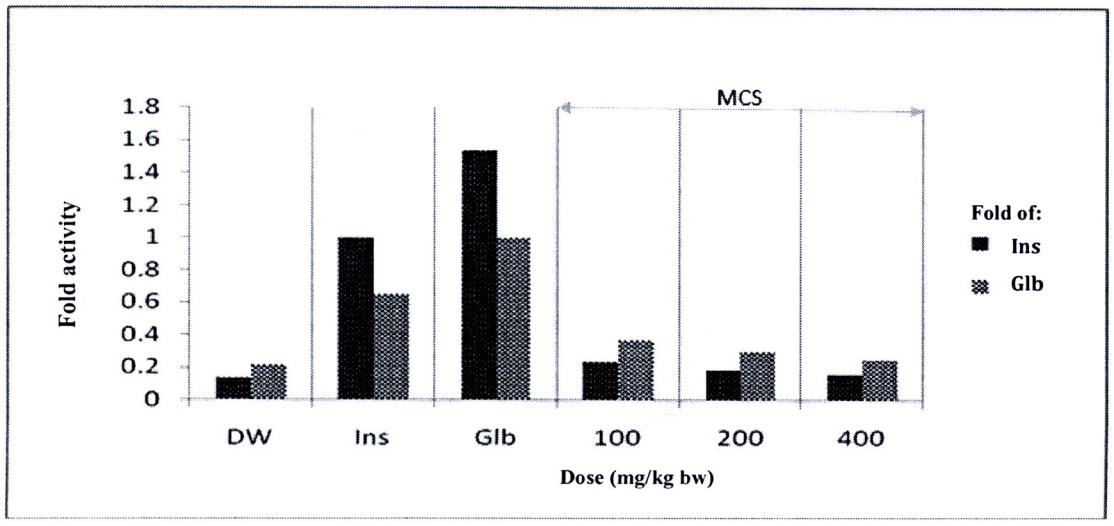
The recipe MCS (100 mg/kg bw) showed 15.72% FBG reduction with the normoglycemic mice at the 4 h (Fig 3.22).



DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, MCS: Maganin Ciwon Suga

Fig 3.22 Hypoglycemic effects of Nigerian medicinal recipe in normoglycemic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*: Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

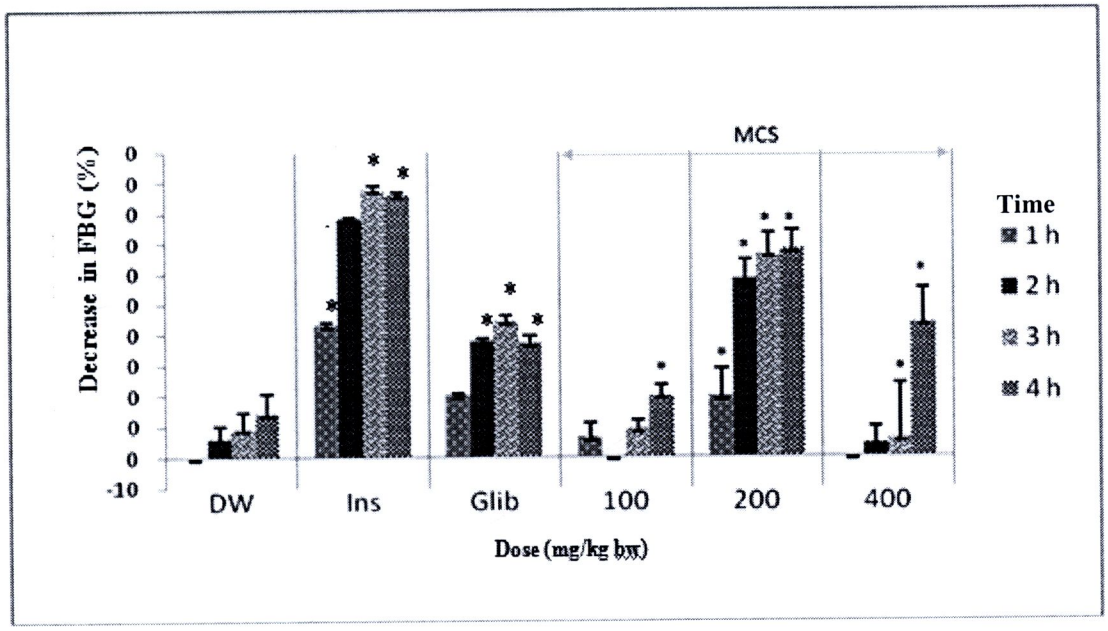
15.72% reduction translates to 0.28 and 0.55 insulin and glibenclamide fold as observed in the normoglycemic mice, respectively (Fig 3.23).



DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, MCS: Maganin Ciwon Suga

Fig 3.23 Hypoglycemic effects of Nigerian medicinal recipe in normoglycemic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations. *: Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

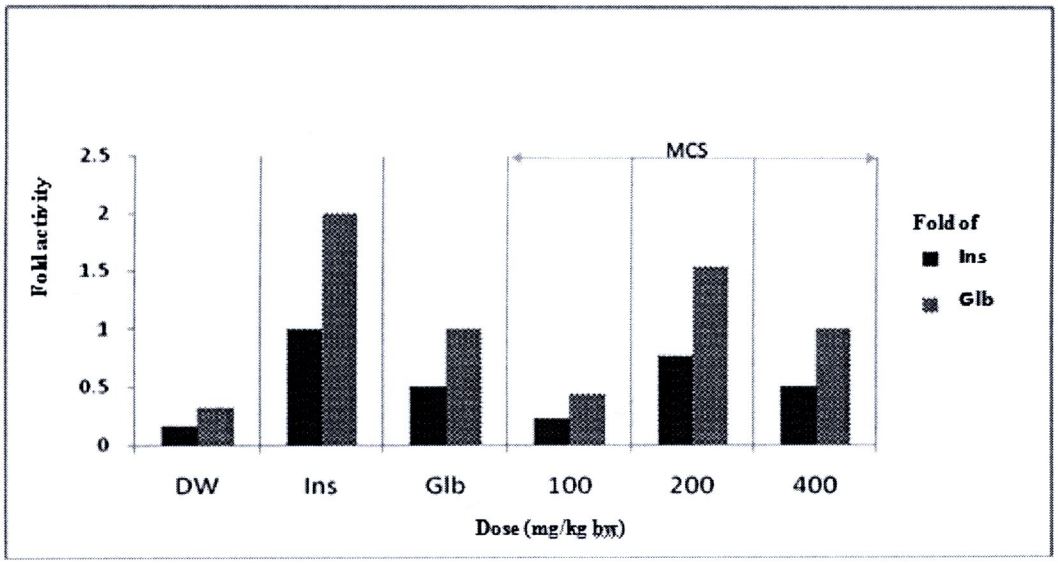
In the alloxan induced mice, the recipe MCS showed 67.91% FBG reduction with the 200 mg/kg bw dose (Fig 3.24).



Abbreviation: DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, MCS: Maganin Ciwon Suga

Fig 3.24 Hypoglycemic effects of Nigerian medicinal recipe in alloxan induced diabetic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations. Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

The significant reduction observed with MCS represents 0.96 and 1.54 fold of insulin and glibenclamide, respectively (Fig 3.25).



DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, MCS: Maganin Ciwon Suga

Fig 3.25 Hypoglycemic effects of Nigerian medicinal recipe in alloxan induced diabetic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations. *Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

3.7 Fractionation and hypoglycemic activity of Thai medicinal plant *Anogeissus acuminata*

Anogeissus acuminata was chosen for this phase. The fractions obtained from the fractionation of the *A. acuminata* extracts were shown in Figure 3.26.

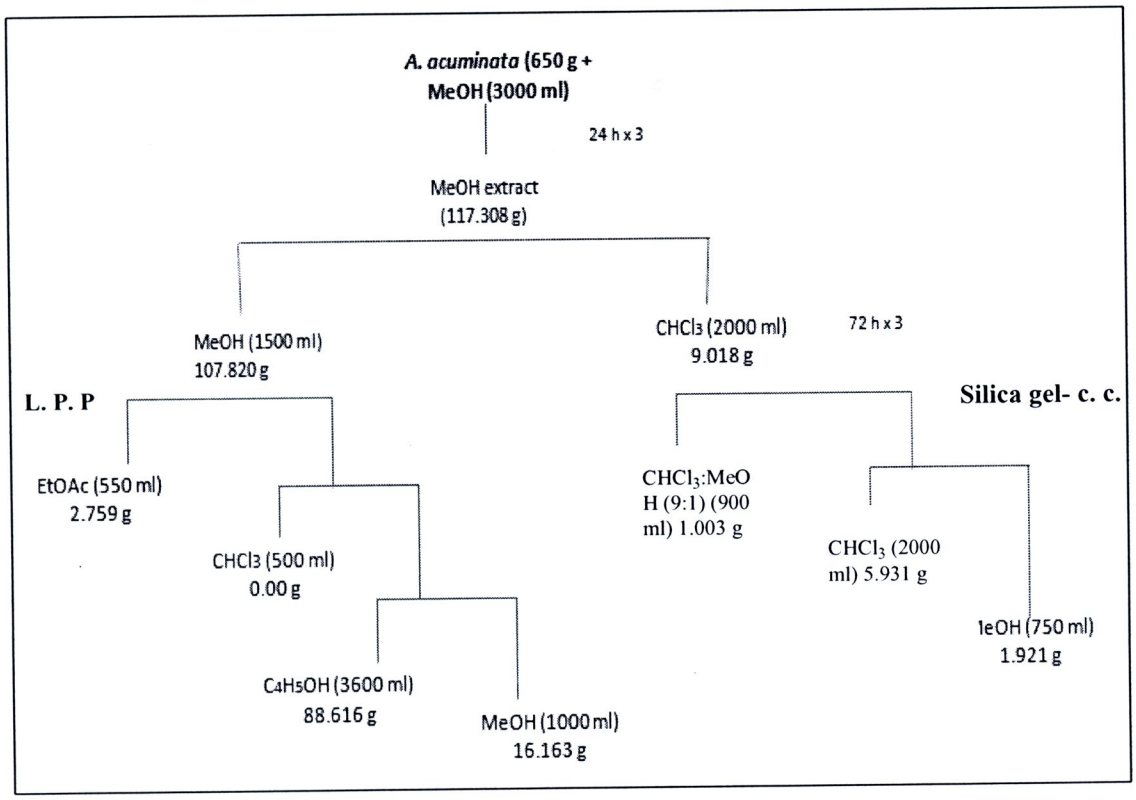


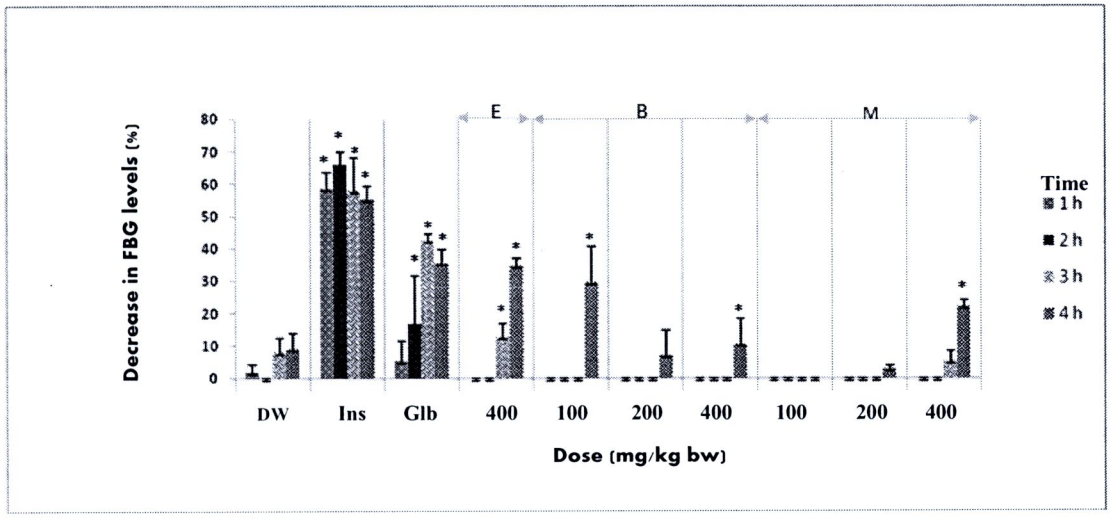
Fig 3.26 Result of the fractionation of methanol extract of *A. acuminata* showing the yields from every solvent used. Solvents used were arrived at after testing by Thin Layer Chromatography (TLC).

