Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2016; 4(3): 29-37

Received: 04-03-2016
Accepted: 01-04-2016

Adedapo AA
Faculty of Veterinary Medicine
University of Ibadan
Ibadan, Nigeria.

Ogunmiluyi IO
Faculty of Veterinary Medicine
University of Ibadan
Ibadan, Nigeria.

Adeoye AT
Faculty of Veterinary Medicine
University of Ibadan
Ibadan, Nigeria.

Ofuegbe SO
Faculty of Veterinary Medicine
University of Ibadan
Ibadan, Nigeria.

Emikpe BO
Faculty of Veterinary Medicine
University of Ibadan
Ibadan, Nigeria.

Correspondence
Dr. Adedapo AA
Faculty of Veterinary Medicine
University of Ibadan
Ibadan, Nigeria
aa.adedapo@ui.edu.ng;
adedapo2a@gmail.com
Tel: +2348162746222

Evaluation of the medicinal potential of the methanol leaf extract of *Chromolaena odorata* in some laboratory animals

Adedapo AA, Ogunmiluyi IO, Adeoye AT, Ofuegbe SO, Emikpe BO

Abstract
Chromolaena odorata is used for its many medicinal properties, especially for external application as in wounds, skin infections, inflammation etc. The antidiabetic property of the methanolic leaf extract of *Chromolaena odorata* was evaluated in Wistar rats by inducing diabetes using a single intraperitoneal (I.P) injection of freshly prepared solution of alloxan monohydrate. Animals with glucose level of 200mg/dl were selected for the study and divided into 5 groups of 5 animals per group. The 6th group was not induced with alloxan. Treatment lasted for 28 days. Phytochemical analysis of the powdered and processed leaves was also carried out. After 28 days blood samples were collected for haematology and serum chemistry profile. Some organs were also collected for histopathology. Phytochemical analysis showed that the plant contained flavonoids, saponins, tannins, and anthraquinones while alkaloids, terpenoids and cardiac glycosides were absent. The group pre-treated with the 200mg/kg of the extract showed moderate changes in the levels of Albumin, ALT, AST, BUN, Cholesterol, Globulin, Glucose, Total Protein and Triglyceride in the animals and this was followed by the rats given 400mg of the extract post-induction. The histopathological changes in the organs were also moderate for the pre-treated group. The level of each of the parameters increased initially for the first 14 days. The effect of the extract is also dose dependent as the 400mg/kg dose post-induction exhibited a higher medicinal potential than the 200mg/kg dose post-induction. Anaemia was found to accompany the development of diabetes mellitus as there was a decrease in the levels of RBC, PCV, HB, MCH, MCV and MCHC when compared to the positive control rats. This anti-anaemic activity of the extract can be attributed to the high iron content of its chlorophyll. In the lipid profile studies, the increased level of cholesterol and triglycerides observed in the diabetic control group and also after induction of diabetes also suggested that the development of diabetes mellitus is usually accompanied by anomalies in body lipid composition. In conclusion, the result of this study showed that the methanol leaf extract of *Chromolaena odorata* exhibited hypoglycemic, hypolipidemic, anti-anaemic and possibly immune-stimulating and prophylactic properties.

Keywords: *Chromolaena odorata*; phytochemistry; medicinal potential; antidiabetic

Introduction
The use of herbs or plants has offered an effective medicine for the treatment of illnesses since the dawn of mankind. Moreover, many conventional/pharmaceutical drugs are derived from both nature and traditional remedies distributed around the world. Plant parts like seeds, berries, roots, leaves, fruits, bark, flowers, or even the whole plants are used in herbal therapy (FALODUN, 2010) [22]. Man was mainly dependent on crude botanical material for medical needs to retain vitality and cure diseases (JACK, 1997) [31], prior to the introduction of aspirin derived from *Spiraea ulmaria* which was already prescribed for fever and swelling in Egyptian papyri and recommended by the Greek Hippocrates for pain and fever. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivores mammals. Many of these phytochemicals have beneficial effect on long-term health when consumed by humans, and can be used effectively to treat human diseases. Not less than 12,000 such compounds have been isolated (LAI and ROY, 2004; TAPSELL *et al*., 2006) [33, 46]. Medicinal plants are highly esteemed as a rich source of new therapeutic agents for the prevention and treatment of diseases. Nowadays, the public acceptance of herbal medicine increases not only in Asian countries (49% in Japan, 45% in Singapore, 70% in China, and 80% in India), but also in western countries (MOULI *et al*., 2009) [39]. Medicinal plants have been used to treat various diseases for thousands of years, and since the 19th century many bioactive pure compounds isolated from these plants became very successful drugs (BEUTLER, 2009) [11].

Moreover, still today natural products are an important source for the discovery and development of new drugs (CRAGG and NEWMAN, 2013) [17]. In fact, natural products possess a high chemical scaffold diversity that are optimized to serve different biological functions, thus conferring on them a high drug-likeness and making them an excellent source for identification of new drug leads (CLARDY and WALSH, 2004; ERTL *et al*., 2008; HEMRICH and BEUTLER, 2013) [15, 21, 27]. The traditional use of plant preparations can often give strong hints for the pharmacological effects of their ingredients.

Chromolaena odorata is being used traditionally for its many medicinal properties, especially for external uses as in wounds, skin infections, inflammation etc. Studies have also shown that the leaf extract of this plant has antioxidant, anti-inflammatory, analgesic, anti-microbial, cytoprotective and many other medicinally-significant properties. The wound healing property of this plant has also been attributed to its antioxidant property which helps in conserving the fibroblast and keratinocyte proliferation on the site (PANDEY and VAISAKH, 2011) [41]. In Vietnam, *Chromolaena odorata* is traditionally used for wound healing. The aqueous extract and decoction from the leaves of this plant have been used for the treatment of soft-tissue wounds, burns, and skin infections (PHAN *et al*., 2001; THANG *et al*., 2001) [42, 47]. Trolox equivalent antioxidant capacity and ferric-reducing antioxidant capacity power assays showed that the antioxidant activities were strongly correlated with total phenols (LUXIMON-RAMMA *et al*., 2002; BHARGAVA *et al*., 2013) [36, 12]. In Thailand traditional medicine, *Chromolaena odorata* is used for the treatment of wounds, rashes, diabetes, and as insect repellent (INTA *et al*., 2013) [29]. Phytochemically, (9S,13R)-12-Oxo-phytodienoic acid in chloroform-soluble extract from the whole plant (DAT *et al*., 2009) [18] and odoratin in the dichloromethane extract (ZHANG *et al*., 2012) [51] have been identified and investigated as sources of peroxisome proliferating activating receptor gamma (PPARγ) ligands (WANG *et al*., 2014) [48].

