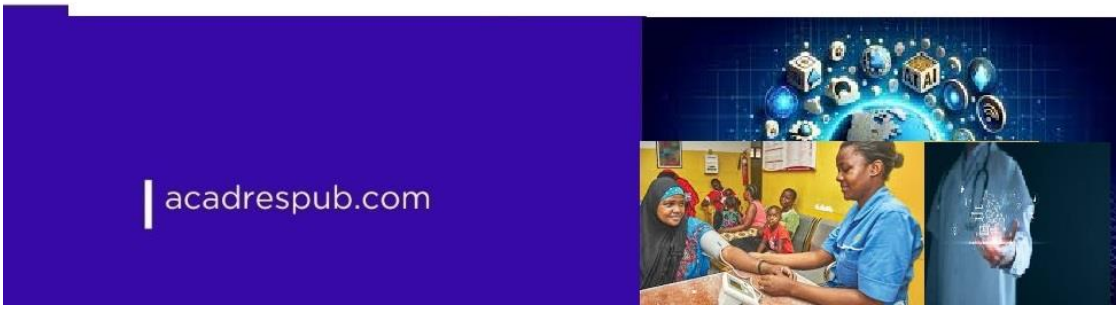
acadrespub.com

OMANARP INTERNATIONAL JOURNAL OF HEALTH SCIENCES



Vol. 2, Issue II, Pp. 26-; 38 JUNE, 2025

INVESTIGATION ON PHYTOCHEMICALS, ORAL ACUTE TOXICITY, AND PROTECTIVE EFFECT OF AQUEOUS EXTRACT OF AG-S90 POLYHERBAL FORMULATION ON ALLOXAN-INDUCED DIABETIC NEUROPATHY PAIN IN WISTAR RATS.

Kokori Bajeh Tijani*[1], Mohammed Garba Magaji[2], Jamilu Ya'u[3], Habib Umar Danmalam[4],

[1]Department of Pharmacology,Faculty of Basic Medical Sciences, College of Health Sciences, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria

[2&3]Department of Pharmacology & Therapeutics Faculty of Pharmaceutical Sciences Ahmadu Bello University, Zaria, Kaduna State, Nigeria. [4]Department of Pharmacognosy & Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

kbtbichempharma@gmail:com +2348154969966

Correspondence Author: Kokori Bajeh Tijani

ARTICLE INFO

Received Date: 30th May, 2025
Date Revised Received: 1st May, 2025
Accepted Date: 20nd June, 2025
Published Date: 27th. June, 2025

Citation: Kokori, BT. et al (2025) Investigation on Phytochemicals, Oral Acute Toxicity, and Protective effect of Aqueous Extract of AG-S90 Polyherbal Formulation on Alloxan-Induced Diabetic Neuropathy Pain in Wistar Rats;: OMANAP INT.J.HEALTH; Vol.2, Issues II Pp.26-38 June,2025.

ABSTRACT

The use of natural product as formulated phytomedicine in Nigeria has significantly increased for mitigating the problem of emerging and re-emerging diseases such as Adayi Gambari Salihu-90 (AG-S90) polyherbal drug. No scientific data-based information, no scientific validation, and enough evidence available on this product for disease management. Aims: The study was aimed at determining phytochemicals, oral acute toxicity and to evaluate the neuroprotective effect of AG-S90 on Alloxan-induced diabetic neuropathic pain model in Wistar rats. Methods: Phytochemical screening was conducted according to standard methods, where acute oral toxicity study was performed following OECD guidelines 425, using *UP-and-DOWN* procedure, 2014 and evaluation of neuroprotective effect of AG-S90 against Alloxan-induced diabetic neuropathy in wistar rats weighing between 150-180 kg p.b.w. Experimental diabetes was induced in Wistar albino rats by single intraperitoneal injection of Alloxan monohydrate (150 mg/kg). Results: result indicated the presence of phytochemical (Alkaloids, Anthraquinones, Anthocyanins, Amino acids/proteins, Carbohydrates, Cardiac glycosides, Saponins, Flavonoids, Polyphenols, Tannins, Terpenoids, Steroids, Fats, and oils). Observational studies revealed normal activities without any behavioural abnormality. There is no death recorded nor any sign of toxicity in acute toxicity after oral administration. Conclusion: Aqueous extract of AG-S90 could be employed as a pain reliever, reversed diabetes to explore its various medicinal activity and very safe for human consumption (at LD50 greater than 5000mg/kg). It is concluded that AG-S90 exhibits rich phytochemicals, significant neuroprotective and lowered blood sugar levels ($p<0.05$) against diabetic neuropathy in rats. Findings, there was an improvement in diabetic state after AG-S90 treatment could be used to mops-up ROS, heals pancreatic gland and good therapeutic candidate for diabetic neuropathic pain.

Key Words: AG-S90, Phytochemicals, Acute toxicity, Alloxan and Diabetic neuropathic pain

Introduction

A collection of metabolic and endocrinological conditions known as diabetes mellitus (DM) are marked by hyperglycemia, altered protein and lipid metabolism, and, in the chronic stage, excruciating pain. (Zimmet *et al*., 2001; Packer *et al*., 2001 and Yusuff *et al*., 2008). But in type-2 diabetes, insulin resistance and beta cell dysfunction gradually develop and are closely linked to obesity and a sedentary lifestyle; in type-1 diabetes, on the other hand, the disease is autoimmune and is characterized by T-cell-mediated death of the pancreatic beta cells. (Liu *et al*., 2006; Zimmet *et al*., 2001; Yusuff *etal*., 2008). According to a review of conducted studies on agonizing diabetic neuropathy, patients with uncontrolled diabetes are more likely to endure an extreme level of neuropathic pain. It is additionally believed that acute biochemical changes in neural tissues may be the result of prolonged hyperglycemia and may play a role in the development of diabetic neuropathy. (Shanmugasundaram *et al*., 2011; Kastrup *et al*., 1987; Brownlee *et al*., 2001).