The final solvent were Ethyl acetate-Butanol-Methanol (8:2:2) for the methanol sub-fraction and Chloroform-Methanol-Formic acid (9:1:0.1) for the chloroform sub-fraction. L.P.P – liquid phase partition, Silica gel c.c.: column chromatography

3.7.1 Hypoglycemic activity of the methanol/chloroform sub-fractions of *A. acuminata*

Normoglycemic group

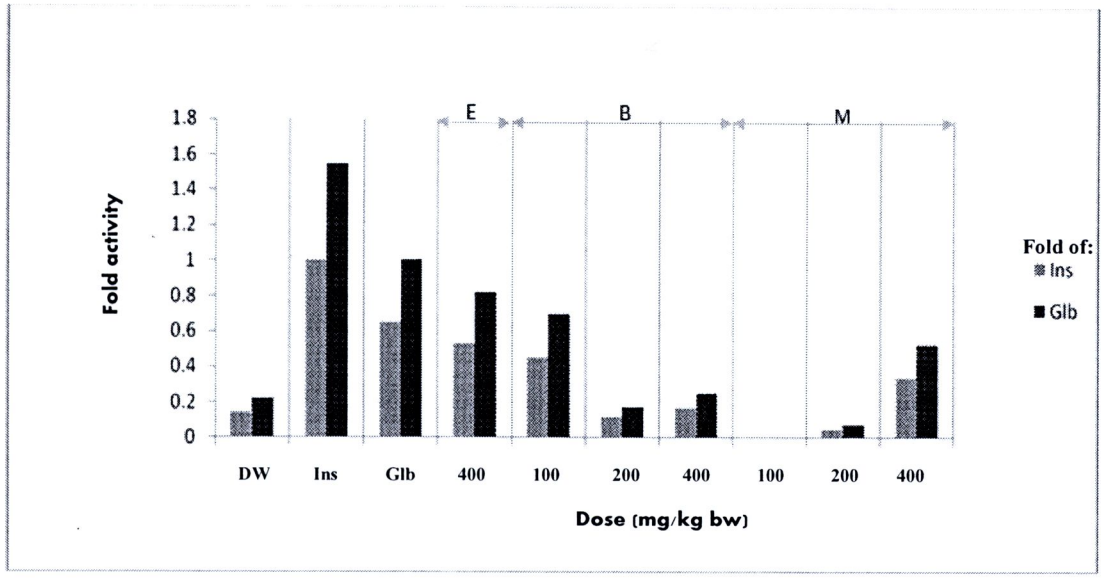
The screening of the various sub-fraction from *A. acuminata* gave the following results. In the normoglycemic mice, the ethyl acetate, butanol and methanolic sub-fractions from the methanol fraction showed significant (p<0.05) FBG reduction of 35.15, 29.91 and 22.43% at the 4 h with 400, 100 and 400 mg/kg bw doses (Fig 3.27).



DW: Distilled water, Insulin, Glb: Glibenclamide: E: Ethyl acetate, B: Butanol, M: Methanol

Fig 3.27 Hypoglycemic effects of methanol sub-fractions of *Anogeissus acuminata* in normoglycemic mice. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

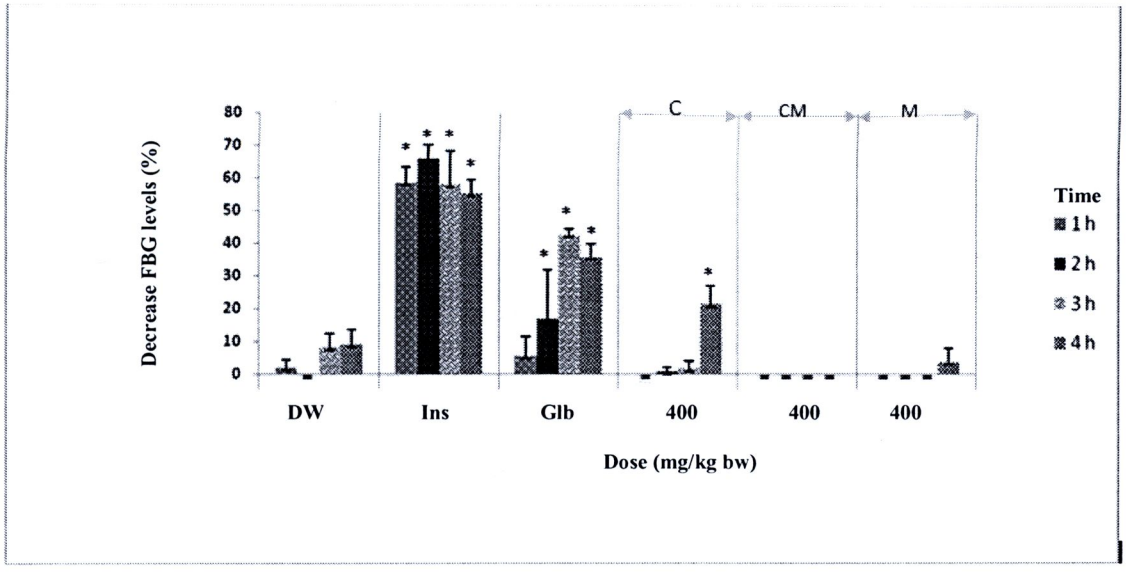
The ethyl acetate, butanol and methanolic sub-fractions from the methanol fraction showed significant (p<0.05) FBG reduction represents 0.53 and 0.82, 0.45 and 0.70, and 0.33 and 0.52 folds compared with insulin and glibenclamide, respectively (Fig 3.28).



DW: Distilled water, Insulin, Glb: Glibenclamide: E: Ethyl acetate, B: Butanol, M: Methanol

Fig 3.28 Hypoglycemic effects of methanol sub-fractions of *Anogeissus acuminata* in normoglycemic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.* Significant decrease (p < 0.05) comparing with FBG at 0 h.

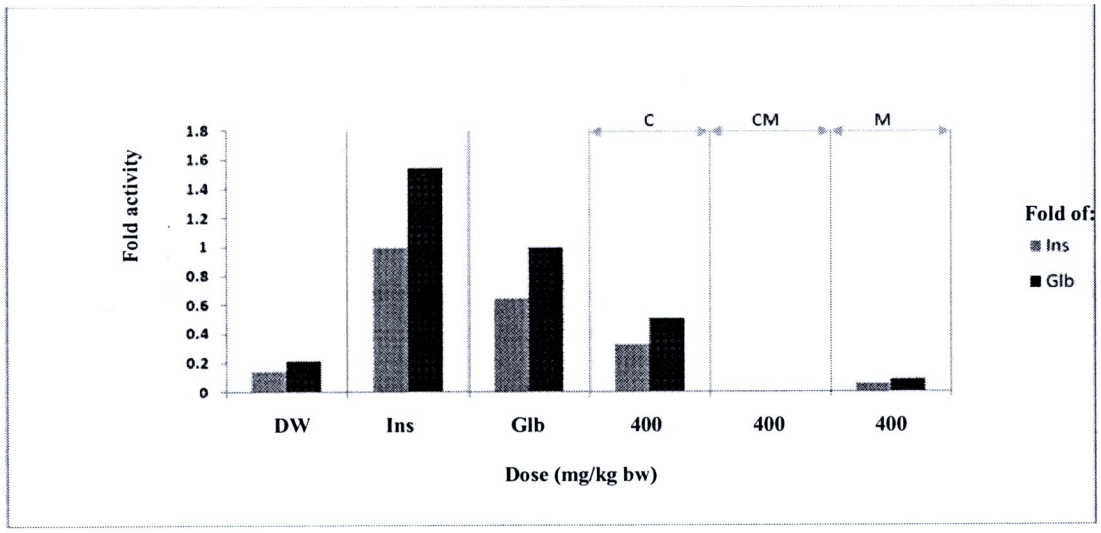
The chloroform sub-fraction showed 21.63% at the 4 h with 400 mg/kg bw dose in normoglycemic mice (Fig 3.29).



DW: Distilled water, Ins: Insulin, Glb: Glibenclamide: C: Chloroform, M: Methanol

Fig 3.29 Hypoglycemic effects of chloroform sub-fractions of *Anogeissus acuminata* in normoglycemic mice. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations. *Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

The chloroform sub-fraction FBG reduction represents 0.33 and 0.51 fold compared of insulin and glibenclamide (Fig 3.30).

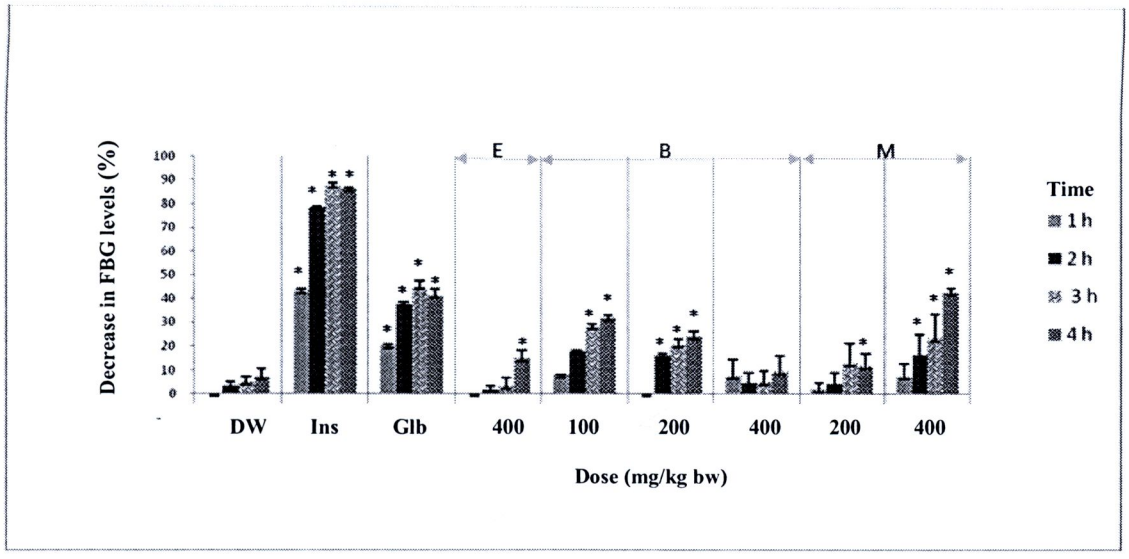


DW: Distilled water, Ins: Insulin, Glb: Glibenclamide: C: Chloroform, M: Methanol

Fig 3.30 Hypoglycemic effects of chloroform sub-fractions of *Anogeissus acuminata* in normoglycemic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.* Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

Diabetic group

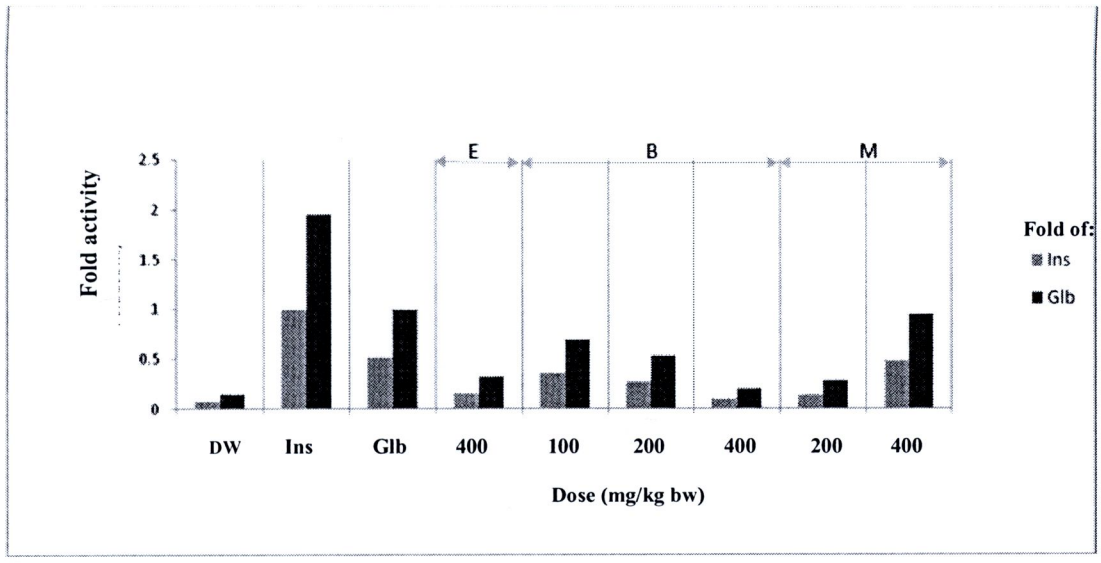
In the alloxan induced diabetic mice, the methanol, butanol and ethyl acetate sub-fractions showed significant ($p<0.05$) FBG reduction of 42.45, 31.46 and 14.59% at the 4 h with 400 and 100 mg/kg bw in the alloxan induced diabetic groups, respectively (Fig 3.31).



Abbreviation DW: Distilled water, Insulin, Glb: Glibenclamide: E: Ethyl acetate, B: Butanol, M: Methanol

Fig 3.31 Hypoglycemic effect of methanol sub-fractions of *Anogeissus acuminata* in alloxan induced diabetic mice. All bars Are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations. *Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

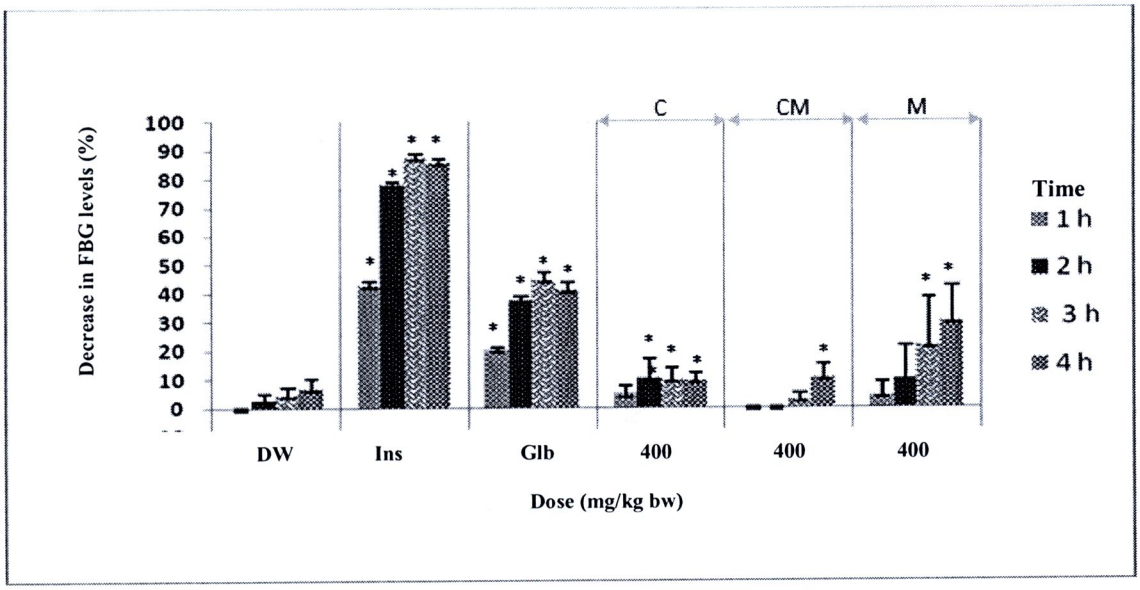
The methanol, butanol and ethyl acetate sub-fractions showed significant ($p<0.05$) FBG reduction in the alloxan induced diabetic groups, represents 0.48 and 0.95, 0.27 and 0.54, and 0.17 and 0.32 folds compared to insulin and glibenclamide, respectively (Fig 3.32).