The medicinal value of the methanolic extract of this plant was therefore evaluated in this study to validate folklore's claim.

Materials and Methods

Plant collection and preparation of extract

Fresh leaves of *Chromolaena odorata* were collected from the campus of the University of Ibadan, Ibadan, Nigeria. The leaves were dried under shade for about 10 days after which they were ground to powder using an electric blender. 300g of the powdered material was soaked in 1 litre of methanol and shaken vigorously. The sample was then filtered after 3days using a Buckner funnel and Whatman No. 1 filtered paper. The extract was further concentrated using a rotary evaporator. The weight of the extract was 14.8grams.

Phytochemical screening

The phytochemical analysis was performed on the food supplement for the identification of the constituents. The constituents tested for were alkaloids, tannins, saponins, anthraquinones, cardiac glycosides and flavonoids (MOODY *et al*., 2006; SAWADOGO *et al*., 2006) [38, 44].

The animals

The animals used in this study were male albino rats weighing between 120 and 150grams. They were kept in the Experimental Animal house of the Faculty of Veterinary Medicine, University of Ibadan throughout the period of this study. They were housed in rats' cages and were fed with standard rat cubes. They were given access to clean water at all times. All experimental procedures were in conformity with the University of Ibadan Ethics Committee on Research in Animals as well as internationally-accepted principles for Laboratory animal upkeep and use.

Antidiabetic study

Diabetes was induced in rats by a single intraperitoneal (I.P) injection of freshly prepared solution of Alloxan monohydrate (160mg/kg). Five days later, the blood samples of the rats were taken in order to check their blood glucose concentration before the commencement of the study. Animals with blood glucose of 200mg/dl and above were selected for the antidiabetic study.

Hypoglycemic effect of the methanol leaf extract of *C. Odorata* in rats

While 5 normal rats were placed in group 1 to serve as the positive control, 20 diabetic ones were divided randomly into 4 groups (2-5) of 5 rats per group. Group 2 which served as the diabetic control (negative control) received no treatment. Groups 3 and 4 animals received 200 and 400mg/kg of *Chromolaena odorata* methanol leaf extract respectively after induction while group 5 was pre-treated with 200mg/kg of the leaf extract before induction. Another group (the 6th group) was the non-diabetic group treated with 200mg/kg methanol leaf extract of *C. odorata*. All treatments were done daily via the oral route and lasted 24 days. Blood glucose level was then measured before (pre-treatment) and on days 14 and 28 post-treatment.

Haematological evaluation

All rats were sacrificed on the 28th day; but before that, blood samples were collected at medial canthus in the supraorbital plexus into EDTA treated bottles to be used for the determination of haematological parameters such as Red blood cell (RBC) counts, Packed cell volumes (PCV), Haemoglobin (Hb) concentrations, White blood cell (WBC) counts, White blood cell differential counts, Platelets counts, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC).

Serum chemistry profile

A portion of each blood sample collected into plain bottles and thereafter centrifuged to obtain clear plasma which was used to estimate total cholesterol, and triglycerides (TG), liver enzymes (ALT, AST), total protein, albumin and globulin, blood urea nitrogen etc using commercial kits and following standard procedures as outlined by the producer.

Histopathology

All rats were sacrificed on the 28th day and after collection of blood from each of the animals, the rats were opened up and a section was taken from four organs which include the liver, kidney, pancreas and heart for histology. The tissue sections were then fixed on slides with haematoxylin and eosin. The stained slides were fixed with mountant, allowed to dry and viewed under the microscope (x400).

Statistical analysis

Results were expressed as mean + SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA), students t-test at 95% level of significance was used to assess significant difference between controls and treated group.

Results

Phytochemical analysis of the leaves of *Chromolaena odorata* showed that tannin, saponin, flavonoid and anthraquinone were present while alkaloid, cardiac glycoside and terpenoid were absent (Table 1). Most of the haematological parameters were within range hence, were said to be normal (Tables 2, 3 and 4).With respect to the effects of the leaf extracts on the liver enzymes such as ALT and AST as well as BUN, varied changes were noted in the level of these parameters and the changes were statistically significant (Table 5).The effect of the extract on total protein (TP), albumin and globulin also experienced some significant changes as shown in Table 6. In Table 7, cholesterol and triglycerides experienced significant increase in their levels after induction but after treatment with the leaf extract, the levels of these parameters were significantly reduced. In the case of platelets, no significant changes were noticed. In this study, the glucose level of the rats experienced significant changes when induced with alloxan monohydrate. This increase was later significantly reduced in the groups treated with the leaf extract (Table 8). Histopathology showed that alloxan induction caused vacuolar degeneration, centrilobular necrosis of hepatocytes, atrophy of hepatic cords and Kupffer hyperplasia in the liver of group B animals. In the kidney of the animals in this group patchy coagulation necrosis of renal tubular epithelium (RTE) and a few casts in tubular lumen were also recorded. Necrosis and atrophy of islet cells were noticed in the pancreas while myofibre degeneration (fragmentation and loss of striation) was noticed in the heart of group B animals. In groups C and D animals, these were far less severe but in groups E and F animals, there were no visible lesions recorded (Figures 1-8).

Table 1: Phytochemical constituents of the leaves of *Chromolaena odorata*

Test	Observation	Inference
Test for Tannins 0.5g of powdered sample + boil in 20ml distilled water, filtered+ few drops of 0.1% $Fecl_3$	A blue black colouration was observed in the test tube.	Tannin is present.
Test for saponins 2g of powdered sample + boil in 20ml distilled water, filtered. 10ml filtrate + 5ml distilled water + vigorous shaking + 3 drops of olive oil.	Formation of froth and presence of emulsion after addition of olive oil.	Saponin is present
Test for Flavonoids Aqueous extract + few drops of 1% NH_3	A yellow colouration is observed	Flavonoid is present but of lesser concentration
Test for Terpenoids 5ml of aqueous extract + 2ml $CHCl_3$ + 3ml conc. H_2SO_4	An interface of a green colouration is observed	Terpenoid is absent
Test for glycosides 5ml of aqueous extract + 2ml glacial acid CH_3CO_2H + 1 drop $FeCl_3$ + 1ml conc. H_2SO_4	Brown ring expected was not seen	Cardiac glycoside is absent
Test for alkaloids 1g of methanolic extract + 5ml of 2M HCl + few drops of Meyers and Wagner's reagent.	Turbidity is not formed, precipitate not observed	Alkaloid is absent.
Test for anthraquinones 0.5g of the extract +10ml benzene which was then filtered. 10ml of 5% ammonia added to the filtrate and shaken.	A pink colour was observed	Anthraquinones is present

