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Evaluation of antidiabetic activity by using alloxan hydrate induced diabetic's model

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Abstract
The primary goal of this work is to evaluate the antidiabetic efficacy of *Piper cubeba* fruits using an Alloxan hydrate-induced diabetic model and to conduct preliminary fruit extraction using petroleum ether and ethanol, followed by fractionation using an ethanolic extract.

Keywords: *Piper cubeba*, Java, botanical

Introduction
Throughout its long history, the botanical species known as *Piper cubeba*-also called Cubeb Pepper or Java Pepper-has been used in several cultural traditions. Many people are interested in the toxicological and pharmacological characteristics of the *Piper cubeba* fruit. Numerous studies have investigated this plant and its constituents in depth, shedding light on its potential benefits and risks. Research into the antimicrobial properties of *Piper cubeba* has uncovered compounds including cubebin and cubebic acid, which show promise against certain types of bacteria and fungus. Curious about the plant's potential anti-inflammatory effects, researchers have discovered fascinating data that suggests bioactive compounds in *Piper cubeba* may possess such qualities. Research on *Piper cubeba* has shown that it has antioxidant activity in addition to antibacterial and anti-inflammatory effects. Many people in the natural medicine community are interested in the plant because of its potential to fight free radicals, which are linked to a variety of health problems.
Analgesic properties of *Piper cubeba* have long been recognised. While anecdotal evidence suggests the plant has been used for pain relief in the past, scientific research has investigated its potential analgesic properties, providing a more complex picture of its effects.
When it comes to respiratory health, *Piper cubeba* has been around for a while. Although the plant's potential to alleviate respiratory issues has been the subject of several investigations, further research is required to establish its efficacy and safety in this particular context.
Piper cubeba may have medicinal uses, but its toxicological characteristics rule it out. Research into the potential toxicity of botanical substances at higher concentrations has been made feasible by cytotoxicity studies. Toxicological assessments must pay special attention to the kidneys and liver. *Piper cubeba* contains chemicals that, according to some research, might harm the liver and kidneys if consumed in large quantities or exposed to them for an extended period of time. This emphasises the need of thoroughly assessing the quantity and duration of its use. Anyone utilising a herbal product runs the risk of experiencing an allergic response. *Piper cubeba* should be used with caution by anyone who have known sensitivities to it. It is also recommended that women who are pregnant or nursing use caution while handling the plant, since there is less evidence on its safety during these periods.

Literature Review
Rai, Gopal & Jain, Nihali & Pandey, Vikas (2023) [1]: The culinary use of herbs and spices (CHS) as seasonings and flavour enhancers is well-known. Because of their many uses in medicine, these plants are highly regarded in traditional Ayurvedic practice in India. Several early studies have shown the potential effects of *Piper cubeba* L. on hypertension, inflammatory reactions, cancer, neuronal lipofuscinogenesis produced by D-galactose in albino rats, and other diseases. *Piper cubeba* (PC), sometimes referred to as tail pepper, has several underappreciated therapeutic properties.

In treating respiratory problems including bronchitis, congestion, and throat infections, the PC includes essential oils that have diverse therapeutic effects. These oils not only help with dyspepsia but also act as diuretics. The main components of the berry oil include sabinene, β-elemene, β-caryophyllene, cubebol, epicubebol, o-cadinene, trans-sabinene hydrate, and cubebol. Also contained in substantial numbers are alkaloids including piperine, glycosides, tannins, flavonoids, anthraquinones, saponins, carbohydrates, tannins, and coumarins. Because of their antioxidant, antibacterial, and anti-inflammatory characteristics, these photo-constituents have beneficial effects on several chronic non-communicable diseases. The greatest phenol content in PC is supposedly due to the polyphenols, which provide it exceptional antimutagenic action. It significantly reduces lipid peroxidation in the liver and restores normal levels of the beneficial antioxidant enzymes CAT and NP-SH (Non-protein thiol).