DW: Distilled water, Insulin, Glb: Glibenclamide: E: Ethyl acetate, B: Butanol, M: Methanol

Fig 3.32 Hypoglycemic effects of methanol sub-fractions of *Anogeissus acuminata* in alloxan induced diabetic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.* Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

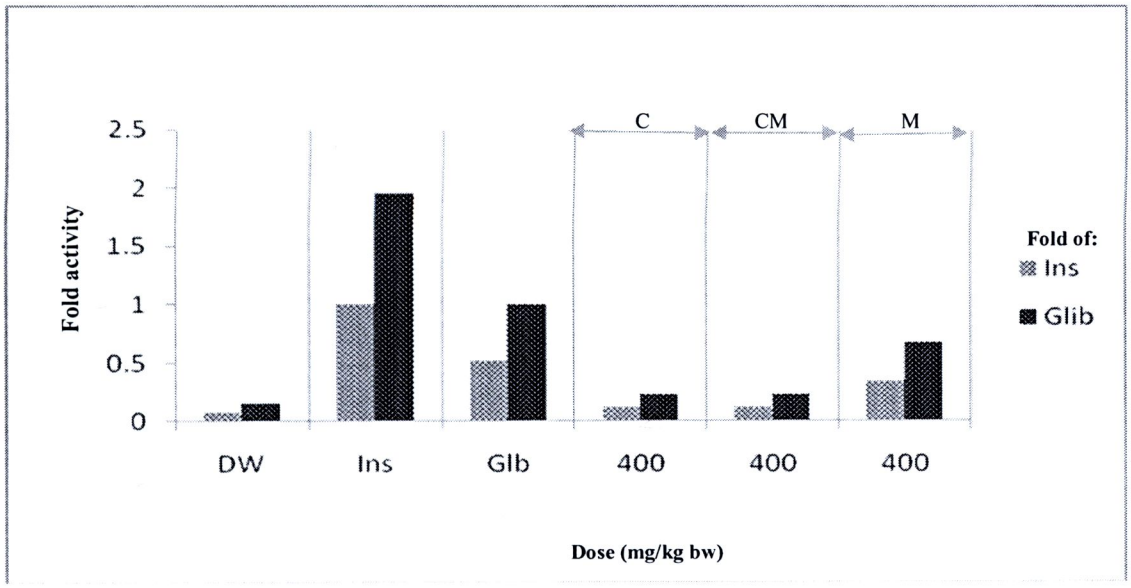
The chloroform sub-fractions, $CHCl_3$, CHCl-MeOH and MeOH sub-fractions also showed significant ($p<0.05$) FBG reduction of 10.49, 10.34 and 29.96% at 2 and 4 h with 400 mg/kg bw, respectively (Fig 3.33).



DW: Distilled water, Insulin, Glb: Glibenclamide: C: Chloroform, M: Methanol

Fig 3.33 Hypoglycemic effects of chloroform sub-fractions of *Anogeissus acuminata* in alloxan induced diabetic mice. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

The chloroform sub-fractions, $CHCl_3$, CHCl-MeOH and MeOH reduction were 0.34 and 0.67, 0.12 and 0.23 folds compared to insulin and glibenclamide, respectively (Fig 3.34).



C: chloroform, CM: chloroform: methanol, M: methanol

Fig 3.34 Hypoglycemic effects of chloroform sub-fractions of *A. acuminata* in alloxan induced diabetic mice in fold comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.* Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

Modified oral glucose tolerance test

The test was performed by feeding the mice with various carbohydrates. The carbohydrates were given 2 h after being fed with the methanol sub-fraction (250 mg/kg *bw*) or glibenclamide (1.00 mg/kg *bw*) * $p < 0.05$. **a**). The methanol sub-fraction was ineffective on the PPBG levels in corn starch fed group, **b**) the methanol sub-fraction effectively reduced the PPBG levels in glucose fed group, **c**) the methanol sub-fraction was ineffective on the PPBG level in the sucrose fed group and **d**) the methanol sub-fraction effectively reduced the PPBG levels in the lactose fed group.

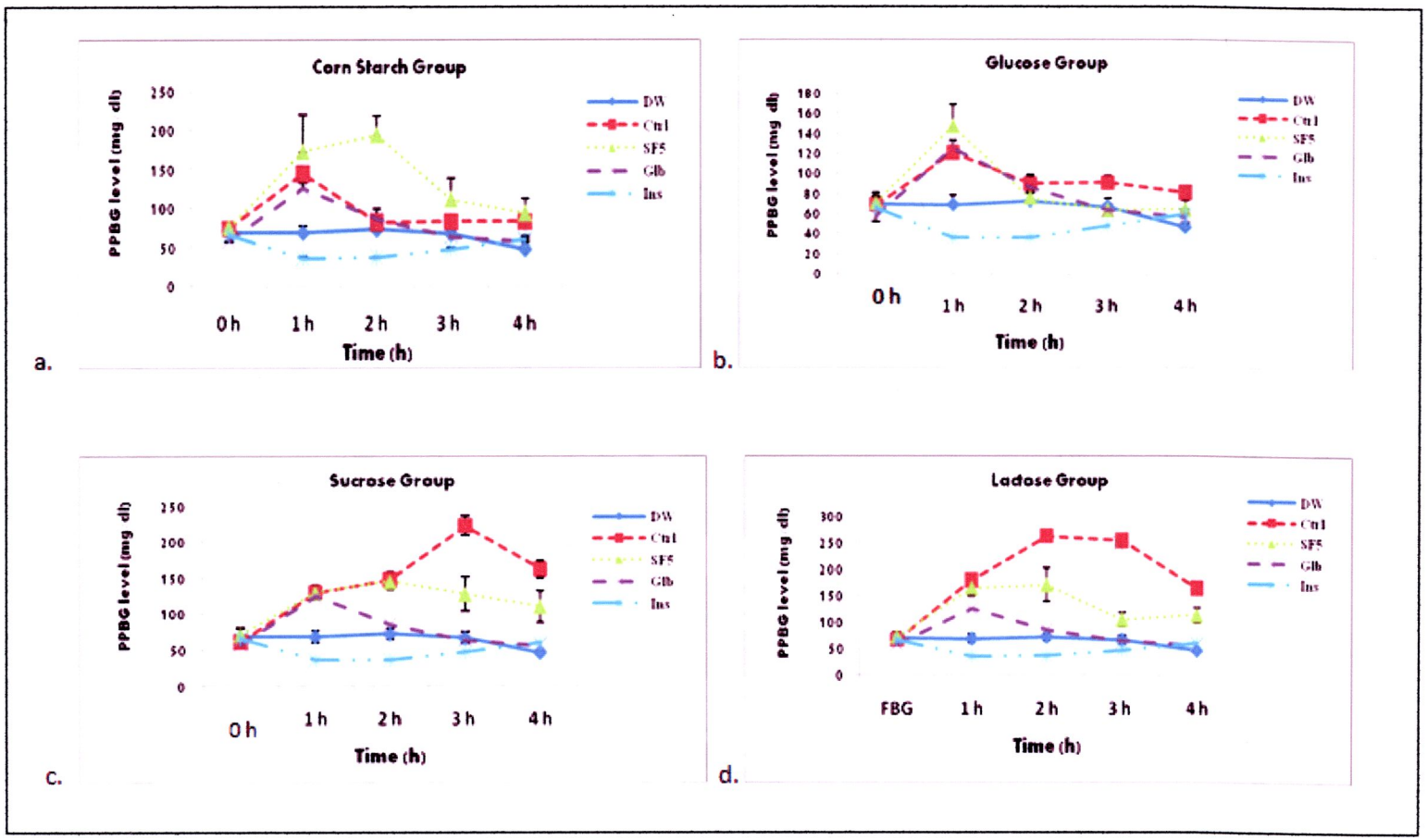
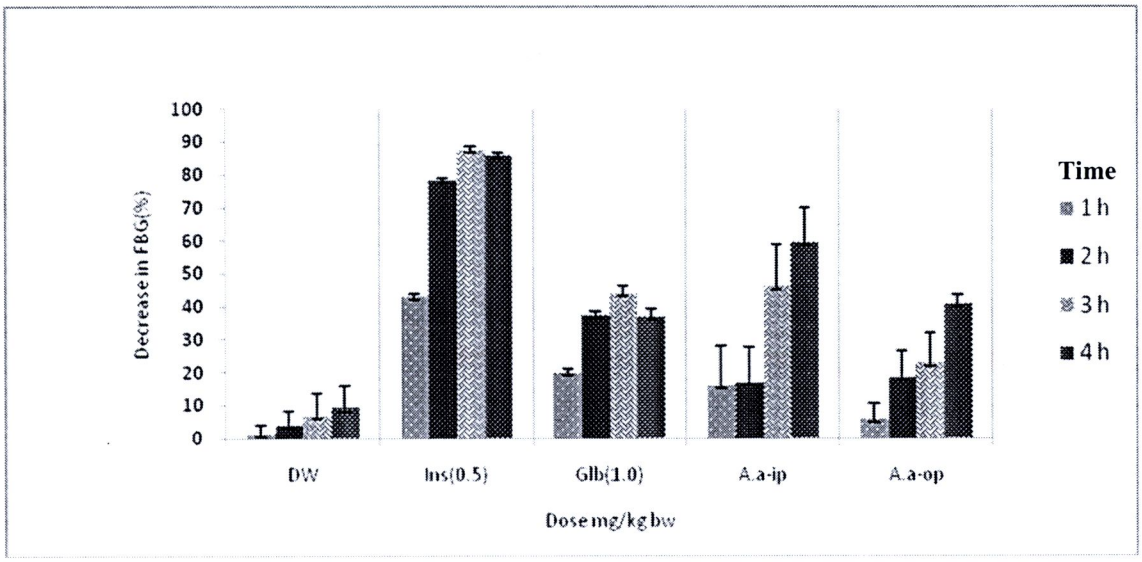


Fig 3.35 Hypoglycemic effect of *A. acuminata* methanol sub-fraction in postprandial glucose (PPBG) levels of normal mice with various carbohydrates. DW: distilled water, Cst: Corn starch, Glu: glucose, Suc: sucrose and Lac: lactose

Oral and intraperitoneal administration of *A. acuminata* methanol fraction

The intraperitoneal administration of the methanol sub-fraction showed hypoglycemic effects at different time intervals. The effect observed in the 62.5 mg/kg *bw, ip* group was higher than those of the glibenclamide (1.0 mg/kg *bw, po*) but lower than the insulin (0.5 iu/kg *bw, iv.*) (Fig 3.36).



A.a-ip: *A. acuminata* – intraperitoneal, A.a-op: *A. acuminata*-oral

Fig 3.36 Comparison between oral and intraperitoneal administration of *A. acuminata* methanol sub-fraction (62.5 or 120 mg/kg) compared with insulin *i.p* (0.5 iu/kg bw) and glibenclamide *o.p* (1.0 mg/kg bw) and distilled water (DW) in diabetic mice.

Effects of calcium or potassium channel regulators on the hypoglycemic activity of *A. acuminata*

FBG levels decreased significantly when treated with the methanol sub-fraction in combination with normal saline, *iv* (methanol sub-fraction) more than the

normal control group (**NC**). The hypoglycemic effects was diminished when the mice were co-treated with a Ca^{2+} ion channel regulator, nifedipine (methanol sub-fraction/**N**) or a K^{+} ion channel regulator, isosorbide dinitrate (methanol sub-fraction/**I**) (Fig 3.37). This indicated that the hypoglycemic activity of the methanol sub-fraction was dependent on the closure of K^{+} and opening of the Ca^{2+} channel. It could therefore be speculated that the sub-fraction possible binding to the receptors on the β-cells of the islet of Langerhans stimulated insulin secretion resulting in the FBG reduction in the diabetic mice.

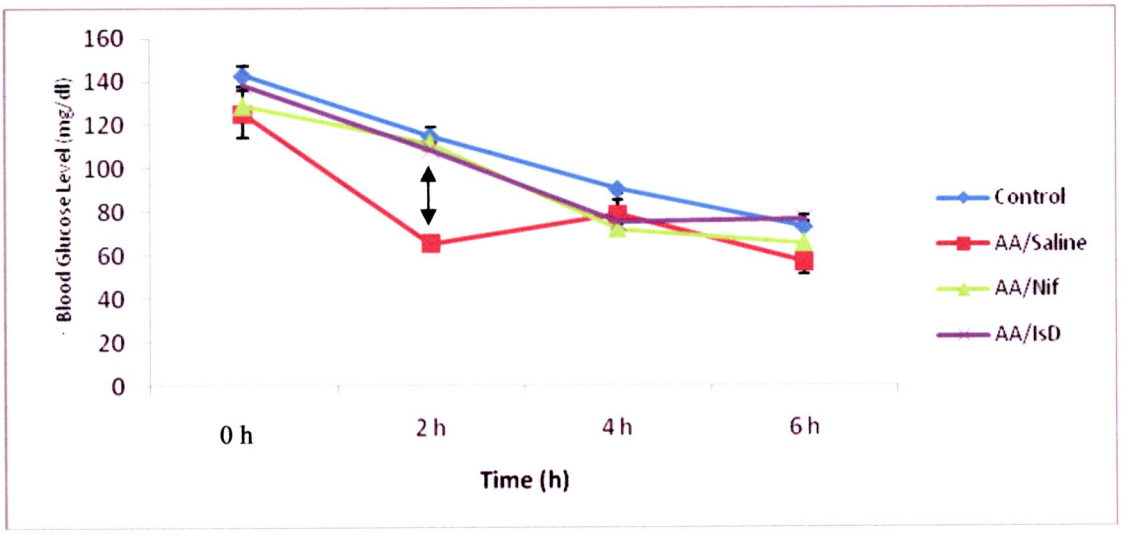


Fig 3.37 Effects of calcium or potassium channel regulators on the hypoglycemic activity of *A. acuminata* methanol sub-fractions in normal mice. NC, normal control treated with distilled water only, AA: treated with *A. acuminata* (250 mg/kg *i.p.*), AAN: treated with *A. acuminata* 250 mg/kg *i.p.*) + nifedipine (13.6 mg/kg, *o.p.*); + AAI: *A. acuminata* (250 mg/kg *i.p.*) + isosorbide dinitrate (6.8 mg/kg, o.p.). $*p < 0.05$ vs. NC group.