Table 2: Effect of methanol leaf extract of *Chromolaena odorata* on PCV, RBC and HB of rats

Treatments	Parameters	Basal	After induction	14 days	28 days
Positive control	PCV (%)	45.00±2.00	37.60±1.39	32.00±1.41	33.00±1.31
	RBC (10^9)	9.75±2.01	11.20±1.87	10.62±3.43	9.22±3.39
	HB	14.88±1.01	12.48±1.49	10.56±0.48	10.70±2.54
Negative control	PCV	36.20±2.2	37.00±3.16	34.25±2.42	37.00±1.73
	RBC	9.32±1.2	10.67±2.11	9.42±1.63	9.01±0.88
	HB	10.25±1.21	12.33±1.11	11.38±1.88	12.37±0.58
200mg	PCV	38.40±2.78	41.00±2.94*	37.00±1.58*	34.75±1.87
	RBC	11.11±1.35	17.78±1.95*	11.06±4.24	7.20±1.16
	HB	12.78±1.59	13.56±1.32	12.22±1.52	11.58±2.66
400 mg	PCV	38.80±0.84	40.60±2.79*	34.40±1.16	36.80±1.89
	RBC	9.72±1.59	10.08±2.32	11.29±2.27	9.12±1.79
	HB	12.88±0.21	13.42±0.93	11.34±1.38	11.84±1.53
Pretreated	PCV	51.40±1.14	39.75±2.87	34.33±1.69	33.00±2.85*
	RBC	10.54±1.49	10.04±0.83	11.74±1.38*	8.73±2.41
	HB	17.08±0.39	13.15±0.96	11.37±1.62	10.93±2.36
Non-diabetic	PCV	24.00±0.70	43.00±3.00*	35.33±2.77*	39.50±2.54*
	RBC	12.28±1.00	10.46±2.05	11.05±1.24	9.19±1.46
	HB	7.90±1.00	14.20±1.00	11.77±1.85	13.05±1.20

Results are expressed in Mean ± S.D, n=5

*when $p < 0.05$ against negative control

Table 3: Effect of methanol leaf extract of *Chromolaena odorata* on MCV, MCH and MCHC of rats

Treatments	Parameters	Basal	After induction	14 days	28 days
Positive control	MCV	46.80±2.69	33.40±1.95	31.60±1.96	35.50±0.71
	MCH	15.20±2.59	10.80±0.84	10.20±2.77	11.50±0.71
	MCHC	33.06±0.00	33.19±0.00	33.00±0.52	32.42±0.71
Negative control	MCV	38.84±2.13	35.50±1.33	36.00±2.53	41.00±2.00
	MCH	11.00±2.10	11.25±3.20	11.50±2.65	13.00±2.00
	MCHC	28.31±1.21	33.32±0.50	33.22±0.50	33.43±0.53
200mg	MCV	35.00±2.63	22.80±1.92*	36.00±1.16	46.75±2.27*
	MCH	11.20±2.86	7.20±0.84*	11.80±3.63	15.25±2.22
	MCHC	33.28±0.22	33.07±0.81	33.03±0.32	33.32±0.23
400 mg	MCV	40.20±2.98	41.20±1.76*	33.40±1.07*	40.60±2.13
	MCH	13.00±2.45	13.20±2.11	10.80±1.03	12.60±1.95
	MCHC	33.20±0.58	33.05±0.42	33.00±0.24	32.17±1.79
Pretreated	MCV	49.20±1.17	39.25±1.91*	28.33±2.43*	37.00±2.00*
	MCH	16.00±2.55	12.75±2.22	8.00±2.65*	11.67±0.58
	MCHC	33.22±0.44	33.08±0.63	32.18±0.58	33.12±0.20
Non-diabetic	MCV	19.00±1.00	42.00±2.54*	32.00±1.94*	43.00±2.83
	MCH	6.00±1.00	13.33±3.51	9.00±3.61	13.50±0.71
	MCHC	32.92±0.10	33.02±0.32	33.31±0.52	33.04±0.54

Results are expressed in Mean ± S.D, n=5
*when $p < 0.05$ against negative control

Table 4: Effect of methanol leaf extract of *Chromolaena odorata* on TWBC, NEUT and LYMP of rats

Treatments	Parameters	Basal	After induction	14 days	28 days
Positive control	TWBC	7.72±1.93	7.92±1.15	10.44±4.45	9.60±1.13
	NEUT	66.40±0.55	74.20±1.30	70.60±0.55	58.50±0.71
	LYMP	33.60±0.55	24.80±0.45	28.40±0.55	41.00±1.00
Negative control	TWBC	5.56±1.52	9.60±1.46	11.05±1.59	11.00±1.56
	NEUT	67.31±1.20	75.50±0.58	51.75±0.50	54.00±0.00
	LYMP	21.83±0.25	23.75±0.50	47.50±0.58	45.67±0.58
200mg	TWBC	7.84±3.17	10.08±2.16	9.36±3.75	10.00±0.73
	NEUT	71.80±0.84	63.80±1.10*	69.20±0.84*	58.75±0.96*
	LYMP	27.40±0.55	35.60±0.55*	30.20±0.45*	40.50±0.58*
400 mg	TWBC	4.16±1.09	8.64±1.51	14.96±2.77*	11.16±2.72
	NEUT	71.60±0.55	64.80±1.48*	68.60±0.55*	59.00±1.00*
	LYMP	27.60±0.55	34.80±1.64*	30.40±0.55*	40.40±0.55*
Pretreated	TWBC	4.72±1.56	6.40±2.23*	9.20±1.20*	9.93±2.32
	NEUT	59.40±0.55	59.00±1.83*	59.67±0.58*	57.67±2.52*
	LYMP	40.60±0.55	40.25±0.92*	40.00±1.00*	42.00±2.65*
Non-diabetic	TWBC	6.80±0.00	5.80±1.78*	8.00±2.12*	10.00±1.13
	NEUT	62.00±0.00	66.67±2.77*	67.67±1.12*	57.50±3.54*
	LYMP	37.00±1.00	33.33±2.77*	31.67±1.69*	42.50±3.54*

Results are expressed in Mean ± S.D, n=5
*when $p < 0.05$ against negative control