Milenkovic, Aleksandra &Stanojević, Jelena (2023) [2]
The commercially relevant essential oils were sourced from the unprocessed fruit of the black pepper plant (*Piper nigrum* L.) and the cubeb pepper plant (*Piper cubeba* L.), which were sourced from the Serbian market. Pepper fruits have a danger of spoilage during transportation and storage, which might reduce their usefulness as feedstocks, because they are exclusively imported to Serbia. Hydrodistillation was used to guarantee the utmost purity of essential oils after manually crushing the fruits at low temperatures, which were cooled immediately upon purchase. There were 42 components in cubeb pepper essential oil (CPEO) and 34 components in black pepper essential oil (BPEO). Comparatively, CPEO inhibited Candida albicans isolate and ATCC strains more efficiently than B. cereus. Bacillus cereus (both isolated and ATCC strains) and Salmonella enterica (isolate) were unaffected by either oil. There was a significant increase in the antioxidant effects after incubation for one hour. The results of this study have significant bearing on the future of the aromatic plant, pharmaceutical, and food sectors, among others.

Alqdeeri, Fatimah &Rukayadi, Yaya & Abas, Faridah (2023) [3]**:** Ligustrum cubeba, belonging to the Piperaceae family, this plant is one of several that are used for medicinal purposes globally. The main objective of this research was to separate the components of P. cubeba L. that have bioactive properties from their respective fractions. To test the antibacterial and antispore characteristics of the compounds, Bacillus cereus ATCC33019, Bacillus subtilis ATCC6633, Bacillus pumilus ATCC14884, and Bacillus megaterium ATCC14581 vegetative cells and spores were administered. The dichloromethane (DCM) and n-hexane fractions of the methanolic extract of P. cubeba L. berries include chemicals that have never been documented previously in isolation. The compound was obtained from the DCM-soluble fraction (2), whereas the n-hexane fraction produced chemical (1). In order to effectively separate these molecules, we used a battery of successive chromatographic methods. Linoleic acid, or 1, is the initial component, and cubebin, or 2, is the end product. The antimicrobial properties of the substances were examined in a Bacillus test. Findings indicate that compound (1) induced a 9.31–9.61 mm wide inhibitory zone in Bacillus sp. vegetative cells. Bacillus sp. had an MBC of 250.0 g/mL and a MIC of 63.0 g/mL. MBC stands for minimum bactericidal concentration. After four hours of incubation, the concentration of the isolated chemical compound (1) was found to be 0.05%. At this concentration, 90.99% (or more than three log10% of the Bacillus sp. spores) became inactive. Furthermore, increasing the concentration of the chemical to 0.1% resulted in the complete elimination of the spores. Compound 1 strongly inhibited the growth of Bacillus sp. vegetative cells and spores in an antibacterial tests. Chemical 2 was still ineffective against the majority of Bacillus strains. The antibacterial properties of compound (1) may be due to its mechanisms of action. You may extend the shelf life of food by adding preservatives to it.

Borges, Alexandre & Pires de Oliveira, Ivan (2023) [4]**:** *In vitro* larvicidal investigations using cubebin, dihydrocubebin, and hinokinin-lignans derived from *Piper cubeba* fruits-were previously performed by our research group. There was a strong larvicidal effect of these compounds against Haemonchuscontortus larvae. Hinokinin demonstrated the highest level of activity, as shown by an EC50 value of 0.34 µg/mL. Scanning electron microscopy revealed that this material strongly affected the cuticle of L3 stage larvae. Using the Haemonchuscontortus enzyme phosphomethyltransferase, in silico investigations were conducted to determine the function of these molecules. Enzymes like this one synthesise phosphocholine, a crucial lipid for worms, by combining the di-domains of two proteins. Because mammals lack this route, targeting this enzyme is crucial for the development of novel anthelmintics. Molecular docking, molecular dynamics, and density functional theory research all came to the same conclusion: the three lignans interact with PMT-1 very little. However, hinokinin has a significant interaction with PMT-2, which deactivates the enzyme and disrupts phosphocholine synthesis. Worms rely on this synthesis to build and maintain their cuticle, a structure that is crucial to their survival. Based on previous laboratory testing and computer-based analysis, it has been suggested that hinokinin might be a potential target for the development of novel anthelmintic drugs against the PMT-2 domain worm Haemonchuscontortus.