Hyperglycemia, or abnormally high blood glucose levels, and dysregulation in the metabolism of lipids and proteins, which are caused by irregularities in both the secretion and action of insulin, are the hallmarks of diabetes mellitus. Known as the "modern-day epidemic," it affects 45 percent of the global population and is a chronic, explosive illness that is predicted to impact over 300 million people by 2025. (Shaw *et al*., 2010). Thus, a projected 366 million sufferers by the year 2030, as against 191 million expected in the year 2000 (Koyuturk, *et al*., 2005; Abdelmoaty *et al*., 2010). In Nigeria, the incidence of diabetes mellitus was put at about 2.8 million in the year 2010 and is projected to rise to over 5.3 million in 2030 (Shaw *et al*., 2010). Diabetes mellitus is a public health concern not only because of the disease associated burden but, also the management of the complications that are the sequel to it. These complications are not only very expensive to manage but incur a substantial economic burden on the healthcare delivery system (Bahia *et al.,* 2019). Underneath these complications are hyperglycemia-induced react oxidative stress and inflammation which destroys micro-vascular and macro-vascular blood vessels (diabetic nephropathy and diabetic retinopathy) and the nervous system (diabetic neuropathy) (Charlton *et al.,* 2020). Diabetic neuropathy is a peripheral nerve system neuro-degenerative condition that disproportionately impacts sensory axons, autonomic axons, and, to a lesser degree, motor axons. The most common type of neuropathy in diabetes mellitus is diabetic polyneuropathy (DPN or DNP), with up to 50% of patients experiencing some degree of painful symptoms and 10% to 20% having symptoms severe enough to warrant treatment (Malik *et al*., 2005). Hyperglycemia-induced process of overproduction of superoxide by the mitochondrial electron transport chain in the body system is overwhelmed with free radical generation elicit spontaneous fenton reaction (Eidi *et al*., 2006). Thus, experimental studies have revealed that reactive oxidant species (ROS) play a significant role in pathophysiology of neuropathic pain in diabetes (Anjaneyulu and Chopra, 2004; Bagri *et al*., 2009). Adequate metabolic control may reduce the symptoms of painful diabetic neuropathy (Kastrup *et al*., 1987).Glycemic homeostasis refers to glucose balance or control within circulation in living organisms. It is normally and largely compromised in diabetes. The compromise when exacerbated, leads to several complications including retinopathy, nephropathy and neuropathy which are collectively known as diabetic complications and are the principal actors in co-morbidity and eventual mortality often associated with diabetes. The ability of therapeutic compounds including medicinal plants to restore glycemic balance or homeostasis in hyperglycemic conditions an index of their antidiabetic nutraceutical function and therapeutic relevance (Jemai *et al*., 2009; Jelodar *et al*., 2005; Jalal *et al*., 2007). Thus, DM occurs either when the pancreas does not produce insulin, or when the body cannot effectively utilize the insulin, it produces. 2 Several findings have revealed that DM is a major global health concern with a projected rise in prevalence from 171 million in 2010 to 366 million 2030 (Shaw *et al*., 2010; Farswan *et al*., 2009). Both the number of cases and the prevalence of DM has steadily been on a rise over the past few decades, and it is regarded to be a silent killer disease, affecting millions of peoples in the world. In Sub-sahara Africa, the number of people with diabetes will increase from 14.2 million in 2015 to 34.2 million in 2040 predominantly populated in some of the region's most populous countries: South Africa, the Democratic Republic of Congo, Nigeria, and Ethiopia due to current trends in the status economy (Lee *et al*., 1990; Kastrup*et al*., 1987 and Brownlee, 2001).

However, numerous conventional medications that have been reported to be in use for diabetes management and its pain, its inaccessibility has also been demonstrated to be huge concern as a result of the relatively high cost and sometimes unavailability particularly for the rural areas. Owing to this, a switch to a readily available and cheaper alternative has become necessary in the form of phytomedicine (Devanjee *et al*., 2008; Kastrup *et al*., 1987). Herbal medicine, also regarded as phytomedicine refers to the use of plants seeds, flowers, roots for medicinal therapeutic purposes. Currently, medicinal plants continue to play an important role in the management of Diabetes mellitus DM, Diabetes neuropathic pain, DNP and Diabetes foot ulcer DFU, especially in developing countries, where many people do not have access to conventional anti-diabetic

therapies (Wainstein *et al*., 2012; Lee *et al*., 1990; Tesfaye *et al*., 2011).

The most common and severe chronic complications of diabetes mellitus is diabetic neuropathy, which is mainly characterized by spontaneous pain and abnormal sensations such as paresthesia, allodynia, and hyperalgesia, at times may result to foot diabetic ulcer (Ragavam *et al*., 2006; Packer *et al*., 2001; Saravanan *et al*., 2010; Yusuff *et al*.,2008). Many neuroanatomical, neurophysiological, and neurochemical (endocrinological) mechanisms are thought to contribute to the development and maintenance of diabetic neuropathic pain (DNP) (Farswan *et al*., 2009; Maiti *et al*., 2004). Current treatment of DNP involves the use of tricyclic antidepressant, selective serotonin reuptake inhibitors (Lee *et al*., 1987), anticonvulsants, opioids and antioxidant protein kinase C inhibitors, COX-2 inhibitors (Kastrup *et al*., 1987) and nonsteroidal anti-inflammatory drugs as mild analgesics, and so on. However, these therapies provide relief only to a fraction of patients and their side effect profiles limit their use (Brownlee, 2001 and Anjaneyulu and Chopra, 2004). Therefore, there is a need to identify an effective and safe clinical treatment for PDN. Complementary medicines have gained in popularity among clinicians in recent years. Many indigenous natural product/herbs have been found to be useful to successfully manage pain in various chronic pain models. World Health Organization (WHO) recognizes traditional medicine as readily easy, accessible, affordable, and culturally acceptable form of healthcare trusted by large numbers of people, which stands out as a way of coping with the relentless rise of chronic non-communicable diseases during soaring health-care costs and nearly universal austerity' (WHO, 2013).AG-S90 poly herbal formulation (20g) is a product of Adayi Gambari Salihu 90 (AG-S90) from Idoma-Okengwe, Okene Local Government Area, Kogi State, Nigeria. Its formulation consists of *Allium sativum* Linn (garlic bulbs), *Nigella sativa* (black seed or caraway) and *Medicago sativa* (alfalfaleaves) in ratio 1:1:1 respectively and without harmful toxic effects. The aqueous extract of AG-S90 and compounds has been found with special peripheral and central nervous effects that could be of pharmacological interest. However, AG-S90 have been used as folk medicine to manage DM and other ailments, lack of scientific data on neuroprotective activity against diabetic neuropathy pain have not been established despite peoples' patronage. Therefore, the present study was designed to evaluate the neuroprotective effects of AG-S90 extract against Alloxan-Induced Diabetic Rats. The aim of the present investigation was to establish and evaluate phytochemicals, oral acute toxicity, and the neuroprotective effect of AG-S90 polyherbal against Alloxan-induced diabetes neuropathic pain and to assess its mechanism of action in Wistarrats. Not enough evidence to support the purported claimed or investigation for the oral acute toxicity for this AG-S90 polyherbal product pharmacological study is not available in the literature and scientific validation. Therefore, AG-S90 phytochemical constituents was carried out according to standard method of Abdullahi *et al*., 2019, oral acute toxicity study was carried out by the OCED guidelines 245 and neuroprotective effect in Alloxan-induced diabetic neuropathic pain to determine and establish the safety for its human usage in the mitigation of the diabetic neuropathy pain. Scientists looked for other drugs to increase the overall analgesic effect without causingun acceptable side effects (Kim and Kim, 2011). AG-S90 polyherbal is used as folkloric herbal medicine for the treatment of pain, inflammation, pyrexia, diarrhoea, dysentery and piles, toothache, rheumatism (arthritis), skin disorders like sores, to boost immune system and as ahypoglycemic agent.