Mean body weight (g) of mice (n=5) after 14 days acute toxicity study

Significant body weight gains were observed in the 5,000 mg/kg *bw* treated groups at day 3 to 14. The body weight gain might be from the effect of the methanol sub-fraction.

Table 3.4.5 Mean body weight (g) of mice (n=5) after 14 days acute toxicity study after feeding by gavage with doses of 2000 and 5000 mg/kg *bw* of the methanolic sub-fraction

Day	Control	Mean body weight (mean + S.E.M)	
		2000 mg/kg	5000 mg/kg
0	25.00 ± 0.00	25.50 ± 0.30*	25.50 ± 0.33*
3	25.25 ± 0.30	25.00 ± 0.47	25.75 ± 0.55
6	25.50 ± 0.43	25.50 ± 0.58	27.75 ± 0.29*
9	25.25 ± 0.28	25.50 ± 0.58	27.60 ± 0.30*
12	25.50 ± 0.22	24.00 ± 0.00	27.50 ± 0.30*
14	25.50 ± 0.30	25.25 ± 0.29	27.00 ± 0.47*

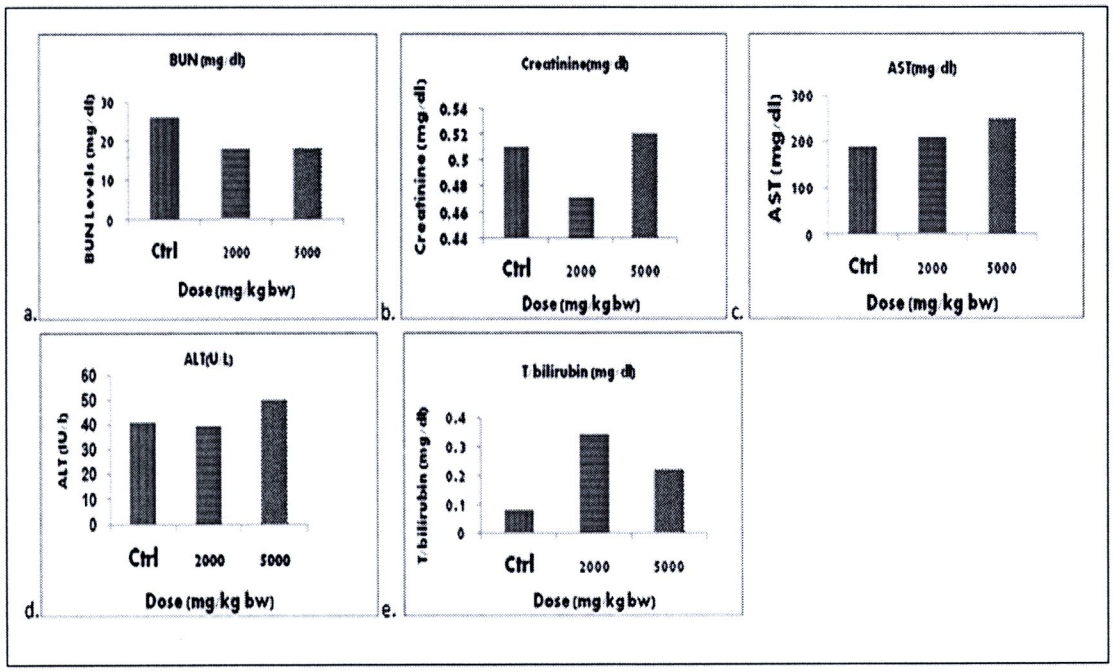
The 2,000 mg/kg *bw* groups gave a significant ($P<0.05$) decrease in organ weights in comparing to the control group while the reverse was the case with the 5,000 mg/kg *bw* (Table 3.4.5 and 3.4.6).

Table 3.4.6 Mean organ weights (g) of mice (n=5) at 14th day after being treated with the SF5 at the doses of 2,000 and 5,000 mg/kg bw

Organ	Control		Fraction of *A. acuminata* (M)			
	Untreated	RW (%)	2000 (mg)	RW (%)	5000 (mg)	RW (%)
Kidney	0.5788 ± 0.02	2.27 ± 0.04	0.5033 ± 0.03*	1.99 ± 0.05*	1.0123 ± 0.12*	3.75 ± 0.04
Liver	1.8698 ± 0.08	7.33 ± 0.09	1.8063 ± 0.22	7.15 ± 0.14*	2.6040 ± 0.30*	9.64 ± 0.09*

*: Significant difference from the control, $p<0.05$, RW: Relative weight = organ weight/body weight x 100

Liver and kidney function tests of the ICR mice fed with 2000 and 5000 mg/kg *bw* of the methanol sub-fraction of *A. acuminata*



BUN: blood urea nitrogen, AST: aspartate transaminase, ALT: alanine transaminase, T/bilirubin: total bilirubin

Fig 3.38 Liver and kidney function tests of the ICR mice fed with 2000 and 5000 mg/kg *bw* of the methanol sub-fraction of *A. acuminata*. **a**). BUN levels, **b**). Creatinine levels **c**). AST levels, **d**). ALT levels and **e**). T/bilirubin levels.

BUN levels decreased in both the 2,000 mg/kg *bw* and 5,000 mg/kg *bw* groups while creatinine decreased in the 2,000 mg/kg *bw* but increased in the 5,000 mg/kg *bw* treated groups (Fig 3.38a and b). However, a dose dependent elevation was observed for AST, ALT and total bilirubin levels of the 2,000 and 5,000 mg/kg *bw* though the 2,000 mg/kg *bw* group was higher (Figs 3.38c - e).

3.8 Fractionation and hypoglycemic activity of Nigerian medicinal plant *A. mannii*

A. mannii was chosen for this phase. The sub-fractions obtained from the fractionation of the *A. acuminata* extracts are shown in Fig 3.39.

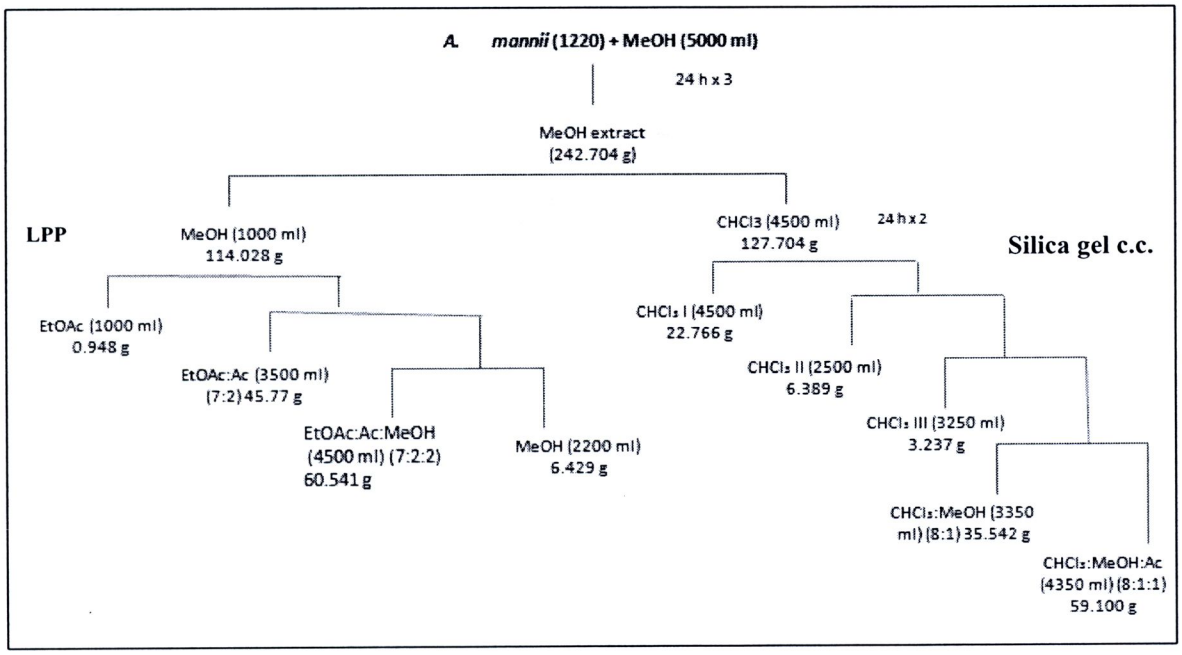


Fig 3.39 Result of the fractionation of methanolic extract of *A. mannii* showing the yields from every solvent used. Solvents used were arrived at after testing by Thin Layer Chromatography (TLC). The final solvent were Ethyl acetate-Acetic acid-Methanol (7:2:2) for the methanol sub-fraction and Chloroform-Methanol-Acetic acid (8:1:1) for the chloroform sub-fraction.

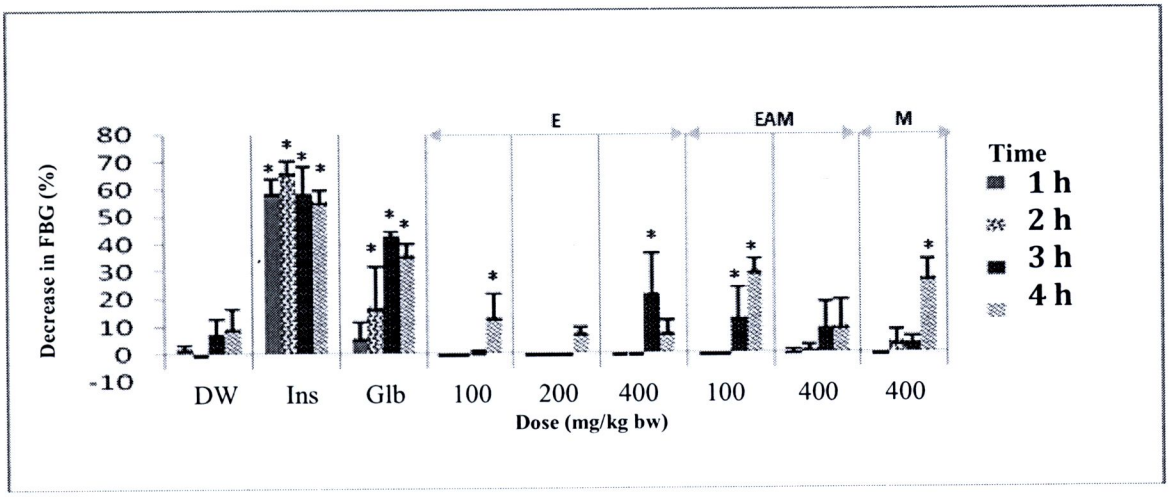
After the reflux, filtration and evaporation of the methanol crude extract of *A. mannii*, the percentage yield was 19.90%. After partitioning, the chloroform fraction was 10.47% and (Fig 3.39) the methanolic fraction was 9.35%. For the elution with the silica gel column chromatography of the chloroform fraction, there were five separate sub-fractions as follows $\mathbf{C}^1$ 1.87% (22.77 g), $\mathbf{C}^2$ 0.52% (6.39 g), $\mathbf{C}^3$ 0.30%

(3.24 g). Three separate bands on the column were observed by using chloroform as an eluant and were eluted and collected separately as C^1, C^2 and C^3, while **CM** 2.91% (8:1) (35.54 g), **CAM** (8:1:1) 4.84% (59.10g) were eluted with chloroform/methanol (8:1) and chloroform/acetic acid/methanol (8:1:1), respectively. For the methanol residue, it gave the sub-fractions of **E** 0.08% (0.95 g), **EA** (7:2) 3.75% (45.77 g), **EAM** 4.96% (7:2:2) (60.54 g) and **M** 0.53% (6.43 g) by liquid phase partitioning.