Weluwanarak, Thekhawet&Changbunjong, Tanasak (2023) [5]**:** Examining the efficacy of the essential oil (EO) derived from the fruit of the *Piper nigrum* L. tree against the stable fly, a blood-feeding insect, was the objective of the experiment. The purpose of this research was to evaluate the insecticidal efficacy of essential oil (EO) by subjecting it to fumigant and contact toxicity tests. To determine the primary components of the essential oil (EO), chemical analysis using gas chromatography-mass spectrometry was performed. The investigation found that the essential oil had the following components: sabinene (24.41%), limonene (23.80%), Π-caryophyllene (18.52%), and α-pinene (10.59%). In the first twenty-four hours after exposure, the data showed that the fly death rate rose with both concentration and duration of EO exposure. For contact toxicity, the LD50 value was 78.37 µg/fly while the LD90 value was 556.28 µg/fly. The studies also revealed that a 90% deadly dose of 45.63 mg/L of air was present, with a median lethal dosage (LD50) of 13.72 mg/L of fumigants. Based on our research, essential oil from P. nigrum fruit might be a good natural insecticide for keeping flies in check. More studies in real-world settings and investigation into the efficacy of nano-formulations are required, as is testing the essential oil extracted from P. nigrum fruit.

***In vivo* antidiabetic activity**

Purpose and Rationale: It is the primary objective of this research to determine the extract's efficacy as a preventive strategy. Against diabetes brought on by Alloxan hydrate. The induction of diabetes in several experimental animals is a common usage of Alloxan Hydrate. Inducing severe diabetes with only one exposure, alloxan hydrate is a very efficient diabetic medication.

Animals: About twenty-five female Wistar rats, ranging in weight from 150 to 200 grammes, were used in the study.

Groups:

Table 1 a: Group of rats for Antidiabetic activity

Group 1	Vehicle treated
Group 2	Alloxan Hydrate Control [130 mg per kg]
Group 3	Ethanolic extract of *Piper cubeba* [250 mg per kg]
Group 4	Ethanolic extract of *Piper cubeba* [500 mg per kg]
Group 5	Pioglitazone (20 mg per Kg)

Procedure

Toxin: The patient was given 130 milligrammes of alloxan hydrochloride per kilogramme of body weight by intravenous injection into the peritoneum.

Standard Drug: Twenty milligrammes of pioglitazone per kilogramme of body mass was the dosage that was administered orally.

Dose selection: In accordance with the OECD recommendations, the extract was deemed safe at dosages as high as 2000 mg/kg. Consequently, the oral dosages of 250 mg/kg (Dose 1) and 500 mg/kg (Dose 2) were chosen for the study.

Methods: In the experimental design, fifteen animals were divided into five groups.

Dosage schedule: The rats were in a freshly fed condition and had fasted for 18 hours before to the experiment. The sodium chloride solution was prepared by dissolving it in water. To induce diabetes in rats, a dosage of 130 mg/kg body weight was given intraperitoneally. The normal water in the feeding bottles was replaced with a 10% glucose solution after an hour of giving Alloxan to control drug-induced hypoglycemia. The hyperglycemic activity of alloxan was assessed in rats three days prior to its administration. A glucometer, most especially the Accu check, was used for this purpose. A sterile needle was used to pierce the tip of the tail in order to extract a drop of blood. Animals were considered diabetics and participated in the research if their blood glucose levels were 300 mg/dl or above after one week of stabilising the diabetes condition. A freshly prepared solution of each drug was orally given to each animal at the prescribed dose.