Materials and Methods

Collection and Authentication of AG-S90 polyherbal Medicine

AG-S90 polyherbal medicine was bought from the herbalist direct at No. 12 Idoma-Okengwe district, Okene Local Government Area, Kogi State, Nigeria. The product was always in a special package of 20g sachet in powdered form.

Chemicals

All the chemicals used were of analytical grade and procured from Sigma chemicals Co, USA.

Animal

Wistar rats weighing 165-180g were obtained from the animal house of College of Health Sciences, Prince Abubakar Audu University, Anyigba. Animals were fed on conventional diets and water *ad libitum* and they were maintained under standard conditions of light (12-hr light: 12-hr dark cycle).The rats were randomly assigned to control and different treatment groups, six animals per group. The Institutional Animal Ethics Committee approved the experimental protocol and the conditions in the animal house approved by Committee for Supervision on Experiments on Animals. The study was conducted in accordance with ABU guidelines (Approval no-ABUCAUC/2022/024). The animals were acclimatized for one week under laboratory conditions.

Phytochemical Screening of AG-S90P

AG-S90P was subjected to standard phytochemical screening investigations to determine various phytoconstituents and its biological functions according to

Abdullahi *et al*., (2019).A phytochemical constituent to be screened includes alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, steroids, terpenoids and tannins. 2.0g of powdered AG-S90 extracts was dissolved in 100ml of distilled water for this screening experiment.

The phytochemical tests carried out include:

Test for Alkaloids (Mayer's Test)
Weigh 0.5g of the AG-S90 extract using weighing balance Mix the weighed sample with 5ml of 1% aqueous HCl in a test tube and warm the preparation on steam water bath.

Filter using whatman No. 1 filter paper.
Treat 1ml of the filtrate with 2-3 drops of Mayers's reagent and a second 1ml portion with Drandorffs' reagent.

Test for the presence of anthraquinones (Bontrager's test)
Weigh 0.5g of the AG-S90 extract
Shake the weighed sample with 10ml of benzene in a test tube.

Filter using Whatman 's No. 1 filter paper
Add 5ml of 10% ammonia solution to the filtrate.
Shake the mixture vigorously

Test for the presence of anthocyanidins (HCl acid test)
Weigh 0.5g of the AG-S90 extract and dissolve in a 10ml of distilled water in a test tube.
Stopper the test tube with a cork.
Shake vigorously for 30 seconds after adding 2-3 drops of Conc. HCl and allow standing for 45 minutes purplish colour to form

Test for saponins (Frothing test)
a. Weigh 0.5g of the AG-S90 extract and dissolve in a 10ml of distilled water in a test tube.
b. Stopper the test tube with a cork.
c. Shake vigorously for 30 seconds and allow standing for 45 minutes.

Test for flavonoids (Shinoda test)
Weigh 0.5g of the AG-S90 extract and add 2-3 drops of 10% ferric chloride solution.
Test for tannins (Ferric Chloride test)
Weigh 0.5g of the AG-S90 extract and dissolve in 5ml of water.
Add 2-3 drops of 10% ferric chloride.

Test for steroidal terpenes (Salkowiski's test)
Add 2ml of acetic anhydride to 0.5g of the AG-S90 extract.
Place 2ml of H_2SO_4

Test for terpenoids (Libermann's test)
Mix 5ml of the AG-S90 extract with chloroform (2ml).
Add 3ml of concentrated. H_2SO_4 to form a layer.

Test for cardiac glycosides (Keller-Kiliani's test)
Weigh 0.5g of the AG-S90 extract and dissolve in 2ml of glacial acetic acid in a test tube.
b. Place 1ml of concentrated H_2SO_4 in the preparation and add one drop of ferric chloride solution.

Test for carbohydrates (Molisch's test)
a. Weigh 0.5g of the AG-S90 extract in a test tube
b. Add 3 drops of molisch's reagent was added followed by concentrated sulfuric acid. The formation of reddish colored ring at the interface indicates the presence of carbohydrates.

Test for amino acids or protein (Biuret or Piotrowski's Test)
a. Weigh 0.5g of the AG-S90 extract and dissolve in a 10ml of distilled water in a test tube.
b. Stopper the test tube with a cork and add 2-3 drops of Biuret reagent.
c. Shake vigorously for 30 seconds and allow standing for 25 minutes for purple colour.

Test for fats and oils (Filter paper's test)
Filter paper soaked in the extract solution or impregnated with extract was allowed to dry and checked for translucence films; that indicate the presence of fat and oils.

Acute toxicity assay
Use of the test that aimed to identify the single lethal dose of a substance that kills half the animals in a test group is called LD_{50} test. In its stead are three recently developed alternative animal tests that significantly improve animal welfare: the fixed dose procedure, the acute toxic class method, and the Up and Down Procedure. These tests have already undergone revision, both to improve their scientific performance and, importantly, to increase their regulatory acceptance. They can now be used within a strategy of acute toxicity testing for all types of test substances and for all regulatory and in-house purposes.

In accordance with OECD Test Guidelines 425 (Up and Down Procedure), both male and female albino wistar rats weighing 165 ± 4 g having age 8–10 weeks were randomly selected. Animals were kept under standard conditions for five days. Limit test was performed at 5000

mg/kg oral gavage as single dose; wistar rats and mice were kept without food for 3–4 hrs, prior to dosing but had access to water *ad libitum.* The dose was administered to both male and female wistar rats and mice according to body weight. The animals were closely observed for the first 30 min, then for 4 hrs. Food was provided after 1–2hrs of dosing and after survival of treated animals, 3 additional wistar rats were administered with the same dose under same conditions. The same procedure was followed for the vehicle treated control group of 5 wistar rats to whom distilled water was administered in same volume as that of treated group. Both the groups were observed closely for any toxic effect within first 6 hrs and then at regular intervals for a total period of 14 days. Surviving wistar rats were observed to determine the toxic reactions onset. Weights of animals were monitored and documented as well.