3.8.1 Hypoglycemic activity of the methanol/chloroform sub-fractions of *A. mannii*

Normoglycemic (mice) groups

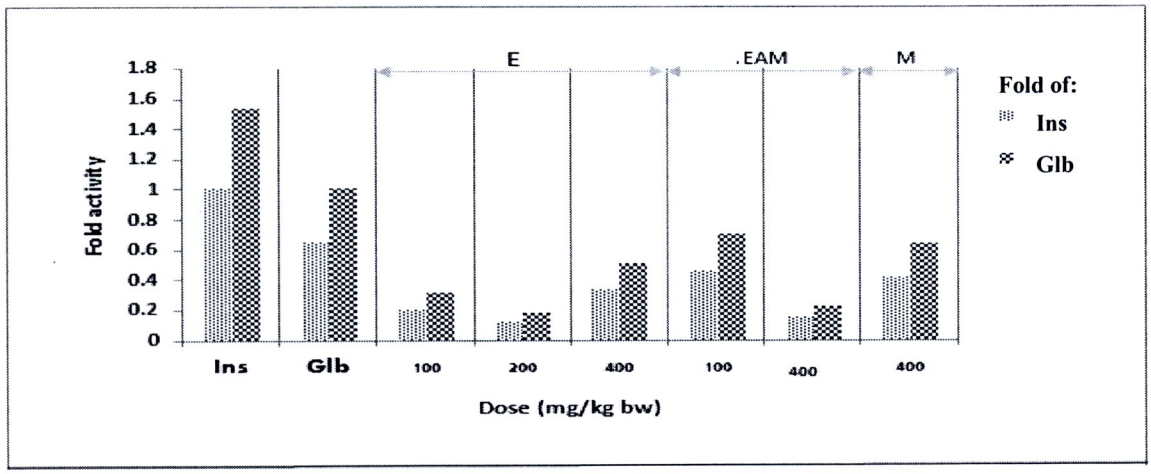
The 100 mg/kg *bw* of ethyl acetate-acetic acid, ethyl acetate-acetic acid-methanol and 400 mg/kg *bw* of ethyl acetate, and methanol sub-fractions gave significant ($p<0.05$) FBG reductions at 3 and 4 h, respectively (Fig 3.40). The highest reduction of 29.78% at 400 mg/kg *bw* of the ethyl acetate- acetic acid-methanol sub-fraction of *A. mannii*.



Distilled Water: **DW**, Insulin: **Ins**, Glibenclamide: **Glb**, Ethyl acetate - **E**, Ethyl acetate-acetic acid-methanol - **EAM** and methanol - **M** fractions.

Fig 3.40 Effect of three sub-fractions of *A. mannii* (methanol fraction) on normoglycemic mice. (↔) showing single fraction. Extracts not shown had no hypoglycemic effect. Values were mean ± s.e.m of five observations. Significant decrease ($p<0.05$) comparing with FBG at 0 h.

Comparing the above effect with standard drugs, the EAM effect which is the highest at the 4 h represents 0.45 and 0.70 folds of insulin and glibenclamide, respectively.

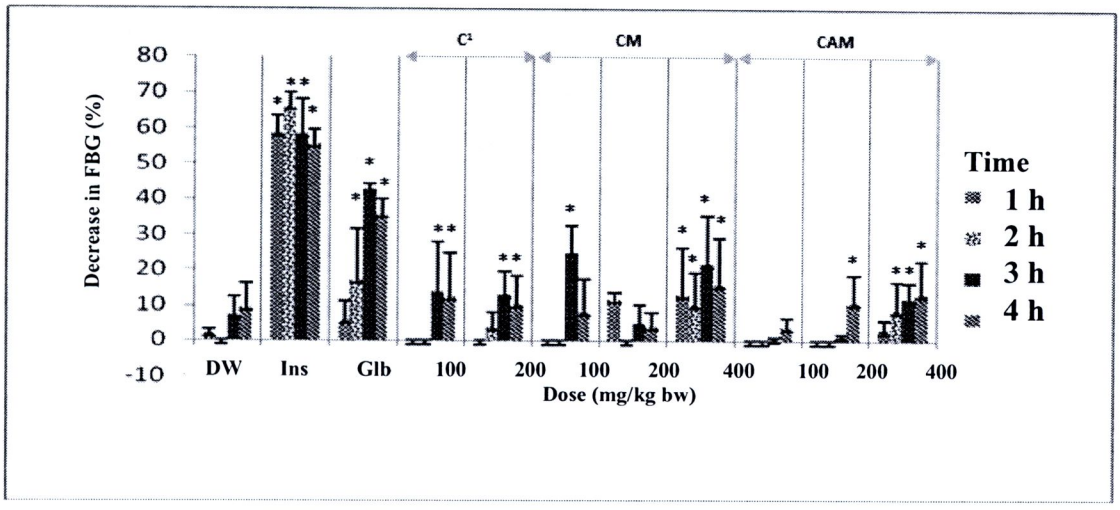


DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, E: Ethylaceatate, A: Acetic acid, M: Methanol

Fig 3.41 Hypoglycemic effects of *A. mannii* methanolic fractions in normoglycemic mice comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

The doses of 100 mg/kg *bw* of chloroform I and chloroform-methanol; 200 mg/kg *bw* of chloroform I, chloroform-methanol and chloroform-acetic acid-methanol

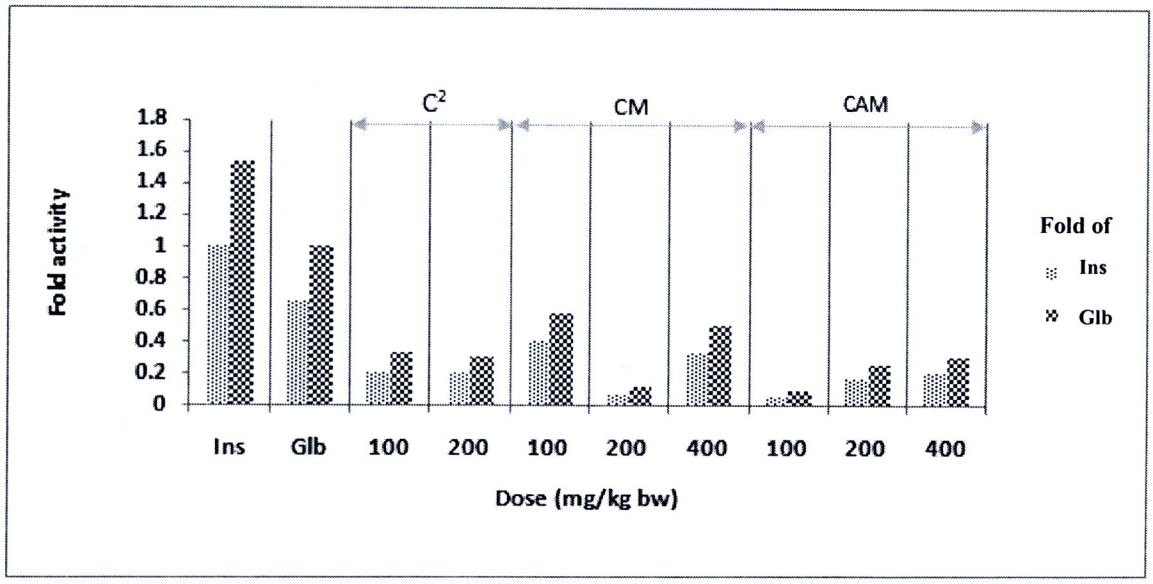
and 400 mg/kg *bw* of chloroform-methanol and chloroform-acetic acid-methanol indicated significant ($p<0.05$) hypoglycemic effect but were lower than glibenclamide and insulin. The CM sub-fraction at 100 mg/kg *bw* showed the highest FBG of 25.01% reduction (Fig 3.42).



Distilled water: DW, Insulin: Ins, C: Chloroform, M: Methanol, A: Acetic acid

Fig 3.42 Effect of *A. mannii* (chloroform fractions) on normoglycemic mice. (↔) showing single extract. Extracts not shown had no hypoglycemic effect. Values were mean ± SEM of five observations. Significant decrease ($p<0.05$) comparing with FBG at 0 h.

Comparing the CM sub-fraction which showed the highest hypoglycemic effect at the 1 h with the standard drugs, the reduction represents 0.38 and 0.58 fold of insulin and glibenclamide.

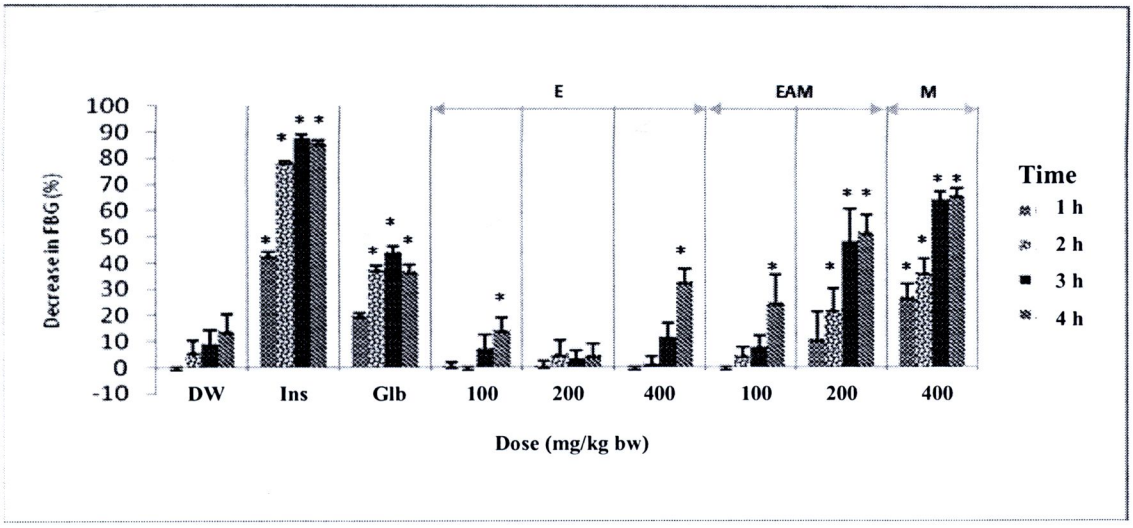


DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, C: Chloroform, A: Acetic acid, M: Methanol

Fig 3.43 Hypoglycemic effects of *A. mannii* Chloroform fractions in normoglycemic mice comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

Diabetic group

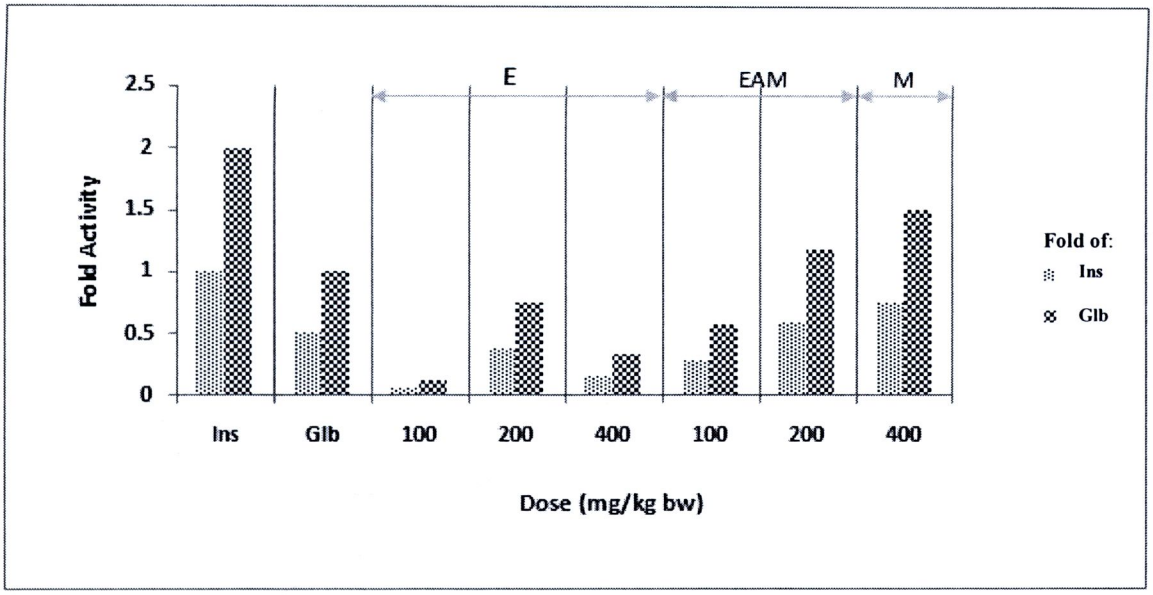
In the alloxan induced diabetic group, the methanol sub-fraction showed 65.57% reduction in FBG in the diabetic group.



Distilled Water: **DW**, Insulin: **Ins**, Glibenclamide: **Glb**, Ethyl acetate - **E**, Ethyl acetate-acetic acid-methanol - **EAM** and methanol - **M** fractions, respectively.

Fig 3.44 Hypoglycemic effect of *A. mannii* methanol fractions on diabetic mice. (↔) showing single extract. Extracts not shown had no hypoglycemic effect. Values were mean ± SEM of five observations. Significant decrease ($p<0.05$) comparing with FBG at 0 h.

In comparing the highest hypoglycemic effect (FBG reduction) of the methanol sub-fraction with the standard drugs, it represents 0.75 and 1.50 fold of insulin and glibenclamide, respectively.