Table 1.b: Dosage schedule of Antidiabetic activity

Groups	Treatment	Day 1 to Day 7	Day 8 to Day 21
I	Vehicle treated	0.75% CMC solution in distilled water	0.75% CMC solution in distilled water
II	Alloxan Hydrate Control	Alloxan Hydrate [130 mg per kg] + 0.75% CMC solution in distilled water	0.75% CMC solution in distilled water
III	Dose 1 [250 mg per kg]	Alloxan Hydrate [130 mg per kg] + Ethanolic extract of *Piper cubeba* [250 mg per kg]	Ethanolic extract of *Piper cubeba* [250 mg/kg]
IV	Dose 2 [500 mg per kg]	Alloxan Hydrate [130 mg/kg] + Ethanolic extract of *Piper cubeba* [500 mg per kg]	Ethanolic extract of *Piper cubeba* [500 mg/kg]
V	Standard	Alloxan Hydrate [130 mg per kg] + Pioglitazone [20 mg per Kg]	Standard

Group I- Vehicle control: One dose of the vehicle was taken orally once day for 21 days in a row. Fasting blood glucose levels were measured on days 14 and 21 using a glucometer.

Group II- Alloxan Hydrate control [130 mg/kg]: Starting on day one and continuing until day seven, take one oral dose of Alloxan Hydrate (130 mg per kg) with 0.75% CMC in distilled water once daily. Every day from day eight through day twenty-one, an oral dosage of 0.75% CMC solution in distilled water was administered without interruption. Blood glucose levels were measured when the subjects were fasting on days 14 and 21, respectively, using glucometers.

Group III- Ethanolic extract a of *Piper cubeba* [250 mg/kg]: from the first day to the seventh, a single oral dose of Alloxan Hydrate (130 mg/kg) and an ethanolic extract of *Piper cubeba* (250 mg/kg) was administered. The ethanolic extract of *Piper cubeba* was administered orally once daily from day eight through twenty-one at a dose of 250 mg per kg. Blood glucose levels were measured when the subjects were fasting on days 14 and 21, respectively, using glucometers.

Group IV- Ethanolic extract a of *Piper cubeba* [500 mg/kg]: oral administration of 130 mg/kg of Alloxan Hydrate and 500 mg/kg of *Piper cubeba* ethanolic extract was given to the participants once daily from the first day to the seventh. From day 8 through day 21, the ethanolic extract of *Piper cubeba* was taken orally once day at a dosage of 500 mg per kilogramme. The fasting blood glucose levels were determined by taking glucometer readings on days 14 and 21.

Group V- Standard control: During the first seven days, a single oral dose of Alloxan Hydrate (130 mg per kg) and Pioglitazone (20 mg per kg) was administered daily. Every day, in a certain order, from day 8 through day 21, patients were given 20 mg per kilogramme of Pioglitazone. On days 14 and 21, fasting blood glucose levels were measured using the glucometer.

Evaluation: On days 14 and 21, when the animals were under ether anaesthesia, blood samples were collected from the retro-orbital plexus using a capillary tube in all groups. Centrifuging the blood to get serum and triglycerides allowed for the estimation of total cholesterol, HDL

cholesterol, and several other lipid profiles.

***In vivo* antidiabetic activity study biochemical parameters monitored / estimated**

Estimation a of Blood a Glucose (By Glucometer)

Table 2a: *Piper cubeba* extract's effect on elevated amounts of glucose in the blood of diabetic rats exposed to alloxan hydrate during fasting

Groups	Fasting Blood Glucose Level (mg per dL)	
	Day 14th	Day 21st
Vehicle control	90.28±3.47	93.34±6.29
Alloxone control	499.88±4.16	510.88±5.21
Test 1 (250 mg/kg)	250.08±4.33	153.34±3.87
Test 2 (500 mg/kg)	211.08±6.95	91.34±4.96
Standard (25 mg/ kg)	105.86±3.07	84.94±3.16