Table 5: Effect of methanol leaf extract of *Chromolaena odorata* on ALT, AST and BUN of rats

Treatments	Parameters	Basal	After induction	14 days	28 days
Positive control	ALT	63.00±1.87	37.40±3.44*	67.20±1.38*	35.00±1.41*
	AST	72.20±1.48	48.80±4.15*	66.60±2.84*	47.00±1.41*
	BUN	4.07±0.06	1.37±0.36*	1.57±0.47*	2.11±0.01*
Negative control	ALT	61.12±1.84	64.50±0.58	77.75±3.10*	37.00±1.44*
	AST	68.21±1.24	75.50±0.58*	87.75±3.55*	91.00±1.13*
	BUN	1.58±0.02	2.35±0.06	3.21±0.07*	3.88±0.41*
200mg	ALT	29.40±5.18	54.60±1.82*	76.20±1.85*	49.00±2.39*
	AST	40.20±2.26	64.40±1.52*	76.40±1.96*	57.00±3.02*
	BUN	1.57±0.50	2.19±0.17*	2.57±1.15*	1.87±0.58*
400 mg	ALT	45.00±0.71	45.80±0.45*	71.00±1.57*	47.60±3.35*
	AST	55.00±0.71	57.60±0.89*	63.40±1.03*	56.80±4.15
	BUN	2.22±0.01	1.28±0.04*	2.82±1.29	1.79±0.54*
Pretreated	ALT	64.20±2.19	52.00±2.32*	61.33±2.08*	46.67±2.86*
	AST	75.00±2.24	69.00±3.42*	65.33±2.43*	54.67±1.02*
	BUN	3.29±0.44	1.08±0.11*	1.25±0.84*	1.52±0.58*
Non-diabetic	ALT	20.00±1.00	61.00±2.12*	77.33±1.58*	48.50±2.78*
	AST	50.00±0.36	67.67±2.98*	88.67±2.18*	63.50±2.12*
	BUN	1.02±0.25	1.95±0.74	2.62±1.57*	1.06±0.06*

Results are expressed in Mean ± S.D, n=5
*when $p < 0.05$ against negative control

Table 6: Effect of methanol leaf extract of *Chromolaena odorata* on TP, ALB and GLB of rats

Treatments	Parameters	Basal	After induction	14 days	28 days
Positive control	TP	5.25±0.11	4.12±0.70	7.75±2.31	4.42±0.03
	ALB	1.20±0.12	1.02±0.05	2.37±1.16	1.12±0.03
	GLB	4.09±0.09	2.70±0.58*	5.38±1.19	3.30±0.00
Negative control	TP	4.32±0.12	5.35±0.06	11.07±0.77*	5.03±0.69
	ALB	1.32±0.18	1.13±0.05	4.20±0.87*	1.02±0.02
	GLB	3.00±0.52	4.18±0.05	6.88±0.64*	3.98±0.65
200mg	TP	3.81±0.43	5.15±0.25*	10.13±2.72*	4.83±1.79
	ALB	1.32±0.44	1.10±0.07	3.72±1.48	1.38±0.52
	GLB	2.68±0.44	4.05±0.28	6.41±1.36*	3.43±1.37
400 mg	TP	4.21±0.06	4.83±0.20	9.91±2.65*	4.89±1.01
	ALB	1.08±0.05	1.41±0.15	3.59±1.55*	1.14±0.15
	GLB	3.13±0.04	3.22±0.13	6.32±1.22*	3.73±1.02
Pretreated	TP	6.65±0.13	3.71±0.54*	8.80±1.20*	4.46±1.30*
	ALB	2.32±0.09	1.15±0.06	2.80±0.59*	1.11±0.11
	GLB	4.33±0.11	2.56±0.50*	6.00±0.98*	3.34±1.23
Non-diabetic	TP	2.45±0.00	5.37±1.36*	9.54±3.81*	3.27±0.21*
	ALB	1.05±0.00	1.91±0.35*	3.91±2.58*	1.02±0.01
	GLB	1.40±0.00	3.47±1.01*	5.63±1.31*	2.21±0.13*

Results are expressed in Mean ± S.D, n=5
*when $p < 0.05$ against negative control

Table 7: Effect of methanol leaf extract of *Chromolaena odorata* on CHOL, TRIGL and PLATELETS of rats

Treatments	Parameters	Basal	After induction	14 days	28 days
Positive control	CHOL	62.80±1.92	67.00±2.83	78.00±1.30	57.00±1.41
	TRIGL	63.40±2.41	137.40±2.11	130.8±2.63	63.00±1.41
	PLATELETS	10.00±1.00	10.80±1.10	10.00±0.00	9.00±4.24
Negative control	CHOL	60.25±1.38	64.50±0.58	99.25±1.50	118.33±2.69
	TRIGL	56.22±1.50	62.00±3.18	156.5±3.74	162.33±3.37
	PLATELETS	9.8±0.12	10.50±1.00	9.50±2.52	10.00±0.00
200mg	CHOL	39.40±4.56	53.40±2.41*	93.00±1.57*	74.00±4.32*
	TRIGL	47.40±2.27	164.0±2.28*	131.6±3.96*	81.00±2.90*
	PLATELETS	10.80±1.10	11.20±1.10	10.80±1.10	10.00±2.83
400 mg	CHOL	43.80±1.10	45.80±0.45*	90.80±1.19*	65.40±2.24*
	TRIGL	54.00±1.41	167.0±2.74*	125.0±3.79	81.80±1.03*
	PLATELETS	10.40±0.89	11.60±0.89	10.40±0.89	10.80±1.10
Pretreated	CHOL	72.60±4.88	59.25±3.22*	86.33±2.50*	43.67±3.21*
	TRIGL	83.20±2.17	236.0±4.64*	100.3±1.62*	48.00±2.21*
	PLATELETS	10.10±1.20	11.00±1.16	10.00±3.46	9.33±3.06
Non-diabetic	CHOL	25.00±0.63	59.67±1.46*	86.67±1.94*	49.00±1.90*
	TRIGL	42.00±0.65	44.67±2.76*	45.0±3.86*	46.50±2.51*
	PLATELETS	6.00±0.80	12.67±1.16*	10.67±1.16	11.00±1.41

Results are expressed in Mean ± S.D, n=5
*when $p < 0.05$ against negative control

Table 8: Effect of methanol leaf extract of *Chromolaena odorata* on the glucose level of rats