Induction of Diabetes

Hyperglycemia was induced by a single i.p. injection of 150 mg/kg of alloxan monohydrate.
(Sigma-Aldrich, U.S.A.) in sterile saline solution. Following injecting rats with alloxan by one hour, wistar rats were allowed to be fed standard pellets and water *ad libitum*. After that, the experimental rats were administered 20% glucose solution for 24 hours to prevent hypoglycemia.

After 72hr of Injection, fasting blood glucose level (estimated by glucometer) was measured according to Ghosh and Suryawanshi, 2001; Gupta *et al*., 2005 and Reshmi *et al*., 2001. Hyperglycemia was confirmed after 5 days of alloxan injection, and hyperglycemic rats (glucoselevel > 200mg/dl) were separated and selected for the study.

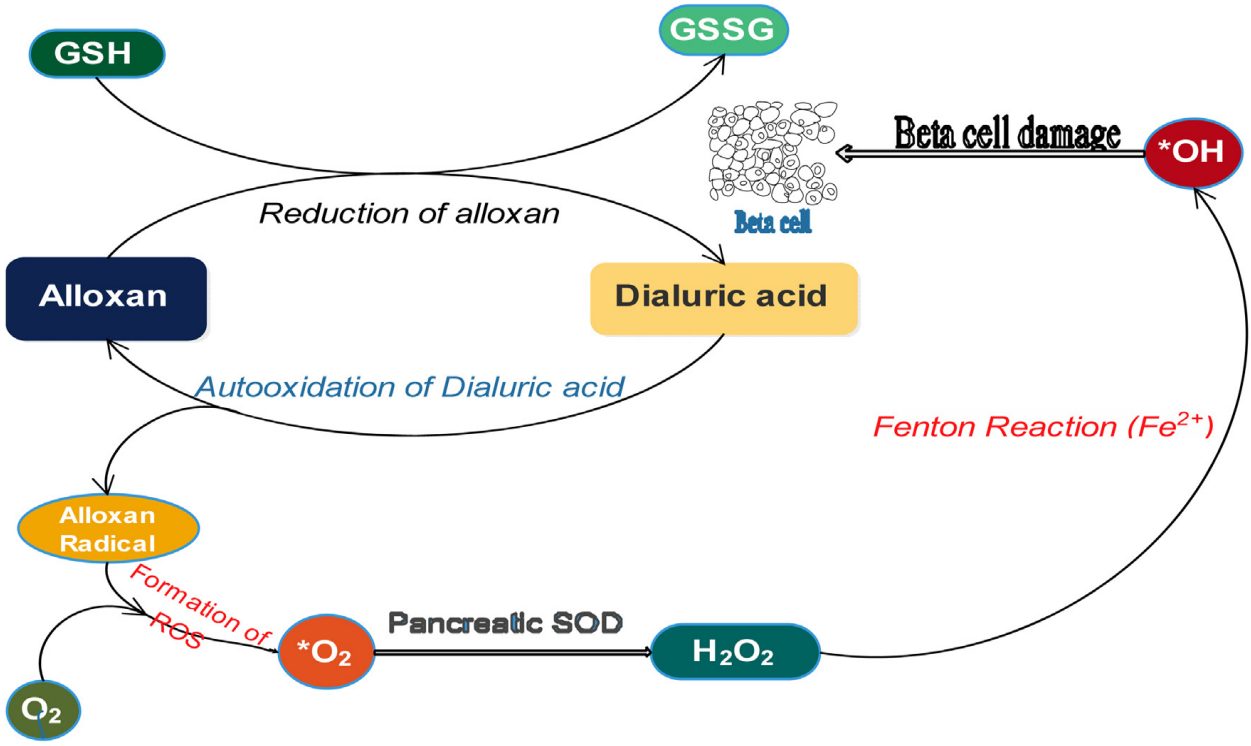


Fig.1- Formation of ROS through redox cycling of alloxan and AG-S90 reversal process (Dzeufiet *et al*., 2017; Ven tatesh *et al*., 2010).

Experimental Design

Total of 30 diabetic surviving and 6 nondiabetic rats were divided in to 5 groups (n=6) as follow according to Pari *et al*., 2002.

Group I	Normal rats received only distilled water (0.5mL/kg body weight) and served as positive control
Group II	Alloxan diabetic rats that received only vehicles (0.5mL/kg body weight) (served as negative control)
Group III	Rats received standard drug Glibenclamide at a dose of 0.5mg/kg orally for 28 days + a single dose of Alloxan
Group IV	Received lower dose of AG-S90 100 mg/kg/d suspended in the vehicle (10ml/kg) for 28days + a single dose of Alloxan
Group V	Received higher dose of AG-S90 200mg/kg/d suspended in the vehicle (10ml/kg) for 28 days + a single dose of Alloxan
Group VI	Received higher dose of AG-S90 300mg/kg/d suspended in the vehicle (10ml/kg) for 28 days + a single dose of Alloxan

For 28 days study period body weight, food and fluid intake of animals were recorded.

Evaluation of Behavioural Activity:
Assessment of hyperalgesia and Allodynia:

Hot water tail immersion test:
In hot water tail immersion test, heat hyperalgesia was measured by immersion of terminal part of the tail (1 cm) in hot water (52.5 ± 0.5°C). The duration of tail withdrawal reflex was recorded, as a response of heat thermal sensation and a cut-off time of 15 seconds was maintained. Shortening of tail withdrawal time is an indication for thermal hyperalgesia (Attal *et al*., 1990).

Cold water tail immersion test:
In cold water tail immersion test, distal 5 cm of tail was immersed in a cold-water container by maintaining a constant temperature (10^{o}C). Duration of time taken for withdrawal of tail from cold water was noted. A cut-off time of 20 sec was maintained to prevent tissue injury. The procedure was repeated three times for each animal and the mean values are taken in consideration. The decrease in tail contact time with cold water was pointing towards nociception, whereas prolonged contact time was noted as anti-allodynic effect (Kanaan *et al*., 1996).

Paw heat- hyperalgesia test (Eddy's hot plate method):
The nociceptive threshold for heat was an index for thermal hyperalgesia. Eddy's hot plate, which is an instrument designed by Eddy and co-workers to assess thermal sensitivity. The plate was preheated and maintained at a temperature of 52.5±2.0°C. The rat was placed on the hot plate and nociceptive threshold, with respect to licking of the hind paw or jumping, was recorded in seconds. The cut-off time of 20 sec was maintained (Kanaan *et al*., 1996).