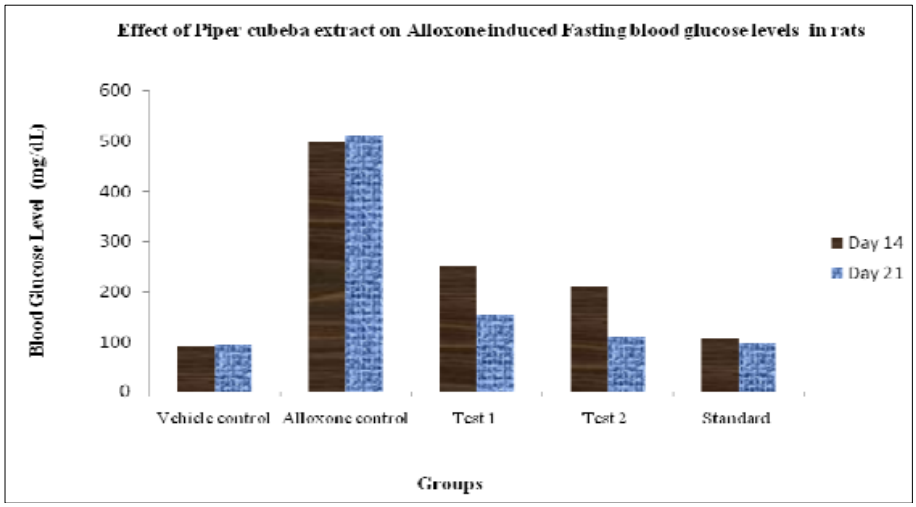

Fig 1: *Piper cubeba* extract's impact on alloxan hydrate-induced fasting blood glucose levels in a rat model of diabetes

After rats were administered the alloxan hydrate toxin, there was a significant increase in serum blood glucose levels. On days 14 and 21, the experimental group compared to the vehicle-treated control group with values of 409.6 mg/dL and 417.54 mg/dL, respectively. *Piper cubeba* extract considerably reduced the alloxan hydrate-induced dose-dependent increase in serum blood glucose levels. The 250 mg/kg dosage decreased serum blood glucose levels by 60.98% on day 14 and 85.63% on day 21, respectively. The 500 mg/kg dosage, on the other hand, inhibited 70.50% and 96.16% on days 14 and 21, respectively. On day 14, pioglitazone showed a 96.19 percent inhibition compared to the alloxan hydrate control group, and on day 21, it showed a 98.85 percent inhibition.

Estimation of Total Cholesterol

Table 2: Impact effects of *Piper cubeba* extract on total cholesterol levels in rats treated with alloxan hydrate to develop diabetes

Groups	Total Cholesterol Level(mg/dL)	
	Day14th	Day21st
Vehicle control	91.53±1.87	89.52±3.38
Alloxone control	199.41±5.61	216.98±6.91
Test1(250mg/kg)	174.07±2.88	150.96±4.76
Test2(500mg/kg)	140.22±3.93	110.94±4.70
Standard(25mg/kg)	120.64±5.99	101.6±4.11

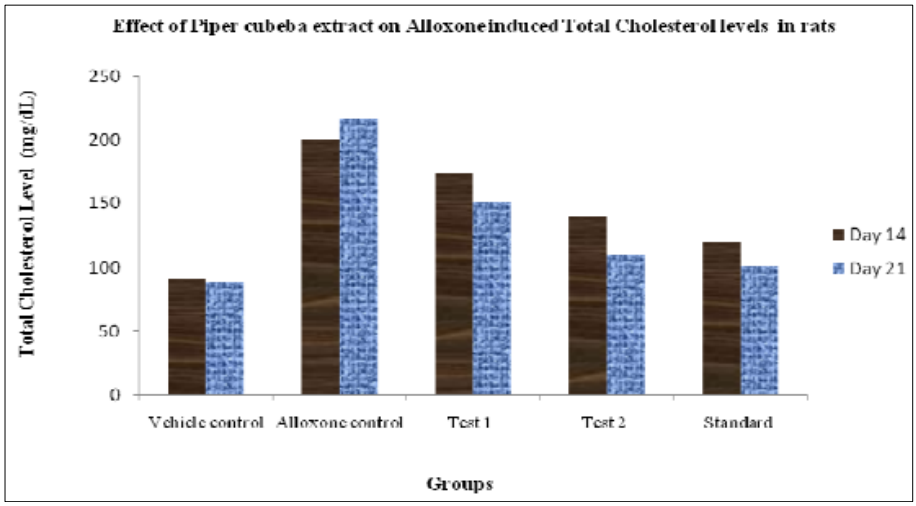

Fig 2: The effect of *Piper cubeba* extract on total cholesterol levels in rats treated with alloxan hydrate to develop diabetes