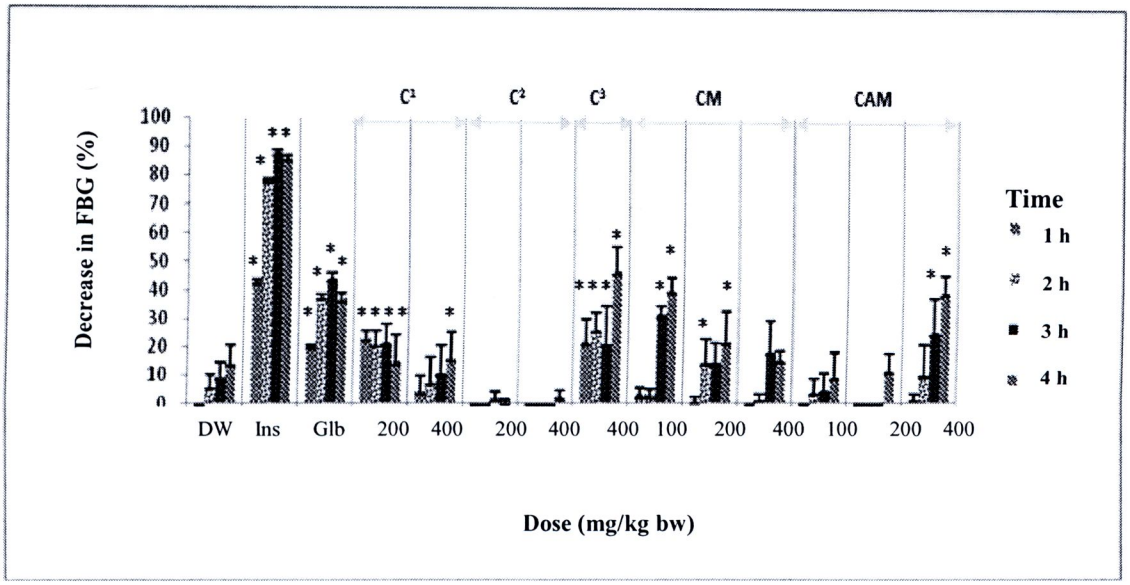


DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, E: Ethyl aceatate, A: Acetic acid, M: Methanol

Fig 3.45 Hypoglycemic effects of *A. mannii* methanolic fractions in alloxan diabetic mice comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

The dose of 400 mg/kg *bw* of C^3 sub-fraction showed significant ($p<0.05$) hypoglycemic effect of 46.89% at the 4 h which was higher in efficiency than glibenclamide. It was followed by 100 mg/kg *bw* of chloroform-methanol showed significant ($p<0.05$) hypoglycemic effect of about 40% which was comparable with glibenclamide at 3 and 4 h. The dose of 200 mg/kg *bw* of Chloroform I and chloroform-methanol gave the significant ($p<0.05$) FBG levels with the highest effect of 23%, while the 400 mg/kg *bw* of Chloroform III, chloroform-methanol and

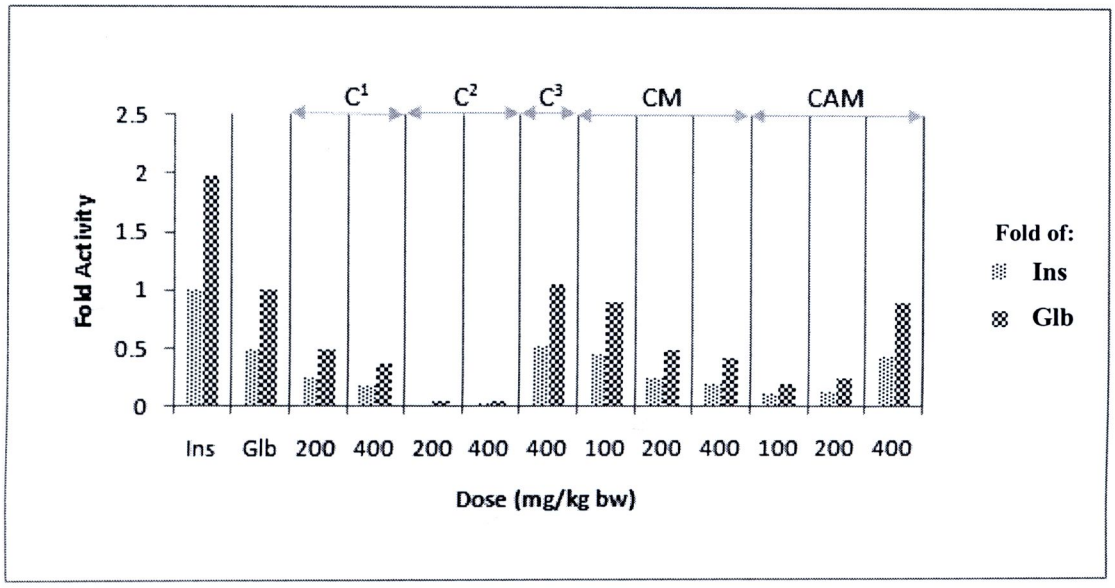
chloroform-acetic acid-methanol sub-fraction showed the effects of about 39% which were comparable to glibenclamide, but lower than insulin (Fig 3.46).



Distilled Water: **DW**, Insulin: Ins, Glibenclamide:**Glib**, Chloroform I- **C^1**, Chloroform II- **C^2**, Chloroform III- **C^3**, Chloroform: methanol - **CM,** Chloroform: acetic acid**:** methanol – **CAM**

Fig 3.46 Effect of *A. mannii* chloroform fractions on diabetic mice. (↔) showing single extract. Extracts not shown had no hypoglycemic effect. Values were mean ± s.e.m of five observations. Significant decrease ($p<0.05$) comparing with FBG at 0 h.

The Chloroform III sub-fraction was the most effective was 0.52 and 1.06 folds of insulin and glibenclamide, respectively. Other sub-fractions which showed no or reverse hypoglycemic effects were not represented. The 400 mg/kg *bw* of the methanol sub-fraction of the methanolic *A. mannii* crude fraction gave the most effective hypoglycemic activity and was used for further experiments (Fig 3.47).



DW: Distilled water, Ins: Insulin, Glb: Glibenclamide, C: Chloroform, A: Acetic acid, M: Methanol

Fig 3.47 Hypoglycemic effects of A. mannii fractions in diabetic mice comparing with the standard drugs. All bars are highest percentage decrease compared with the FBG at 0 h. Values are means ± SEM of groups of five observations.*Significant decrease ($p < 0.05$) comparing with FBG at 0 h.

Modified oral glucose tolerance test

The test was performed by feeding the mice with various carbohydrates (Fi 3.48). The carbohydrates were given 2 h after being fed with the methanol sub-fraction (250 mg/kg *bw*) or glibenclamide (1.00 mg/kg *bw*). *$p < 0.05$. **a)** the methanol sub-fraction was ineffective on the PPBG levels in corn starch fed group, **b)** the methanol sub-fraction effectively reduced the PPBG levels in glucose fed group, **c)** the methanol sub-fraction was ineffective on the PPBG level in the sucrose fed group and

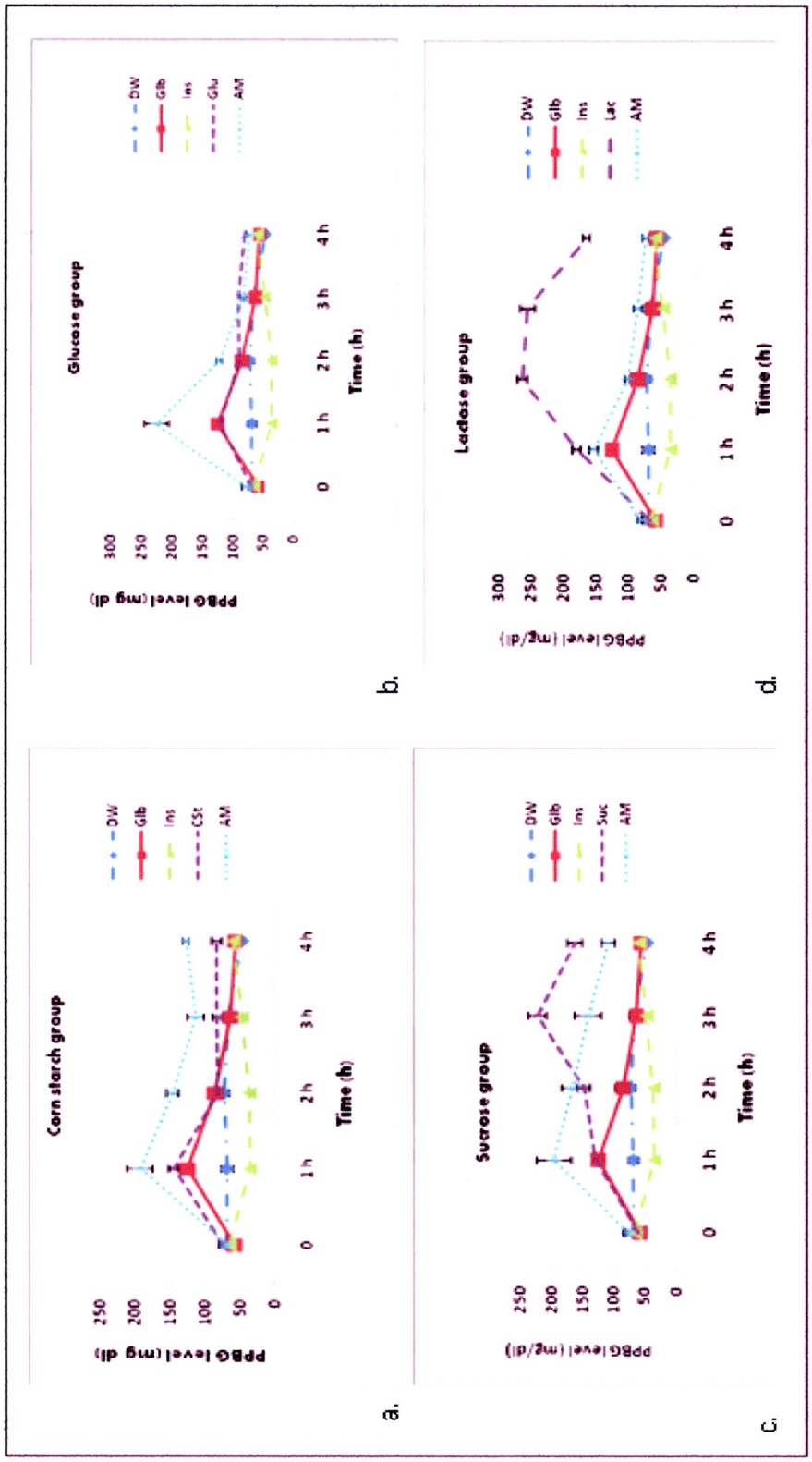


Fig 3.48 Hypoglycemic effect of *A. mannii* methanol sub-fraction in postprandial glucose (PPBG) in normoglycemic mice with various carbohydrates

d) the methanol sub-fraction effectively reduced the PPBG levels in the lactose fed group. DW: distilled water, Cst: corn starch, Glu: glucose, Suc: sucrose, Lac: lactose, AM: *A. mannii* sub-fraction, Glib: glibenclamide, Ins: insulin, PPBG: postprandial blood glucose. The hypoglycemic effects of the methanol sub-fraction in postprandial hyperglycemia in normal mice fed with various carbohydrates were shown in Fig 3.48. Oral administration of the methanol sub-fraction (250 mg/kg *bw*) inhibited the increase, but stimulated the decrease in post prandial glucose (Fig 3.48a to d).

Effect of oral and intraperitoneal administration of *A. mannii*

Both oral and intraperitoneal administration of the M sub-fraction showed similar

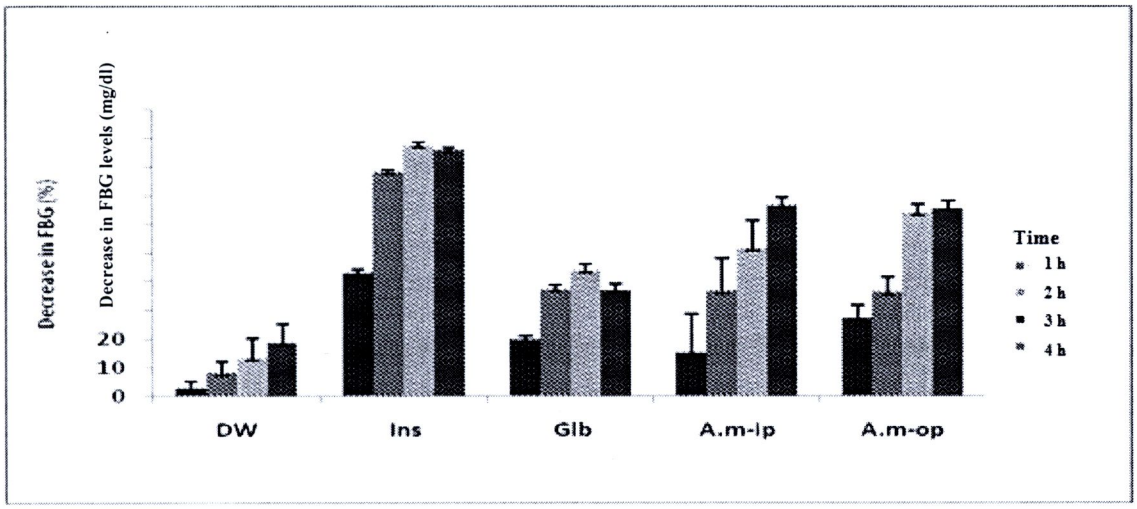


Fig 3.49 Effects of oral and intraperitoneal administration of the methanol sub-fraction of *A. mannii* (62.5 or 120 mg/kg) on FBG compared with insulin (0.5 I.U/kg bw- *ip*) and glibenclamide (1.0 mg/kg bw – *op*) in diabetic mice. DW: distilled water, Ins: Insulin, Glb: glibenclamide, A.m-ip: *A.mannii*-itraperitoneal, A.m-op: *A.mannii*-per oral hypoglycemic effects (Fig

3.49). The hypoglycemic effect of a single dose of the M sub-fraction (62.5 mg/kg *bw*, *ip* and 120 mg/kg *bw*, *po*) were compared to the standard drugs (insulin - 0.5 iu/kg *bw* by *iv* and glibenclamide 1.0 mg/kg *bw* by po). The sub-fraction showed higher efficiency than glibenclamide, but lower than insulin.