Glucose level on different days				
Treatments	**Basal**	**5 days**	**14 days**	**28 days**
Positive control	81.20±1.79	89.00±2.34	89.49±3.80	75.00±1.41
Negative control	65.10±1.62	225.00±2.83	242.75±2.22	252.67±1.37
200mg	59.60±3.85	228.6±3.78	136.80±1.79*	65.50±1.36*
400mg	64.00±2.35	238.0±4.06*	138.0±2.00*	63.60±3.54*
Pretreated	82.80±2.68	236.75±3.59*	122.7±2.57*	65.67±2.34*
Non-diabetic	63.40±0.00	59.00±4.78*	56.7±2.58*	58.50±2.16*

Results are expressed in Mean ± S.D, n=5
*when $p < 0.05$ against negative control

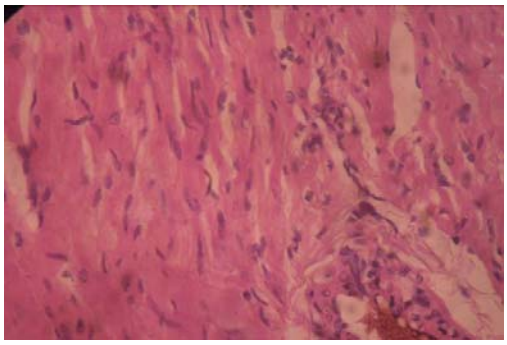

Fig 1: Heart section of group A animals (X 400) – no visible lesion

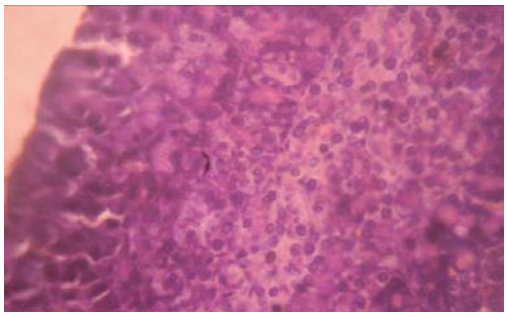

Fig 2: Pancreas section of group A animals (X 400) - no visible lesion

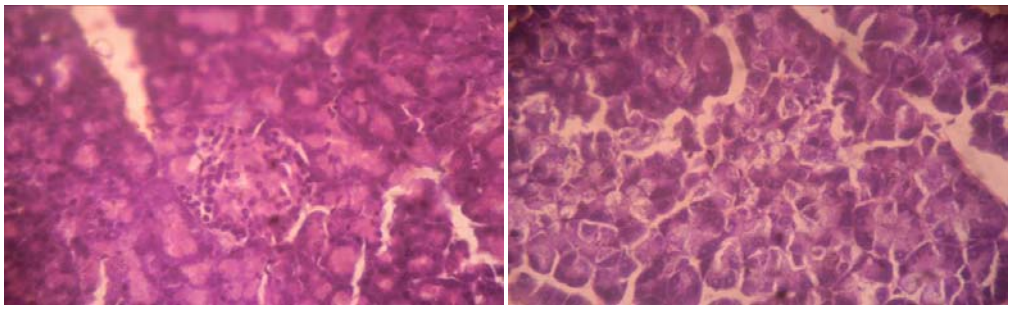

Fig 3: Pancreas section of group B animals (X 400) - Necrosis & atrophy of islets

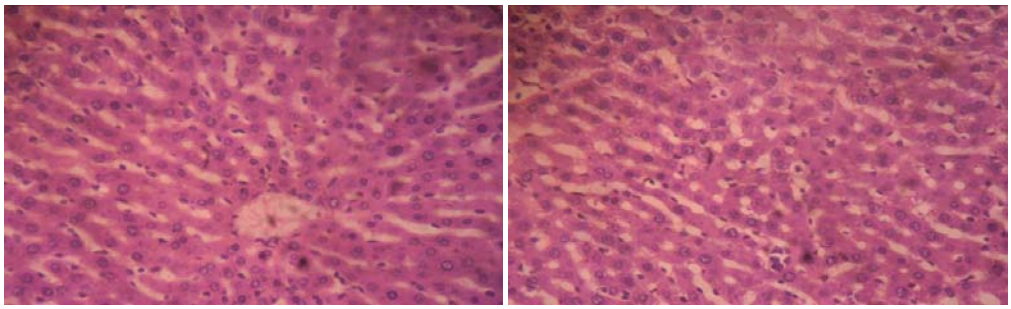

Fig 4: Liver section of group B animals (X 400) - Hepatocellular degeneration and atrophy, and accentuation of sinusoids

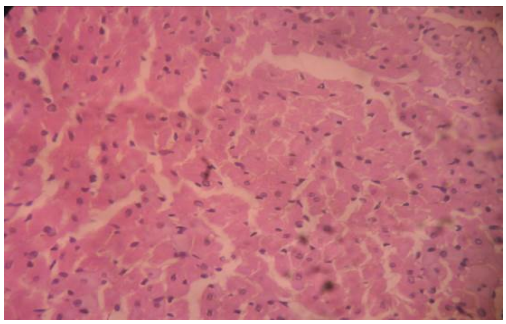

Fig 5: Heart section of group B animals (X 400) - Myofibre degeneration and necrosis

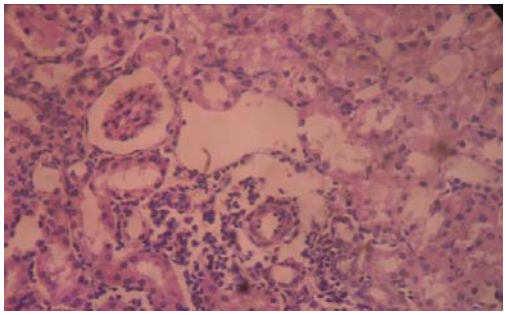

Fig 6: Kidney section of group B animals (X 400) - coagulation necrosis of tubular epithelia and vasculitis

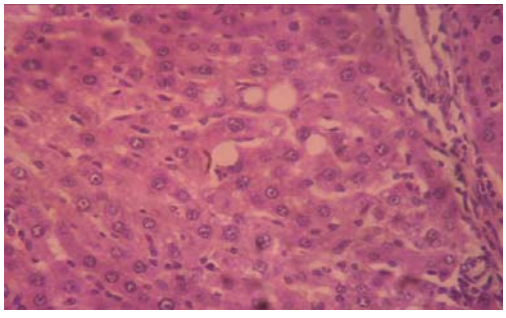

Fig 7: Liver section of group C animals (X 400) - Moderate vacuolar degeneration of hepatocytes