Alloxan hydrate toxin treatment significantly increased blood total cholesterol levels in rats. The level surpassed that of the control group on day 21, having reached 127.46 mg/dL on day 14, after reaching 107.87 mg/dL on day 14. Reduced alloxan hydrate-induced increases in blood total cholesterol levels were seen to be dose-dependently affected by *Piper cubeba* extract. At a dosage of 250 mg/kg, the suppressed blood total cholesterol level was 23.48% on day 14 and 51.79% on day 21. However, on days 14 and 21, the 500 mg/kg dosage inhibited 54.86% and 83.19%, respectively. Pioglitazone exhibited an inhibition of 74.01% and 90.52%, respectively, on days 14 and 21, in contrast to the alloxan hydrate control group, which exhibited no inhibition whatsoever.

Estimation of HDL Cholesterol

Table 3: Study on the effects of *Piper cubeba* extract on HDL cholesterol levels in rats treated with alloxan hydrate for diabetes

Groups	HDL Cholesterol Level(mg/dL)	
	Day14th	Day21st
Vehicle control	44.51±0.31	47.02±0.74
Alloxone control	17.61±0.33	15.50±0.31
Test1(250mg/kg)	29.47±0.29	32.59±0.34
Test2(500mg/kg)	35.60±0.27	41.53±0.33
Standard(25mg/kg)	42.86±0.54	44.93±0.58

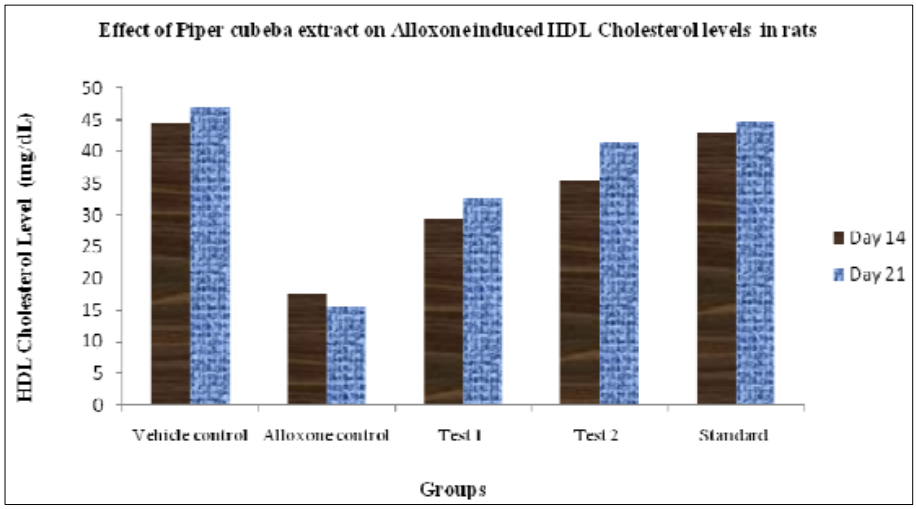

Fig 3: The effect of *Piper cubeba* extract on high-density lipoprotein (HDL) cholesterol levels in alloxan-induced diabetic rats

After being exposed to the alloxan hydrate toxin, rats' blood levels of healthy cholesterol (HDL) drop considerably. On days 14–21, the level increased from 26.9 mg/dL in the control group to 31.51 mg/dL in the experimental group. The potential of alloxan hydrate to reduce blood HDL cholesterol levels was suppressed in a dose-dependent manner by *Piper cubeba* extract when given orally. At a dosage of 250 mg/kg, the blood HDL cholesterol level rose 44.10 percent on day 14, and 54.23 percent on day 21. On the other hand, the 500 mg/kg dosage increased by 82.58% and 66.89% between days 14 and 21, respectively. Blood HDL cholesterol levels increased by 93.37% on day 21 and 93.87% on day 14 when pioglitazone was compared to the alloxan hydrate control group.