Effects of calcium (nifedipine) and potassium (isosorbide) channel regulators on hypoglycemic activity of the methanol sub-fraction of *A.mannii*

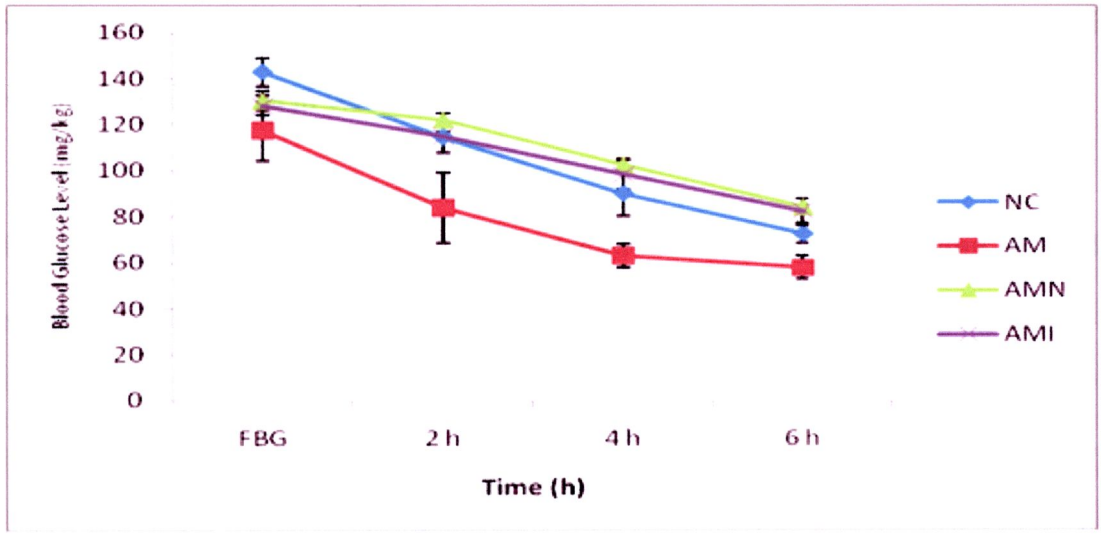


Fig 3.50 Effects of calcium (nifedipine) and potassium (isosorbide) channel regulators on hypoglycemic activity of the methanol sub-fraction of *A.mannii* in normal mice. NC, normal control treated with distilled water only, AM: treated with the sub-fraction M at 250 mg/kg *ip*, AMN: treated with the M subfraction at 250 mg/kg *ip* plus nifedipine (13.6 mg/kg, *o.p.*); AMI: treated with the M sub-fraction (250 mg/kg *ip*) plus isosorbide dinitrate (6.8 mg/kg, o.p.). $*p < 0.05$ *vs*. NC group. AM: *A. mannii*, AMN: *A. mannii*/Nifedipine, AMI: *A. mannii*/Isosorbidide dinitrate, NC: Normal control

Mean body weights (g) of mice (n=5) at 14 days after being fed with methanol sub-fraction of *A. mannii*

The body and organ weights of mice fed with *A. mannii* methanol sub-fraction at the doses of 2,000 and 5,000 mg/kg *bw* were shown in Tables 3.4.5 and 3.4.6. There were no animal deaths in any of the groups. Significant body weight gains were observed both in the 2,000 and 5,000 mg/kg *bw* treated groups at day 3 to 14. The relative body weights in the 2,000 mg/kg *bw* group were surprisingly higher than those in the 5,000 mg/kg *bw* group. The body weight gains might be from the effect of the methanol sub-fraction. The mean ± SEM of kidney and liver weights with their relative organ weights in comparing to the body weights were shown in Table 3.4.8.

Table 3.4.7 Mean body weights (g) of mice (n=5) at 14 days after being fed with methanol sub-fraction of *A. mannii* at the doses of 2,000 and 5,000 mg/kg *bw*

Day	Control	Mean body weight (me: ± SEM)	
		2,000 mg/kg	5,000 mg/kg
0	24.20 ± 0.86	23.40 ± 0.52	24.40 ± 0.66
3	23.40 ± 0.88	24.80 ± 0.26*[a]	24.80 ± 0.26
6	24.20 ± 0.63	24.60 ± 0.32*[a]	25.20 ± 0.63*[a]
9	24.60 ± 0.52	25.20 ± 0.26*[a]	25.60 ± 0.52*[a]
12	24.20 ± 0.48	24.80 ± 0.26*[a]	25.80 ± 0.63*[a]
14	25.20 ± 0.26	25.40 ± 0.32[a]	26.00 ± 0.58*[a]

*Significant difference from the control, $p<0.05$. [a] Significant difference from day 0 – 14, $p<0.05$. Each group was given the M *A. mannii* sub-fraction and normal saline sequentially in the first day and observed the body weights for 14 days.

Table 3.4.8 Mean organ weights (g) of mice (n=5) at 14 days after being fed with the M sub-fraction of *A. mannii* at the doses of 2,000 and 5,000 mg/kg *bw*

Organ	Control		Fraction of *A. mannii* (M)			
	Untreated	RW (%)	2000 (mg)	RW (%)	5000 (mg)	RW (%)
Kidney	0.3925 ± 0.02	1.5699 ± 0.09	0.3306 ± 0.04*	1.3223 ± 0.17*	0.3484 ± 0.01*	1.3937 ± 0.03*
Liver	1.4778 ± 0.06	5.9113 ± 0.24	1.3379 ± 0.06*	5.3517 ± 0.23*	1.3750 ± 0.01*	5.50 ± 0.02*

*Significant difference from the control, $p<0.05$. RW: Relative weight = organ weight/body weight x 100.

Liver and kidney function tests of the ICR mice fed with 2000 and 5000 mg/kg *bw* of the methanol sub-fraction *A. mannii*

All of the treated groups gave a significant ($P<0.05$) decrease in organ weights in comparing to the control group. The BUN and creatinine levels decreased in the methanol sub-fractions both at the 2,000 and 5,000 mg/kg *bw* treated groups (Fig 3.51a and b). However, a dose dependent elevation was observed for AST, ALT and total bilirubin levels.

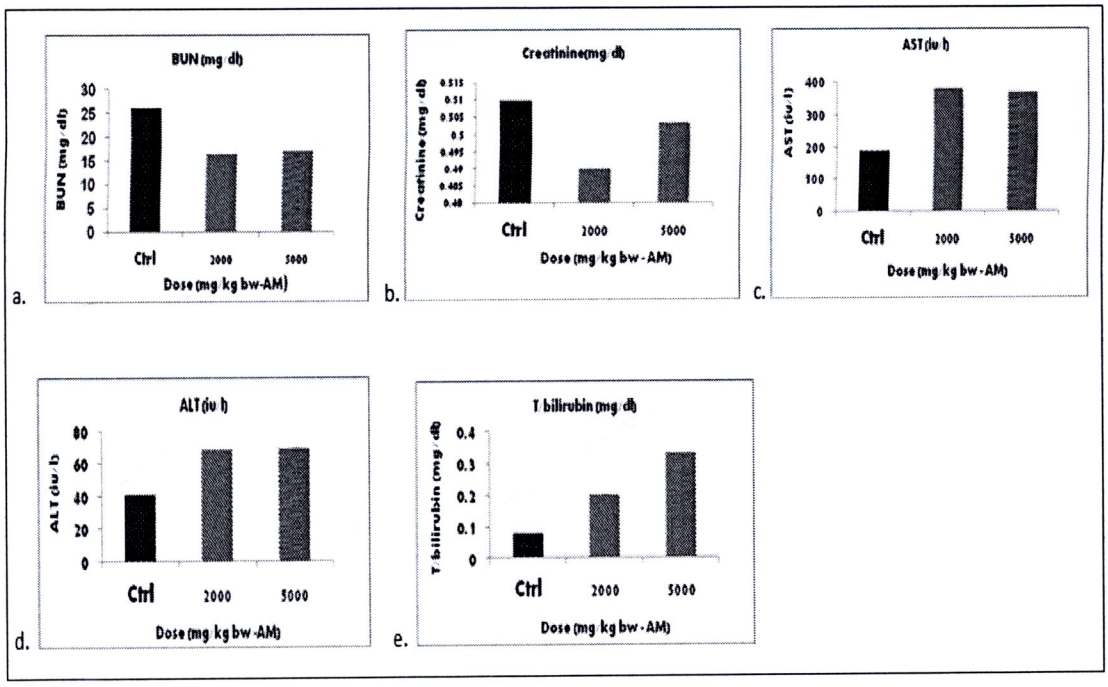


Fig 3.51 Liver and kidney function tests of the ICR mice fed with 2000 and 5000 mg/kg *bw* of the methanol sub-fraction *A. mannii* in ICR mice. **a**). BUN levels, **b**). creatinine levels **c**). AST levels, **d**). ALT levels and **e**). T/bilirubin levels. BUN: Blood Urea Nitrogen, AST: Aspartate transaminase, ALT: Alanine transaminase, T/bilirubin: Total Bilirubin.

The histopathological examinations

The histopathological examinations indicated no abnormality of the liver and kidney cells (Fig 3.52).

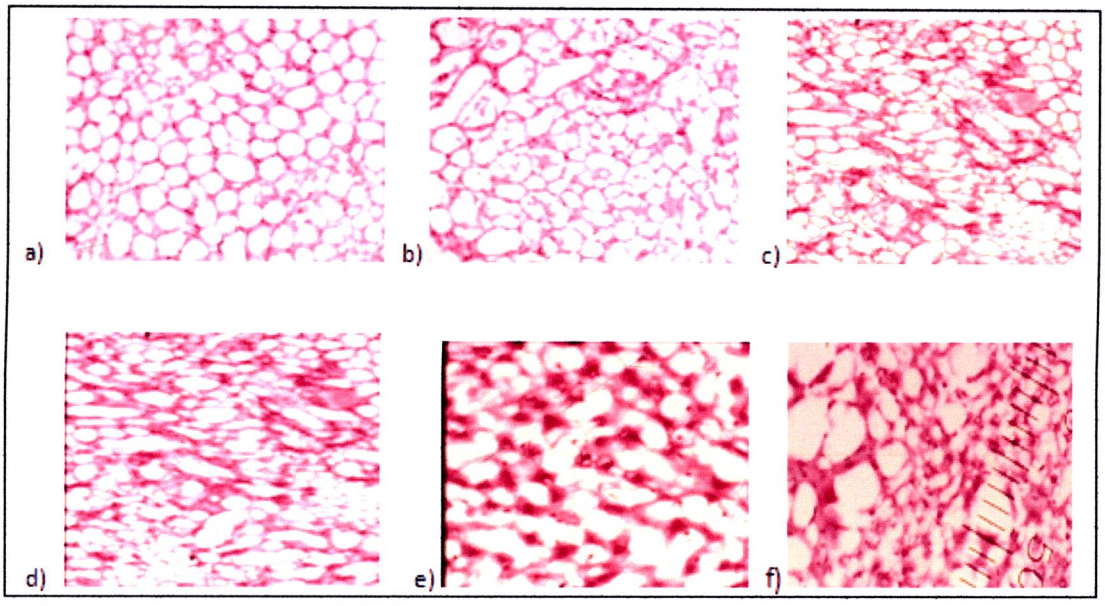


Fig 3.52 Pathology examination of kidney **a**. control, **b**. 2 000 mg/kg *bw* and **c.** 5 000 mg/kg *bw* treated and liver **d**. control, **e**. 2 000 mg/kg *bw* and **f.** 5 000 mg/kg *bw* treated from mice groups at 14 days.

Part 4: Purification and isolation of the selected fractions

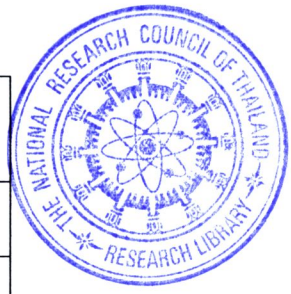
A. Thai hypoglycemic medicinal plant (methanolic sub-fraction of *A. acuminata*)

Table 3.4.9 The methanol sub-fraction of *A. acuminata* (8.0 g) was loaded unto ODS gel in a 90 x 4.5 cm column and semi-pure or pure fractions were eluted with methanol-water, 300 ml collected at intervals as follows:

Eluant	**Fraction**
MeOH:H_2O (1:9)	1 – 7
MeOH:H_2O (2:8)	8 – 17
MeOH:H_2O (3:7)	18 – 27
MeOH:H_2O (4:6)	28 – 34
MeOH:H_2O(100%)	35

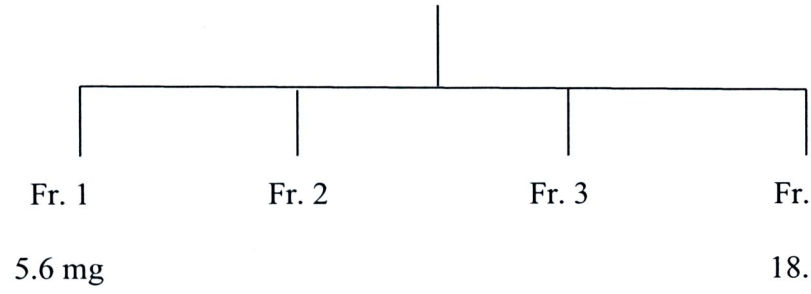
All fractions were monitored by analytical TLC and combined according to their composition as follows:

Table 3.4.9.1 Poling of the various fractions of *A. acuminata* based on the analytical TLC



S/No.	Fraction (g)	Isolated compound
1.	1(2.6250)	Not purified
2.	2(0.7859)	Not purified
3.	3(0.4883)	Sephadex (5 g)
4.	4-5(0.3605)	Not purified
5.	6-8(0.3564)	Not purified
6.	9(0.4918)	Not purified
7.	10(0.3956)	Not purified
8.	11-12(0.4660)	Not purified
9.	13-18(0.5727)	Not purified
10.	19(0.9717)	Not purified
11.	20(0.4236)	HPLC
12.	21-22(0.3929)	Not purified
13.	23-27(0.5444)	Not purified
14.	28(0.7003)	Sephadex gel
15.	29-34(0.2466)	
16.	35(0.1101)	Not purified