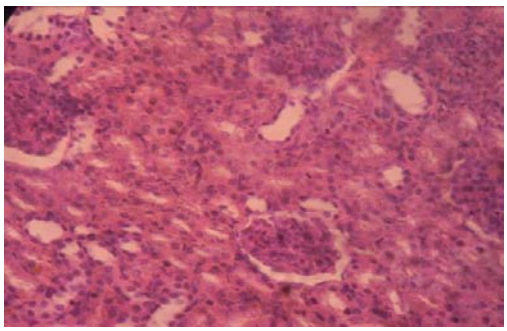

Fig 8: Kidney section of group E animals (X 400) - no visible lesion

Discussion

In this study, the pulverized leaves of *Chromolaena odorata* were found to contain tannin, saponin, flavonoids, and anthraquinones which all have medicinal properties. Alkaloids, terpenoids and cardiac glycosides on the other hand were absent. The absence of alkaloids, terpenoids and cardiac glycosides in this study is contrary to a study in which the aqueous extract of the pulverized leaf contained these phytochemicals (AKINMOLADUN *et al*., 2007; HARINI *et al*., 2014) [5, 25].

Anaemia was found to accompany the development of diabetes mellitus as there were slight lowering of RBC, PCV, HB, MCH, MCV and MCHC values when compared to the normal control rats. In diabetes mellitus, anaemia has always been observed particularly the hypochromic type, due to fall in the iron content of the body resulting from oxidation stress associated with the condition (AKINDELE *et al*., 2012; SALIU, 2012; COLAK *et al*., 2012) [4, 43, 16]. The anti-anaemic activity of the extract in this can be attributed to the high iron content of its chlorophyll, as seen in other green, leafy vegetables (SALIU *et al*., 2012) [43] and/or the ability to improve bone marrow functions (ADENEYE and AGBAJE, 2008) [2].

The animals that were pre-treated with the methanol extract of *Chromolaena odorata* showed the best results in terms of serum profile. In this regards the levels of Albumin, ALT, AST, BUN, cholesterol, Globulin, Glucose, Total Protein and

Triglyceride were within the normal range for these parameters even after induction with alloxan. This was then followed by the rats given 400mg/kg dose of the extract and lastly the 200mg/kg dose of the extract. The effect of the extract is thus dose-dependent. The level of each of the parameters increased initially for the first 14 days of the treatment before decreasing which implies that the extract does not have an immediate effect. There is also the possibility of the extract having a prophylactic property due to the fact that the value of most of the parameters for the pre-treated group even after induction seems not to increase. This possibly suggests that the extract contains substances with hypoglycemic properties and tends to agree with OGBONNA *et al* (2010) [40], that the hydro-ethanolic extract of *Chromolaena odorata* lowered glucose levels when administered to rats. This hypoglycemic effect may have been achieved by increasing insulin secretion and peripheral utilization of glucose in diabetic rats, inhibition of endogenous glucose production, inhibition of intestinal glucose absorption and/or regenerating existing β cells. These mechanisms have all been reported to be responsible for lowering blood sugar levels (EDDOUKS *et al*., 2003; BAKIREL *et al*., 2007; ADENEYE and AGBAJE, 2008) [20, 9, 2].

In this study, the increased level of cholesterol and triglycerides observed in the diabetic control group and also after induction of diabetes suggest that the development of diabetes mellitus is usually accompanied by anomalies in body lipid composition. This is in consonance with existing report that the development of diabetes mellitus is usually followed by marked increase in blood cholesterol, triglycerides, LDL-C, VLDL-C and a reduction in HDL-C (AKAH *et al*., 2009) [3]. The lowering of cholesterol and triglycerides observed in the study indicates the presence of principles with hypolipidemic properties in the extract. The presence of saponin and flavonoids has been reported in the leaf extract of *Chromolaena odorata,* and these have been implicated in the lowering of blood cholesterol (IKEYI *et al*., 2013) [28].

The antidiabetic effects (α-amylase and α-glucosidase inhibition activities) of tannins extracted from some cereals, legumes, oil seeds, and vegetables have been studied (CATHERINE *et al*., 2011) [13] and results have shown encouraging effects. Flavonoids have also been reported to suppress glucose level significantly and the typical flavonoid, luteolin, has been found to be a strong inhibitor of α-glucosidase (KIM *et al*., 2000) [32]. Saponin, an abundant secondary metabolite in the seed of *Entada phaseoloides* was reported to have dramatically reduced fasted blood glucose and serum insulin levels and alleviates hyperglycemia associated oxidative stress in type 2 diabetes (ZHENG *et al*., 2012) [52]. In another study, it was reported that saponin extract from the root of *Garcinia kola* (bitter cola) demonstrated remarkable antidiabetic activity even more than a standard antidiabetic drug, metformin in alloxan-induced diabetic rats (ALLI *et al*., 2012) [8]. The saponin is known to elicit serum cholesterol lowering activity and may be classified as a direct hypoglycemic agent, in contrast to the indirect agents such as the sulphonylureas that act by stimulating the pancreatic beta cells to release more insulin (ABDEL-HASSAN *et al*., 2000; CHANGYONG *et al*., 2010) [1, 14].

Diabetes mellitus was induced by a single intraperitoneal (I.P) injection of freshly prepared solution of alloxan monohydrate where β cells of Islets of Langerhans of the pancreas are destroyed resulting in a decrease in endogenous insulin secretion leading to decrease utilization of glucose by body tissues (YAMAMOTO *et al*., 1981) [49]. It results in elevation of blood glucose level, decreased protein content, increased levels of cholesterol and triglycerides (DHANABAL *et al*., 2007) [19]. The diabetic animals treated with methanol extract of *Chromolaena odorata* showed decrease in blood glucose level.

Several factors such as oxidative stress (Hayden *et al*., 2005) [26], chronic hyperglycemia (LEUNG and LEUNG, 2008) [35] and autoimmune (YOSHIDA *et al*., 1995) [50], fibrocalculous (MOHAN *et al*., 2008) [37] or types of chronic pancreatitis, damage the pancreas and impair insulin secretion and hence glycaemic control. Results of histological studies on pancreas isolated from treated diabetic rat showed that the extract may have repaired the pancreas damaged by alloxan. Alloxan causes diabetes by destruction of ß-cells of the islet (SZUDELSKI, 2001; FRODE and MEDEIROS, 2008) [45, 23] which consequently impairs insulin secretion and gives rise to hyperglycemia.