Estimation of Triglycerides

Table 4: The effect of *Piper cubeba* extract on the triglyceride levels in rats driven to diabetes by alloxan hydrate

Groups	Triglyceride Level(mg/dL)	
	Day14th	Day21st
Vehicle control	72.19±2.45	71.74±3.12
Alloxone control	272.61±1.65	277.90±5.24
Test1(250mg/kg)	182.0±2.25	145.13±3.71
Test2(500mg/kg)	135.30±3.28	84.81±3.22
Standard(25mg/kg)	97.67±1.51	74.30±3.09

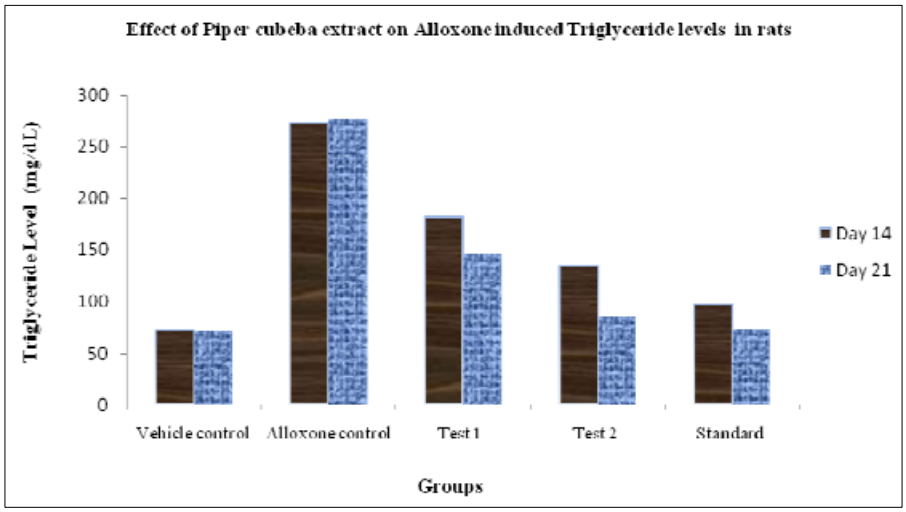

Fig 4: The effect of *Piper cubeba* extract on the triglyceride levels in rats driven to diabetes by alloxan hydrate

Administering the alloxan hydrate toxin to rats resulted in noticeably elevated triglyceride levels. This group's triglyceride level increased from 200.42 mg/dL on day 14 to 206.16 mg/dL on day 21, compared to the vehicle-treated control group. After taking *Piper cubeba* extract, the increase in blood triglyceride levels caused by alloxan hydrate was reduced in a dose-dependent manner. By day 21, the serum triglyceride levels had been reduced by 64.40 percent at a dose of 250 mg/kg, and by day 14, the reduction had been 45.21 percent. On day 14, the 500 mg/kg dose blocked 68.50% of the target, and on day 21, it increased to 93.66%. On day 14, pioglitazone inhibited 98.76% of the alloxan hydrate group, while on day 21, the alloxan hydrate group had an inhibition of 87.28%.

Conclusion

The many bioactivities found in *Piper cubeba* fruits have earned them great respect in traditional medicine. Unfortunately, this drug's antidiabetic effects have not been validated by extensive research. In this follow-up investigation, rats that had been artificially rendered diabetic using alloxan were tested to see whether an ethanolic extract of *Piper cubeba* fruits had any antidiabetic effects. Notable antioxidant, hepatoprotective, and antidiabetic activities are shown by the ethanolic extract of *Piper cubeba* fruits. The results of this study provide credence to long-held assumptions about *Piper cubeba* and highlight the plant's promising future as a source of the bioactive compounds thought to have antidiabetic, antioxidant, and hepatoprotective properties.

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