Purification of *A. acuminata* sub-fraction 3



Fraction 4 was precipitated wit excess EtOAc and subjected to NMR spectrum

Fig 3.53 Purification of *A. acuminata* sub-fraction 3 (0.4883 g)

Table 3.4.9.2 HPLC analysis of *A. acuminata* sub-fraction 11 (0.4236 g) using MeCN 30% at UV 253 nm

S/No.	Sample Wt (g)	Remark
1.	0.3861	Not purified
2.	0.0064	RI
3.	0.0050	Not purified
4.	0.0041	Not purified
5.	0.0072	RI
6.	0.0031	Not purified
7.	0.0007	Not purified

RI: refractive index detector

Table 3.4.9.3 RI Detection for sub-fractions 11 of *A. acuminata* using MeCN 30%

Fraction 11/2 (0.0064 g)

S/No.	Sample Wt(g)	Remark
1.	MeCN 30% - 0.0037	Not clear
2.	MeCN 30% - 0.0003	Not tested
3.	MeCN 30% - 0.0005	Not tested

Table 3.4.9.4 Purification of *A. acuminata* sub-fraction 11/5 (0.0072 g)

S/No.	Sample Wt(g)	Remark
1.	MeCN 30% - 0.0066	Not tested
2.	MeCN 30% - 0.0002	Not tested
3.	MeCN 30% - 0.0005	Not tested

Isolation of pure compound from the MeOH sub-fraction (3/4) of *A. acuminata*

Table 3.4.9.5 ^{1}H – NMR (400 MHz) Spectroscopic Data for Castalagin (D_2O)

Position	$\delta(H^{o})$
CH (1)	**5.65** (brs)
CH (2)	**5.06** (d, J = 7.3)
CH(3)	**5.00** (d, J = 6.9)
CH(4)	**5.09** (d, J = 6.9)
CH(5)	**5.49** (d, J = 6.9)
CH2 (6)	**4.12** (d, J = 12.8)
	4.90 (d, J = 11.9)
CH (2')^{iii}	**6.90** (s)
CH (2')^{iv}	**6.75** (s)
CH (2')^{v}	**6.70** (s)

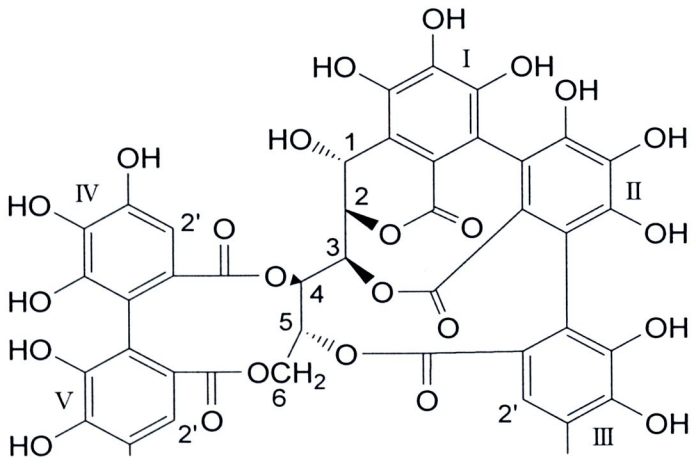


Fig 3.54 Chemical structure of Castalagin

(1, 2, 3, 5-nonahydroxytriphenoyl-4, 6-hexahydroxydiphenoyl-glucose)

B. Nigerian hypoglycemic medicinal plant (methanolic sub-fraction of *A. mannii*)

The methanolic sub-fraction of *A. mannii* (4.5335 g) was loaded unto ODS gel in a 90 x 4.5 cm column and eluted with methanol-water and 300 ml fraction were collected at intervals:

Table 3.5 The methanolic sub-fractions of *A. mannii* (Manosrin)

S/No	Eluant	Fraction
1.	MeOH:H_2O (1:9)	1 – 6
2.	MeOH:H_2O (2:8)	7 – 10
3.	MeOH:H_2O (3:7)	11 – 13
4.	MeOH:H_2O (4:6)	14 – 18
5.	MeOH:H_2O (5:5)	19 – 22
6.	MeOH:H_2O (6:4)	23 – 25
7.	MeOH:H_2O (1:9)	26 – 27
8.	MeOH (100%)	28

All fractions were monitored by analytical TLC and combined according to their composition as follows:

Table 3.5.1 Poling of the various sub-fractions of *A. mannii* based on the analytical TLC

S/No.	**Fractions (g)**	**Isolated Compounds**
1.	1-3(2.5378)	Not purified
2.	4-7(0.0598)	Not purified
3.	8-11(0.0749)	Not purified
4.	12-14(0.0602)	Not purified
5.	15-19(0.1948)	Not purified
6.	20-22(0.2411)	Not purified
7.	23-27(0.3916)	RI (MeOH 56%)
8.	28(0.5241)	Not purified

Table 3.5.2 RI Detection for sub-fractions 7 (0.3916 g) of *A. mannii* using 56% MeOH

S/No.	**Fractions (g)**	**Isolated Compounds**
1.	MeOH:H_20 (56%)	2.1430*
2.	MeOH:H_20 (56%)	0.0050
3.	MeOH:H_20 (56%)	0.0186*
4.	MeOH:H_20 (56%)	0.0076
5.	MeOH:H_20 (56%)	0.0071

*Further purification by RI detection

Table 3.5.3 RI Detection for sub-fractions 7/1 (2.1430 **g) of *A. mannii* using MeOH 56%**

S/No.	Sample wt(mg)	Isolated Compound
1.	0.20	Not tested
2.	0.30	Not tested
3.	0.70	Not tested

Table 3.5.4 RI Detection for sub-fractions 7/3 (0.0186 **g) of *A. mannii* using MeOH 56%**

S/No.	Sample wt(mg)	Isolated Compound
1.	214.30	Not tested
2.	0.80	Not tested
3.	3.60	Not tested
4.	0.00	---
5.	2.70	NMR/MS

Table 3.5.5 ^{13}C (100 MHz), spectroscopic data (δ values, CD 30D) for Fr. 7-3-4

NO	Position	Δ(C)	No	Position	Δ(C)	No	Position	Δ(C)
	Aglycone moiety			Aglycone moiety			Aglycone moiety	
1.	CH_2	39.0	11.	CH_2	24.7	21.	CH_2	35.2
2.	CH_2	26.9	12.	CH	123.7	22.	CH_2	33.0
3.	CH	83.6	13.	C	145.6	23.	CH_2	64.8
4.	C	43.0	14.	C	42.9	24.	CH_3	13.4
5.	CH	48.1	15.	CH_2	26.3	25.	CH_3	16.7
6.	CH_2	18.9	16.	CH_2	22.6	26.	CH_3	17.6
7.	CH_2	33.2	17.	C	37.7	27.	CH_3	26.6
8.	CH	41.1	18.	CH	43.9	28	CH_2	77.9
9.	CH	48.8	19.	CH_2	47.6	29.	CH_3	33.8
10.	C	37.6	20.	C	31.8	30	CH_3	24.2

Table 3.5.6 ^{1}H NMR (100 MHz) C- 3

Position	Sugar moeity Δ(C)	Position	Sugar moeity Δ(C)	Position	Sugar moeity Δ(C)
C – 1'	Glc – 105.7	C – 1''	Glc -105.3	C – 1'''	Xyl -105.5
C – 6'	Glc – 70.2	C – 6''	Glc – 69.6	C – 6'''	Xyl – 66.9
C – 28					
C – 1'	Glc – 104.8	C – 1''	Glc – 104.8		
C – 6'	Glc – 69.9	C – 6''	Glc – 62.7		

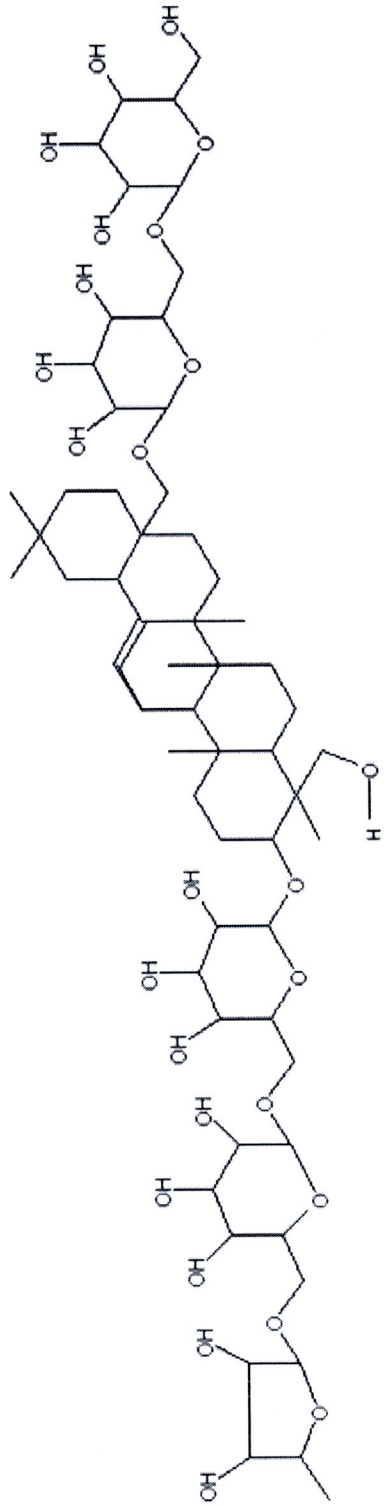


Fig 3.55 Structure of the pure compound from the methanolic sub-fraction of *A. mannii*

(3, 23, 28–Trihydroxy-12-oleanen-3-*O*-(β-D-glucopyranosyl-(1,6)-β-D-glucopyranosyl-(1,6)-β-D-xylopyranosyl)-28-*O*-β-D-glucopyranosyl-(1, 6)-β-D-glucopyranoside) or (Manosrin)

3.8 Part 5: Hypoglycemic effect of the isolated compounds

Table 3.8.1 ***Anogeissus acuminata*** **(Castalagin)**

Group/Time	0 h	1 h	2 h	3 h	4 h	FBG reduction (%)
DW (0.5 ml)	233.20 ± 14.37	247.60 ± 13.74	268.20 ± 12.33	234.60 ± 23.45	246.00 ± 16.55	0.00
Ins (0.5 iu/kg)	313.00 ± 84.54	1330 ± 27.78^{a,b,c}	90.60 ± 26.06^{a,b,c}	87.80 ± 12.79^{a,b,c}	88.40 ± 25.06^{a,b,c}	71.95
Glb (1.0 mg/kg)	237.60 ± 17.61	193.60 ± 21.54^{a,b,c}	146.40 ± 8.61^{a,b,c}	129.20 ± 6.37^{a,b,c}	130.80 ± 13.73^{a,b,c}	45.62
N	212.40 ± 13.43	265.00 ± 52.82	329.40 ± 55.02	275.60 ± 29.42	207.20 ± 36.76	2.45
2N	290.00 ± 37.77	367.00 ± 53.41	406.80 ± 43.31	402.80 ± 43.31	370.40 ± 28.28	0.00
5N	314.80 ± 25.86	412.20 ± 24.34	354.80 ± 48.06	143.60 ± 19.27^{a,b,c}	110.00 ± 12.85^{a,b,c}	65.06

Hypoglycemic effect of the methanolic pure compound (Castalagin) isolated from *Anogeissus acuminata compared* to distilled water: DW and standard drugs, insulin: Ins and glibenclamide: Glb and monitored 4 h post treatment. **N**: 0.32 µg/kg bw, **4N**: 1.60 µg/kg bw, **5N**: 4.00 µg/kg bw. Significant a: $p<0.05$, b: $p<0.01$, c: $p<0.001$

Table 3.8.2 *Anisopus mannii* **(Manosrin)**

Group/Time	0 h	1 h	2 h	3 h	4 h	FBG reduction (%)
DW (0.5 ml)	233.20 ± 14.37	247.60 ± 13.74	268.20 ± 12.33	234.60 ± 23.45	246.00 ± 16.55	0.00
Ins (0.5 iu/kg)	313.00 ± 84.54	1330 ± 27.78[a,b,c]	90.60 ± 26.06 [a,b,c]	87.80 ± 12.79 [a,b,c]	88.40 ± 25.06 [a,b,c]	71.95
Glb (1.0 mg/kg)	237.60 ± 17.61	193.60 ± 21.54 [a,b,c]	146.40 ± 8.61 [a,b,c]	129.20 ± 6.37 [a,b,c]	130.80 ± 13.73 [a,b,c]	45.62
N	222.20 ± 15.51	221.20 ± 31.52	215.40 ± 16.40	209.20 ± 16.16	207.80 ± 17.02	6.48
4N	310.80 ± 69.56	308.80 ± 78.02	292.80 ± 50.49	281.40 ± 82.94	268.20 ± 27.55	14.71
10N	257.80 ± 76.02	246.20 ± 50.41	197.40 ± 82.64	174.60 ± 50.18	141.40 ± 8.50[a]	**45.15**
100N	260.40 ± 78.97	210.80 ± 68.24	151.40 ± 54.11[a,b]	107.00 ±32.62[a,b,c]	83.40 ± 22.74[a,b,c]	**67.97**
1000N	295.60 ± 73.09	309.00 ± 62.54	245.60 ± 57.81	209.80 ± 54.69	210.20 ± 61.02	29.03

Hypoglycemic effect of the methanolic pure compound isolated from *Anisopus mannii* compared to distilled water: DW and standard drugs, insulin: Ins and glibenclamide: Glb and monitored 4 h post treatment. **N**: 0.128 µg/kg bw, **4N**: 1.28 µg/kg bw, **10N**: 3.20 µg/kg bw. **100N**: 32.00 µg/kg bw, **1,000N**: 320 µg/kg bw, Significant a: $p<0.05$, b: $p<0.01$, c: $p<0.001$