Treatment with the extract may have restored the integrity and perhaps, functions of the damaged pancreatic tissues. Also, the extract was able to restore the damaged kidney and liver to their normal architecture. The precise mechanism of this tissue repair is not known. However, due to the large implication of oxidative stress (HAYDEN *et al*., 2005; LEUNG and LEUNG, 2008) [26, 35] in damage to the pancreas, it seems reasonable to suggest that the antioxidant (IROBI, 1992; BAMBA *et al*., 1993; THANG *et al*., 2001; DAT *et al*., 2009; BHARGAVA *et al*., 2013) [30, 10, 18, 47, 18, 12] and radical scavenging (ALISI and ONYESE, 2008; ALISI *et al*., 2011) [6, 7] effects of this plant may play a key role in protecting pancreatic tissues from oxidants including that generated by alloxan. Alloxan destroys insulin-producing pancreatic ß-cells through the formation of reactive oxygen species that cause tissue damage (LEE *et al*., 2008) [34].

The heart, liver and pancreas of the diabetic and non-treated animals were also affected in this study while those of other groups were mildly affected or in some cases no visible lesion was observed. It thus showed that the extract from this plant has some protective properties for these organs. The group of animals that were pre-treated with the 200 mg/kg dose of the extract particularly showed very good results and thus justifying its chemopreventive ability.

The results obtained from this study indicate that *Chromolaena odorata* leaves contain substances with hypoglycemic properties and at low doses could be a safe and potent agent to be employed in the treatment of diabetes mellitus and resulting haematological and lipid profile anomalies. Meanwhile, DAT *et al* (2009) [18] has shown that (9S, 13R)-12-Oxo-phytodienoic acid in chloroform-soluble extract from the whole plant and odoratin in the dichloromethane extract (ZHANG *et al*., 2012) [51] have been identified and investigated as sources of peroxisome proliferating activating receptor gamma (PPARγ) ligands. PPARγ has attracted significant scientific and clinical interest because of its role in macronutrient metabolism. It is a target of the synthetic insulin sensitizers – thiazolidinediones and these are used in the treatment of type 2 diabetes mellitus (GRYGIEL-GORMIAK, 2014) [24]. The study supports the traditional use of the plant for the purposes already mentioned.

References

1. Abdel-Hassan IA, Abdel-Barry JA, Mohammeda ST. The hypoglycemic and antihyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. J Ethnopharmacol. 2000; 71;325-330.
2. Adeneye AA, Agbaje EO. Pharmacological Evaluation of Oral Hypoglycemic and Antidiabetic effects of fresh leaves ethanol extract of *Morinda Lucida Benth*. In normal and alloxan-induced diabetic Rats. Afr. J Biomed Res. 2008; 11:65-71.
3. Akah PA, Alemji JA, Salawu OA, Okoye TC, Offiah NV. Effects of *Vernonia amygdalina* on biochemical and hematological parameters in diabetic rats. Asian J Med Sc. 2009; 1(3):108-113.
4. Akindele OA, Babatunde AI, Chinedu FM, Samuel OA, Oluwasola CA, Oluseyi AA. Rat model of food induced non-Obsessed Type-2 diabetes mellitus: comparative pathophysiology and histopathology. Int. J Physiol. 2012; 4(1):51-58.
5. Akinmoladun AC, Ibukun EO, Dan-Ologe IA. Phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata.* Sc. Res. Essay 2007; 2(6):191-194.
6. Alisi CS, Onyese GOC. Nitric oxide scavenging ability of ethyl acetate fraction of methanolic leaf extracts of *Chromolaena odorata* (Linn.). Afr. J Biochem Res. 2008; 2(7):145-150.
7. Alisi CS, Onyese GOC, Ojiako OA, Osuagwu CG. Evaluation of the protective potential of *Chromolaena odorata* Linn. Extract on carbon tetrachloride-induced oxidative liver damage. Int. J Biochem Res Rev. 2011; 1(3):69-81.
8. Alli SYR, Adanlawo IG, Oni OS. Hypoglycemic Effect of Saponin from the Root of Garcinia kola (bitter kola) on alloxan-induced diabetic rats. J Drug Delivery Ther. 2012; 2(6):9-12.
9. Bakirel T, Utku B, Oya UK, Sinem GU, Hasret Y. In vivo assessment of anti-diabetic and antioxidant activities of Rosemary (*Rosmarinus officinalis*) in alloxan-diabetic rabbits. J Ethnopharmacol. 2007; 116:61-73.
10. Bamba D, Bessiere JM, Marion C, Pelissier Y, Fourate I. Essential oil of *Eupatorium odoratum*. Planta Med. 1993; 59:184-185.
11. Beutler JA. Natural products as a foundation for drug discovery. Curr. Protoc. Pharmacol 2009; 9:9-11.
12. Bhargava D, Mondal CK, Shivapuri JN, Mondal S, Kar S. Antioxidant properties of the leaves of *Chromolaena odorata* Linn. J Inst Med. 2013; 35(1):53-56.
13. Catherine NK, Jasper KI, Michael O, Clare M, Han KB, Vellingiri V. Antioxidant and antidiabetic properties of condensed tannins in acetonic extract of selected raw and processed indigenous food ingredients from Kenya. J Food Sc. 2011; 76(4):C560-567.
14. Chang-Yong Y, Jing W, Yuan Z, Li S, Xing J, Zhen-Guo X**.** Anti-diabetic effects of *Panax notoginseng* saponins and its major anti-hyperglycemic components. J Ethnopharmacol. 2010; 130:231-236.
15. Clardy J, Walsh C. Lessons from natural molecules. Nature 2004; 432:829-837.
16. Colak S, Geyikoghu F, Aslan A, Deniz GY. Effects of Lichen Extracts on Hematological Parameters of Rats with Experimental insulin –dependent diabetes mellitus. Toxicol Ind. Health Publishers, 2012.
17. Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. Biochim. Biophys. Acta. 2013; 1830:3670-3695.
18. Dat NT, Lee K, Hong YS, Kim YH, Minh CV, Lee JJ. A peroxisome proliferator−activated receptor-gamma agonist and other constituents from *Chromolaena odorata*. Planta Med 2009; 75:803-807.
19. Dhanabal SP, Raja MK, Ramanathan M, Suresh B. Hypoglycemic activity of *Nymphaea stellata* leaves ethanolic extract in alloxan induced diabetic rats. Fitoterapia 2007; 78:288-291.
20. Eddouks M, Jonad H, Maghrani M, Lemhadri AB. Inhibition of endogenous glucose products accounts for hypoglycemic effects of *Spergularia purpurea* in Streptozotocin induced- diabetes mellitus in mice. Phytomed 2003; 6(7):594-599.
21. Ertl P, Roggo S, Schuffenhauer A. Natural product-likeness score and its application for prioritization of compound libraries. J Chem. Inf Model. 2008; 48:68-74.
22. Falodun A. Herbal medicine in Africa-distribution, standardization and prospects. Res. J Phytochem. 2010; 4(3):154-161.
23. Frode TS, Medeiros YS. Animal models to test drugs with potential antidiabetic activity. J Ethnopharmacol. 2008; 115:173-183.
24. Grygiel-Gorniak B. Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications –a review. Nutrit J. 2014; 13:17.
25. Harini K, Jerlinshowmya J, Geetha N. Phytochemical constituents of different extracts from the leaves of *Chromolaena odorata* (L.) King and Robinson. Int. J Pharm Sc Bus Mg. 2014; 2(12):13-20.
26. Hayden MR, Tyagi SC, Kerklo MM, Nicolls MR. Type 2 diabetes mellitus as a conformational disease. J Pancreas (Online). 2005; 6(4):287-302.
27. Henrich CJ, Beutler JA. Matching the power of high throughput screening to the chemical diversity of natural products. Nat. Prod. Rep. 2013; 30:1284-1298.
28. Ikeyi AP, Ogbonna AO, Inain DE, Ike AO. Phytochemical analysis of pineapple fruit (*Ananas comosus)* and fluted pumpkin leaves (*Telfaria occidentalis)*. World J Pharm. Res 2013; 2(4):712-719.
29. Inta A, Trisonthi P, Trisonthi C. Analysis of traditional knowledge in medicinal plants used by Yuan in Thailand. J Ethnopharmacol. 2013; 149:344-351.
30. Irobi ON. Activities of *Chromolaena odorata* (Compositae) leaf extract against *Pseudomonas aeruginosa* and *Streptococcus faecalis*. J Ethnopharmacol. 1992; 37:81-83.
31. Jack DB. One Hundred Years of Aspirin. The Lancet 1997; 350(9075):437-439.
32. Kim JS, Kwon CS, Son KH. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. Biosci. Biotechnol. Biochem 2000; 64(11)2458-2461.
33. Lai PK, Roy J. Antimicrobial and chemopreventive properties of herbs and spices. Curr. Med. Chem. 2004; 11:1451-1460.
34. Lee JH, Park JW, Kim JS, Park BH, Rho HW. Protective effect of Amomi semen extract on alloxan-induced pancreatic ß-cell damage. Phytother. Res 2008; 22:86-90.
35. Leung KK, Leung PS. Effects of hyperglycemia on the Angiotensin II receptor type 1 expression and insulin secretion in an INS-1E pancreatic Beta-cell line. J Pancreas (Online). 2008; 9(3):290-299.
36. Luximon-Ramma A, Bahorun T, Soobrattee MA, Aruoma OI. Antioxidant activities of phenolic, proanthocyanidins, and flavonoid components in extracts