Mechanical hyperalgesia (Pin prick test):
According to Erichsen and Blackburn-Munro, the surface of the injured hind paw was touched with a point of the bent gauge needle at 90°, without piercing deep into tissue. The intensity was sufficient to produce a reflex withdrawal response in normal control animals. The duration of the paw withdrawal was recorded in seconds using 20 sec as cut-off time (Kanaan *et al*., 1996).

Ethical Considerations
The study was approved to be carried out with ABU Ethical Committee on Animal Use and Care of Ahmadu Bello University, Zaria. The permission to conduct the study was granted with Approval No: ABUCAUC/2022/024 to maintain and care for the Experimental Animals.

Statistical Analysis
Results obtained in the experiment were expressed as mean ± SEM and subjected to one-way analysis of variance (ANOVA) and where significant differences exist, by using the SPSS software (version 11.5). $P< 0.05$ was considered as highly significant.

Results:

Phytochemical Analysis of AG-S90 powder.

The phytochemical analyses of AG-S90 powder were tested positive; for alkaloids, anthraquinones, anthocyanins, amino acid/protein, cardiac glycosides, flavonoids, saponins, steroids, terpenoids and tannins.

Table 1: Effects of the extract on body weight of wistar rats in acute toxicity study.

Groups	1st Day Body Weight (g)	7thDay Body Weight (g)	14th Day Body Weight (g)
Control	165.21 ± 0.9	170.11 ± 0.7	180.03 ± 0.4
	0/3	0/3	0/3
5000 mg/kg AG-S90	167.31 ± 0.8	172.16 ± 0.5	183.04 ± 0.6
	0/3	0/3	0/3

AG-S90 aqueous extract; Values are presented as mean ± SEM; N = 3; No death occurs.

Table 2: Behavioral pattern of Wistar rats in extract tested (AG-S90) 5000 mg/kg P.O. and control group

Parameters	Observation of control and AGS-90 treated groups											
	30 mins		4 hrs		24 hrs		48 hrs		7 days		14 days	
	CG	TG	CG	TG	CG	TG	CG	TG	CG	TG	CG	TG
Fur and Skin	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Respiration	N	N	N	N	N	N	N	N	N	N	N	N
Urination (colour)	N	N	N	N	N	N	N	N	N	N	N	N
Faeces consistency	N	N	N	N	N	N	N	N	N	N	N	N
Somatomotor activity and behavioral pattern	N		N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Mucous membrane	N	N	N	N	N	N	N	N	N	N	N	N
Convulsion and tremor	N.F	N	N.F	N	N	N.F	N.F	N.F	N.F	N.F	N.F	N.F
Itching	N	P	N	N	N	N	N	N	N	N	N	N
Coma	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	NF
Mortality	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	NF

Key: GC-Vehicle Control Group, T-AG-S90 aqueous etract treated groups, N-Normal, P-Present, N.F- Not found

Effect of AG-S90 extract on behavioural parameters:

Hot and cold-water tail immersion test:

Alloxan induced hyperglycaemia results in progressive heat hyperalgesia and cold allodynia which was showed by the shortening of tail withdrawal latency in comparison with normal rats.

AG-S90 treated rats started to show early significant improvement in tail withdrawal latency at dose200 mg/kg from 21st day and 300 mg/kg showed the effect on 14th day for hot water tail immersion test depicted in the experimental therapeutic (Table-4.2.3). In cold water tail immersion both the doses 200 and 300 mg/kg significantly improved tail withdrawal latency in a dose dependent manner (Table-4.2.4).

Paw heat- hyperalgesia test:

Alloxan induced Diabetic rats showed significant reduction in paw withdrawal latency when compared with normal rats (group 1). Standard drug Glibenclamide at a dose of 0.5 mg/significantly ($P<0.001$) improved the paw withdrawal latency from 14th day. Whereas 200 and 300 mg/kg of AG-S90 significantly ($P<0.05$) improved the paw withdrawal latency from 14th and 21st day in comparison with diseased control rats (Table-4.2.5).

Pinprick test:

In pinprick test the hyper responsiveness to a noxious stimulus was observed with significant rise in paw withdrawal latency in diabetic control as compared with normal control. Whereas treatment with 200 and 300mg/kg of AG-S90 caused significant reduction in paw withdrawal latency in a dose dependent manner. The anti-nociceptive effect of AG-S90 was found similar effective to the reference standard Glibenclamide (Table-4.2.6).

Body weight, food intake and water intake

Body weight of Alloxan induced diabetic control rats was found to be significantly ($p<0.05$) less compared to normal control rats. After 4 weeks of treatment with AG-S90 body weight significantly ($p<0.05$) increased compared to diabetic control groups. Food intake was significantly high in diabetic control rats as compared to normal control. At the end of 28 days of treatment food intake of treated groups significantly ($p<0.01$) decreased as compared to diabetic control. Significant decrease ($p<0.01$) in water intake was observed in treated groups as compared to diabetic control at the end of study period. (Table: 3).

Group I	Normal rats received only distilled water (0.5mL/kg body weight) and served as positive control
Group II	Alloxan diabetic rats that received only vehicles (0.5mL/kg body weight) (served as negative control)
Group III	Rats received standard drug Glibenclamide at a dose of 0.5mg/kg orally for 28 days + a single dose of Alloxan
Group IV	Received lower dose of AG-S90(100 mg/kg/d) suspended in the vehicle (10ml/kg) for 28days + a single dose of Alloxan
Group V	Received higher dose of AG-S90 (200mg/kg/d) suspended in the vehicle (10ml/kg) for 28 days + a single dose of Alloxan
Group VI	Received higher dose of AG-S90 (300mg/kg/d) suspended in the vehicle (10ml/kg) for 28 days + a single dose of Alloxan

For 28 days study period body weight, food and fluid intake of animals were recorded.

Table 4: Effect of AG-S90 aqueous extract on wistar rats subjected to hot water tail immersion test.