of Cassia fistula. J Agric. Food Chem. 2002; 50(18):5042-5047.
37. Mohan V, Farooq S, Deepa M. Prevalence of fibrocalculous pancreatic diabetes in Chennai in South India. J Pancreas (Online) 2008; 9(4):489-492.
38. Moody JO, Robert VA, Connolly JD, Houghton PJ. Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae). J Ethnopharmacol. 2006; 104:87-91.
39. Mouli KC, Vijaya T, Rao SD. Phytoresources as potential therapeutic agents for cancer treatment and prevention. J Global Pharm Tech 2009; 1(1):4-18.
40. Ogbonna SO, Mbaka GO, Anyika EN, Osegbo ON, Igbokwe NH. Evaluation of acute toxicity in mice and subchronic toxicity of hydro-ethanolic extract of *Chromolaena odorata* in rats. Agric. Biol. J N. Am. 2010; 1(5):859-863.
41. Pandey A, Vaisakh MN. The invasive weed with healing properties: A review on *Chromolaena odorata*. Int. J Pharm Sc Res. 2011; 3(1):80-83.
42. Phan TT, Wang L, See P, Grayer RJ, Chan SY, Lee ST. Phenolic compounds of *Chromolaena odorata* protects cultured skin cells from oxidative damage. Implication for cutaneous wound healing. Biol. Pharma. Bull 2001; 24:1373-1379.
43. Saliu JA, Elekofehinti OO, Komolafe K, Oboh G. Effect of some green leafy vegetables on the hematological parameters of diabetes rat. J Nat Prod Plant Res. 2012; 2(4):482-485.
44. Sawadogo WR, Boly L, Lompo M, Some N. Anti-inflammatory, analgesic and antipyretic activities of *Dicliptera verticillata*. Int. J Pharmacol. 2006; 2:435-438.
45. Szudelski T. The mechanism of alloxan and streptozotocin action B cells of the rat pancreas. Physiol. Res 2001; 50:536-546.
46. Tapsell LC, Hemphill I, Cobiac L, Patch CS, Sullivant DR. Health benefits of herbs and spices: the past, the present, the future. Med. J Austr. 2006; 185:S4-S24.
47. Thang PT, See P, Lee ST, Chan SY. Anti-oxidant effects of the extracts from the leaves of *Chromolaena odorata* on human dermal fibroblasts and epidermal keratinocytes against hydrogen peroxide and hypoxanthine−xanthine oxidase induced damage. Burns. 2001; 27:319-327.
48. Wanga L, Waltenbrger B, Pferschy-Wenzig E, Blunder M, Liu X, Malainer C *et al*. Natural product agonists of peroxisome proliferator-activated receptor gamma (PPARg): a review. Biochem. Pharmacol. 2014; 92:73-89.
49. Yamamoto H, Uchigata Y, Okamoto H. Streptozocin and alloxan induce DNA strand breaks and poly (ADP-ribose) synthetase in pancreatic islets. Nature 1981; 294:284-286.
50. Yoshida K, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N. Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. Dig. Dis. Sci 1995; 40:1561-1568.
51. Zhang ML, Irwin D, Li XN, Sauriol F, Shi XW, Wang YF. PPAR gamma agonist from *Chromolaena odorata*. J Nat. Prod. 2012; 75:2076-2081.
52. Zheng T, Shu G, Yang Z, Mo S, Zhao Y, Mei Z. Antidiabetic effect of total saponins from Entada phaseoloides (L.) in type 2 diabetic rats. J Ethnopharmacol. 2012; 139(3):814-821.