Treatment	Reaction Time (sec)				
	Day 1	Day 7	Day 14	Day 21	Day 28
Group I	9.67± 0.02	9.52± 0.21	9.53± 0.62	9.54±0.78	9.56± 0.79
Group II	6.81± 0.51	6.79± 0.62	6. 74± 0.87	6.81±0.90	6.90± 0.45
Group III	6.72± 0.62	7.82± 0.87	8.93± 1.24***	10.67±1.24***	12.26±1.35***
Group IV	6.56± 0.71	7.80 ± 0.74	8.56± 1.34*	9.35±1.27**	9.45±1.42**
Group V	6.87± 0.80	7.87± 0.60	9.23± 1.44***	9.87±1.32***	11.50±1.40***
Group VI	6.97± 0.97	7.92± 0.54	10.35±1.54***	10.641.40***	12.35±1.62***

All values are presented as Mean ± SD, (n = 6), $p<0.05$*, $p<0.01$**,$p<0.001$*** when compared to disease group.

Table 5: Effect ofAG-S90 aqueous extract on rats subjected to cold water tail immersion test

Treatment	Reaction Time (sec)				
	Day 1	Day 7	Day 14	Day 21	Day 28
Group I	11.67±0.51	11.42± 1.02	11.54± 0.22	11.35±0.32	11.49±0.11
Group II	7.56±0.61	8.01±0.20	7.48±0.03	6.96±0.40	5.88±0.34
Group III	7.63±0.67	8.60±0.02	10.23±0.22***	11.41±0.37***	13.20±0.41***
Group IV	7.65±0.71	8.64±0.40	8.90±0.10*	9.30±0.03**	10.10±0.50**
Group V	7.67±0.69	8.74±0.03	9.65±0.32***	10.04±0.43***	11.97±0.20***
Group VI	7.69±0.81	8.80±0.01	10.87±0.41***	11.90±0.60***	12.85±0.68***

All values are presented as Mean±SD, (n = 6), $p<0.05$*, $p<0.01$**, $p<0.001$*** when compared to disease group.

Table 6: Effect of AG-S90 aqueous extract on rats subjected to paw heat- hyperalgesia test

Treatment	Reaction Time (sec)				
	Day 1	Day 7	Day 14	Day 21	Day 28
Group I	10.42±0.30	10.47± 0.02	10.54± 0.22	10.56±0.32	10.60±0.07
Group II	7.67±0.60	7.56±0.34	7.04±0.31	6.56±0.27	5.06±0.21
Group III	6.87±0.78	7.75±0.05	8.45±0.02***	10.87±0.12***	12.04±0.51***
Group IV	6.77±0.68	7.34±0.16	8.21±0.17*	9.02±0.01***	10.34±0.28***
Group V	6.82±0.56	7.80±0.08	8.30±0.23**	10.01±0.13***	11.10±0.56***
Group VI	7.02±0.60	7.98±0.01	8.90±0.43***	11.20±0.25***	13.38±0.76***

All values are presented as Mean±SD, (n = 6), p<0.05*, p<0.01** p<0.001*** when compared to disease group.

Table 7: Effect of AG-S90 aqueous extract on rats subjected to pinprick test

Treatment	Reaction Time (sec)				
	Day 1	Day 7	Day 14	Day 21	Day 28
Group I	2.07±0.50	2.42± 0.11	2.54± 0.62	2.57±0.61	2.60±0.58
Group II	11.21±0.23	12.07±0.31	13.08±0.12	14.12±0.24	15.17±0.44
Group III	11.05±0.87	10.67±0.50	8.51±0.46	6.89±0.35	5.22±0.11
Group IV	11.30±0.61	11.27±0.42	10.68±0.29***	9.36±0.24***	8.25±0.01***
Group V	11.20±0.50	11.10±0.37	9.60±0.25**	7.82±0.18***	6.14±0.07***
Group VI	11.11±0.30	10.65±0.05	8.90±0.11***	6.76±0.07***	5.29±0.04***

All values are presented as Mean±SD, (n = 6), p<0.05*, p<0.01**, p<0.001*** when compared to disease group.

Table 8: Effect of AG-S90 aqueous extract on body weight, food and water intake in Alloxan diabetic rats

Experiment Groups	Body Weight(g)		Food Intake(g/24hrs)		Water Intake (ml/24 hrs)	
Group I	Initial	Final	Initial	Final	Initial	Final
	165.23±6.20	169.28±2.12	16.02±0.21	18.07±0.02	12.01±0.52	12.21±0.60
Group II	175.31±5.10	150.12±3.05	19.04±0.22	23.15±0.31	47.09±0.16	64.04±0.32
Group III	172.40±2.50	177.16±2.17	21.01±0.30	17.23±0.72	49.12±0.34	18.10±0.41
Group IV	180.43±3.12	184.50±2.41	23.03±032	18.16±0.09	53.34±0.45	36.09±0.62
Group V	179.20±2.03	183.41±3.09	25.05±0.40	I7.32±0.19	57.12±0.32	27.19±0.12
Group VI	183.19±3.40	187.23±4.05	28.04±0.24	16.17±0.16	64.81±0.44	21.70±0.26

Values are the mean ± S.E.M. of six rats/treatment.

Discussion

There is effective and strong relationship exists between hyperglycemia and diabetic neuropathy and microvascular complications in both type 1 and type 2 diabetes (Brownlee, 2001). Generation of superoxide due to oxidative stress in diabetes may be responsible for vascular (hypertension) and neuronal complications of painful neuropathy (Anjaneyulu and Chopra, 2004). Alloxan is acyclic urea compound, which induces permanent diabetes. It is a highly reactive molecule, which produces free radical damage to beta islet cells & causes cell death (Burkart *et al*., 1999). When islets are exposed *in-vitro* to alloxan, it exhibits exceptional beta cell specificity, the other islets cells remaining largely unaffected by both its inhibitory and cytotoxic effects. Alloxan is a specific toxic substance that destroys the β-cells of Islet of Langerhan in the pancrease provoking a state of primary deficiency of insulin without affecting other islet types (α-cells) (Claudia *et al*., 2006). The damage occurs in nerves as neurons over fired due reactive oxygen stress (ROS) generation; hence, alloxan was selected to induce diabetes in the present study.

In this study aqueous AG-S90 extract was given for the prevention as well as treatment of neuropathic pain in Alloxan induced diabetic rats. The development of neuropathy was observed at 7th day after induction of diabetes, which was consistent with previous reports (Anjaneyulu and Chopra, 2004; Aubel *et al*., 2006).The behavioural parameters such as thermal and cold hyperalgesia; and allodynia were assessed by using hotplate, pin prick, hot and cold-water tail immersion tests. In behavioural examination, diabetic rats were shown significant reduction in tail and paw withdrawal latency than the normal control rats, are an indication for decreased nociceptive threshold to heat resulting hyperalgesia and allodynia. Similar models of thermal hyperalgesia and tail flick latency have been reported previously in Alloxan induced diabetic animals. The delay in tail withdrawal response depicts the involvement of spinal reflex arc and delay in paw withdrawal latencies to noxious thermal stimuli depicts the involvement of supra spinal sensory pathways. The hyperalgesic response to a noxious stimulus (pin prick) in diabetic rats were shown significant rise in hind paw withdrawal latency than the normal rats. Rats treated with 200 and 300 mg/kg were shown improvement in mops up free radicals at 14th and 28th day, and heals pancreatic cells respectively. This indicates its protective role against damage to the neurons like the findings of Choi *et al*., 1975 and Dobretsov *et al*., 2009.

Conclusions

The results of AG-S90P revealed strong and well loaded with antioxidant phytochemicals such as anthraquinones, flavonoids, terpinoids, tannins, saponins including others;

practically AG-S90P was very safe tolerability with LD50 greater than 5000mg/kg. The oral administration of aqueous extract of AG-S90 polyherbalnot only attenuated the Alloxan- induced diabetic condition but also detoxified and reversed the neuropathic pain. The probable mechanism could be via enhancing insulin production and decreasing the glucagon production (cellular glucose modulation and restoration). In treated wistar rats with AG-S90 extract shown lactate dehydrogenase (LDH), glutathione (GSH) content, glutathione peroxidase (GPx) activity, glutathione reductase (GR) activity, catalase (CAT) activity, and glutathione S transferase (GST) activity up-regulated and integrity, and decreased Lipid peroxidation (LPO) were similar to the findings described by Wheeler*et al*.., 1990; Habig *et al*., 1974; Carlberg and Mannervik, 1974 and Laight*et al*., 1999. AG-S90 polyherbal detoxifies toxic metabolites in the cells, repairs damaged cells and boosts immunity lead to reversal mechanism of this disease conditions (Ven katesh *et al*., 2010; Achrekar *et al*., 1994).

The onset of neuropathic complications could be prevented by early glycemic controls. Thus, from this study we conclude that AG-S90 polyherbal exhibits significant neuroprotective activities against Alloxan-induced diabetic neuropathy in rats. However, further studies are needed for theAG-S90 polyherbal formulation for better understanding of the mechanism of action.

Acknowledgments
The authors are grateful to all colleagues for their extensive support.

Authors' contributions
MGM, JY, KBT and HUD designed the study and interpreted the results. KBT, JY did statistical analysis of the results. KBT, MGM and JY did the experiments. KBT, MGM and HUD wrote the manuscript. All authors read and confirmed the last edition of the manuscript and confirmed it for publication.

Conflicts of interest
Authors declare that no competing interest exists.

Ethical considerations
Approval for the use of animals in the study was obtained from the Animal Ethics Committee of the Ahmadu BelloUniversity, Zaria (ABUCAUC/2022/024).
Funding/Support
This research was carried out with personal funds from the authors.

References

Abdelmoaty MA, Ibrahim MA, Ahmed NS, Abdelaziz MA. Confirmatory studies on the antioxidant and antidiabetic effect of quercetin in rats. Indian J Clin Biochem 2010;25 (April (2)):188–92.

Abdullahi Attah Alfa, Orukotan Abimbola Ayodeji, Goji Anthony Donatus Teru, and Kokori Bajeh Tijani (2019). Studies on the Phytochemical Compounds in the Ethanolic Leaf Extract (ELE), Ethanolic Bark Extract (EBE) and Ethanolic Root Extract (ERE) of *Bridelia ferruginea* Benth (Euphorbiaceae). *Asian Journal of Biochemistry, Genetics and Molecular Biology*; 2(4):1-8.

Anjaneyulu, M., and Chopra, (2004). K. Quercetin attenuated thermal hyperalgesia and cold allodynia in STZ-induced diabetic rats, *Indian Journal of Experimental Biology*. 42, 766-769.

Attal, N., Jazat, F., Kayser, V., Guilbaud, G. Further evidence for pain-related behaviours in a model of unilateral peripheral mononeuropathy. *Pain*. 41 (1990) 235-251.

Aubel B. et al., Antihyperalgesic effects of cizolirtine in diabetic rats: *behavioral and biochemical studies, Pain.* 110 (2004) 22-32.

Bagri P, Ali M, Aeri V, Bhowmik M, Sultana S. Antidiabetic effect of Punica granatum flowers: effect on hyperlipidemia, pancreatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes. Food Chem Toxicol 2009;47(January (1)):50–4.

Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications, Nature. 414(6865), 813-820.

Burkart V, Koike T, Brenner HH, Imai Y, Kolb H. Dihydrolipoic acid protects pancreatic islet cells from inflammatory attack. Inflamm Res 1993;38:60–5.

Carlberg, I. and Mannervik, B. (1975) "Glutathione reductase levels in rat brain, "*The Journal of Biological Chemistry*, vol. 250, no. 14, pp. 5475–548,.

Charlton, A., Garzarella, J., Jandeleit-Dahm, K. A., and Jha, J. C. (2020). Oxidative stress and inflammation in renal and cardiovascular complications of diabetes. *Biology*, *10*(1), 18.

Choi Y. *et al*., (1995) .Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain, *Pain.* 59, 369-376.

Claudia, E.N.M., Julius, E.O., Dagobert, T. Etienne, D. (2006). Antidiabetic and Hypolipidemic effects of Laporteaovalifolia (URTICACEAE) in alloxan induced diabetic rats, *Afr. J. Tradit. Complement. Altern. Med.* 3(1) , 36-43.

Dewanjee S, Bose S, Sahu R, Mandal S. Antidiabetic effect of matured fruits of Diospyros peregrina in

alloxan-induced diabetic rats. Int. J Green Pharm 2008;2 (April (2)):95.

Dobretsov, M., Hastings, S. L., Stimers, J. R. and Zhang, J.-M (2009). "Mechanical hyperalgesia in rats with chronic perfusion of lumbar dorsal root ganglion with hyperglycemic solution," *Journal of Neuroscience Methods*, vol. 110, no. 1-2, pp. 9–15, 200.

Eidi A, Eidi M, Esmaeili E. Antidiabetic effect of garlic (Allium sativum L.) in normal and streptozotocin-induced diabetic rats. Phytomedicine 2006;13 (November (9):624–9.

Farswan, M., Mazumder, P.M., Percha, V. (2009). Protective effect of *Cassia glauca* Linn. On the serum glucose and hepatic enzymes level in streptozotocin induced NIDDM in rats, *Indian J. Pharmacol*. 4, 19-22.

Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, Watal G. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of Annona squamosa L. in experimental animals. J Ethnopharmacol 2005;99(May (1)):75–81.

Habig, W. H., Pabst, and Jakoby, W. B. (1974)."Glutathione S transferases. The first enzymatic step in mercapturic acid formation," *Journal of Biological Chemistry*, vol. 249, no. 22, pp. 7130–7139,.

Jalal R, Bagheri SM, Moghimi A, Rasuli MB. Hypoglycemic effect of aqueous shallot and garlic extracts in rats with fructose-induced insulin resistance. J Clin Biochem Nutr 2007;41(3):218–23.

Jelodar G, Maleki M, Motadayen M, Sirus S. Effect of fenugreek, onion and garlic on blood glucose and histopathology of pancreas of alloxan-induced diabetic rats. Indian J Med Sci 2005;59(February (2)):64.

Jemai H, El Feki A, Sayadi S. Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from olive leaves in alloxan-diabetic rats. J Agric Food Chem 2009;57 (September (19)):8798–804.

Kanaan, S.A. *et al*., (1996). Endotoxin-induced local inflammation and hyperalgesia in rats and mice: A new model for inflammatory pain, *Pain*. 66, 373-379.

Kastrup J. et al., (1987). Intravenous lidocaine infusion – a new treatment of chronic painful diabetic neuropathy?, *Pain*. 28, 69-75.

Kim, S.O., Kim, H.J., (2013). Berberine ameliorates cold and mechanical allodynia in a rat model of diabetic neuropathy, *J. Med. Food*. 16: 511-517.

Koyuturk, M., Ozsoy-Sacan, O., Bolkent, S. and Yanardag, R. (2005). Effect of glurenorm on Immunohisto chemical changes in pancreatic β-cells of rats in experimental diabetes. *Indian Journal of Experimental Biology*, 43: 268-271.

Laight, D.W., Gunnarsson, P. T. Kaw, A. V., ¨Angg˚ard, E. E. and Carrier, M. J. (1999). "Physiological microassay of plasma total antioxidant status in a model of endothelial dysfunction in the rat following experimental oxidant stress in vivo," *Environmental Toxicology and Pharmacology*, vol. 7, no. 1, pp. 27–31.

Lee, J.H., Cox, D.J., Mook, D.G., McCarty, R.C. (1990). Effect of hyperglycemia on pain threshold in alloxan-diabetic rats, *Pain*. 40, 105-107.

Liu CT, Wong PL, Lii CK, Hse H, Sheen LY. Antidiabetic effect of garlic oil but not diallyl disulfide in rats with streptozotocin-induced diabetes. Food Chem Toxicol 2006;44 (August (8)):1377–84.

Maiti R, Jana D, Das UK, Ghosh D. Antidiabetic effect of aqueous extract of seed of Tamarindus indica in streptozotocin-induced diabetic rats. J Ethnopharmacol 2004;92 (May (1):85–91.

Malik, R. A., Tesfaye, S., Newrick, P. G., Walker, D., Rajbhandari, S. M., Siddique, I., Sharma, A. K., Boulton, A. J., King, R. H., Thomas, P. K., and Ward, J. D. (2005). Sural nerve pathology in diabetic patients with minimal but progressive neuropathy. *Diabetologia,* 48(3):578-85.

Novo Nordisk (2023). Activeadvocacy and educational initiative awareness about diabetes and its complications associated with diabetes.

Packer, L., Kraemer, K.. and Rimbach, G. (2001). Molecular aspects of lipoic acid in the prevention of diabetes complications, *Nutrition* 17, 888-895.

Pari L, Amarnath Satheesh M. Antidiabetic effect of Boerhavia diffusa: effect on serum and tissue lipids in experimental diabetes. J Med Food 2004;7(December (4)):472–6.

Pari L, Saravanan G. Antidiabetic effect of Cogent db, a herbal drug in alloxan-induced diabetes mellitus. Compar Biochem Physiol C: Toxicol Pharmacol 2002;131(January (1)):19–25.

Ragavan B, Krishnakumari S. Antidiabetic effect of T. arjuna bark extract in alloxan induced diabetic rats.Indian J Clin Biochem 2006;21 (September (2)):123.

Reshmi, C.R., Fatima, A., Sinilal, B., Latha, M. S. (2001).Antidiabetic effect of herbal drug in Alloxan diabetic rats, Indian Drugs. 38, *International Journal of Pharmacology, Phytochemistry and Ethnomedicine* Vol. 4 59, 319-322.

Shanmugasundaram R, Devi KV, Soris TP, Maruthupandian A, Mohan VR. Antidiabetic, anti hyperlipidaemic and antioxidant activity of Senna auriculata (L.) Roxb. leaves in alloxan induced

diabetic rats. Int J Pharm Tech Res 2011;3(2):747–56.

Shaw,J.E.,Sicree,R.A.,Zimmet,P.Z.,2010.Global estimates of the prevalence of diabetes for 2010 and 2030.*Diabetes Research and Clinical Practice* 87,4–14.

Tesfaye et al., (2011). Painful diabetic peripheral neuropathy: Consensus recommendations on diagnosis, assessment and management, *Diabetes Metabol. Res. Rev*. 27, 629-638.

Venkatesh S, Reddy BM, Reddy GD, Mullangi R, Lakshman M. Antihyperglycemic and hypolipidemic effects of Helicteres isora roots in alloxan-induced diabetic rats: a possible mechanism of action. J Nat Med 2010;64 (July (3)):295–304.

Wainstein J, Ganz T, Boaz M, Bar Dayan Y, Dolev E, Kerem Z, et al. Olive leaf extract as a hypoglycemic agent in both human diabetic subjects and in rats. J Med Food 2012;15 (July (7)):605–10.

Wheeler, C. R., Salzman, J. A., Elsayed, N. M., Omaye, S. T. and Korte Jr., D. W. "Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity," *Analytical Biochemistry*, vol. 184, no. 2, pp. 193–199, 1990.

WHO, 2013.In:World Health Organization (Ed.), WHO Traditional Medicine Strategy2014–2023. WHO Press, Geneva, Switzerland.

Yu, L.-N. , Yang, X.-S. Hua, Z. and Xie, W. "Serum levels of pro-inflammatory cytokines in diabetic patients with peripheral neuropathic pain and the correlation among them," *Zhonghua Yi Xue Za Zhi*, vol. 89, no. 7, pp. 469–471, 2009

Zimmet, P., Alberti, K.G.M., Shaw,J.,2001.Global and societal implications of the diabetes epidemic. Nature 414,782–787.