

Research Article

Studies on the effects of *Adansonia Digitata* Lin's fruits on diabetes

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Article History

Received: 12 Aug 2023

Revised: 10 September 2023

Accepted: 27 October 2023

ABSTRACT:

The point of this study is to look into how *Adansonia digitata* Linn plants can help people with diabetes. The bioactive extract was chosen by using the Haemoglobin Glycosylation Inhibition Assay and α -Amylase Inhibition Assay in a lab setting. So, the ethanolic solution was the most effective at fighting diabetes. In vivo study was done on it because of this. The extract was determined to be safe up to a level of 5000 mg/kg in a trial on acute poisoning. Anti-diabetic action was tested using a model of diabetes mellitus caused by streptozotocin. The factors that were looked at were changes in body weight and blood sugar levels. It was determined that following significant necrotic alterations, a damaged β -cell population, and smaller cells, the cells in the diabetes control group developed atrophy and fibrosis. The group that got the test amount, on the other hand, had normal pancreatic cells, more and bigger

CC License CC-BY-NC-SA 4.0	islets, and no necrosis or fibrotic changes. These were about the same amounts as those found in people who took Glibenclamide, which is the common drug. The analysis of phytochemicals showed that flavonoids exist. These chemicals may be what give <i>Adansonia digitata</i> Linn's fruits their ability to help people with diabetes. The current study says that ethanolic extract greatly lowers the chance of getting diabetes in both lab-based and real-life settings. KEYWORDS: <i>Adansonia Digitata</i> Lin, anti-diabetic activity, Diabetes mellitus, α-Amylase Inhibition Assay,
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INTRODUCTION:

Diabetes mellitus is a metabolic disease that can hurt or destroy many organs, depending on how bad it is [1]. The free radicals that are made as the disease progresses can damage DNA as well as lipid peroxidation, protein change events, and transglycation of key proteins [2]. People who have the chronic form of the disease have worsening hyperglycemia, and high reactive stress causes a number of health problems that make their quality of life worse [3]. Even though a lot of study has been done on the subject for decades [4, 5], no effective therapy method has yet been found.

Herbal treatments that have been shown to work in the past are likely to work as diabetes medicines. Malvaceae is the family name for *Adansonia digitata* and species that are linked to it. Although the tree is native to Africa, it is valued for its health benefits. Different parts of the tree are used to treat a wide range of illnesses because they are such great antioxidants and pain relievers [6–8]. A recent phytochemical study of the leaves [9] found that they contain many different chemicals, such as reducing sugars, alkaloids, steroids, terpenoids, saponins, tannins, flavonoids, anthraquinones, resins, phenols, and cardiac-active glycosides. Minerals like potassium, calcium, iron, phosphorus, magnesium, manganese, and zinc are found in large amounts in the leaves. The leaves also have mucilage, carbs, protein, fiber and fat [10]. Talari et al. [11] had already found that the phytochemical parts of the tree's different parts were very good at getting rid of free radicals in methanolic extracts. This property makes it seem like these methanolic products could be used to make antioxidant medicines that can help with a number of illnesses [12].

In many places, different parts of the tree have been used for a long time to treat diarrhea and dysentery. It has also been said that some parts of trees can kill pests, ease pain, boost the immune system, and reduce inflammation [13]. It's interesting that the leaf extract is ten times more powerful as vitamin C at fighting free radicals. The leaf extract also lowers the production of the anti-inflammatory iNOS gene. This may have something to do with getting rid of peroxyl radicals and stopping NF- κ B from sending signals. Its leaf extract is a strong antioxidant that may protect against cancers that are linked to inflammation [14]. It has already been found that a methanolic product of the bark of its stem can lower blood sugar in people who have diabetes caused by streptozotocin (STZ) [15]. Additionally, some investigations have demonstrated that the plant's pulp and leaves contain a methanolic extract that lowers cholesterol levels [16], indicating that the plant's various parts may be utilized to treat various illnesses [17].

A recent study also found that drugs from plants are thought to be safer and better compatible with biological systems than synthetic medicines because they have fewer negative effects. Because of this, a lot of scientific research is focused on making new medicines for difficult

diseases like diabetes that are based on natural or nature-identical substances that come from herbs [18]. In this study, STZ was used to give lab rats diabetes mellitus, which was meant to mimic the pathophysiology of the human disease [19]. The study's goal was to find out how well the leaf extract reduced the oxidative stress, hyperlipidemia, and hyperglycemia that STZ caused in albino rats [20].

MATERIALS AND METHODS

Plant Collection: *Adansonia digitata* Linn.'s fresh fruits were collected from the Tamil Nadu.

Plant Material Authentication and Identification: The authenticity of the plant material was confirmed by NMKRV College for Women, Bengaluru, Karnataka, India. The plant specimens were stored in the herbarium of the NMKRV College for Women, Bengaluru, Karnataka, India. The fruits were employed for additional research after being coarsely pulverized and shade dried.

MATERIALS AND METHODS

Using the appropriate chemical tests, to ascertain the kind of phytoconstituents a plant contains, phytochemical analysis is employed. Studying the plant's pharmacological actions is crucial. It can be accomplished by confirmation using several chromatographic methods, such as TLC and HPTLC. As a result, a thorough analysis is needed to qualitatively and quantitatively identify the phytoconstituents [21, 22].

Preparation of Extracts:

The first stage in the phytochemical research is extraction. Depending on its polarity, it draws the metabolites into the extraction solvent [23].

Extraction:

In the first step, different liquid extracts were made. First, petroleum ether was used in a Soxhlet device to extract 500g of dried, coarsely powdered *Adansonia digitata* Linn. fruits. Next, liquids like ethanol and ethyl acetate that became more polar were added (60–70°C). Each extract was made stronger with a rotating vacuum evaporator. Before they were put through more thorough phytochemical and pharmacological screening [24], the color, consistency, and yield percentage of these extracts were recorded.

PRELIMINARY PHYTOCHEMICAL SCREENING: The fruit and dried powder extracts of *Adansonia digitata* Linn. were subjected to chemical analyses as demonstrated below in order to identify several phytoconstituents. The results were written down [25].

PHARMACOLOGICAL STUDIES

INVITRO ANTIDIABETIC STUDIES

α -Amylase Inhibition Assay:

The Bernfeld method was used to study amylase blockage in vitro. To put it briefly, 100 μ l of the test extract was combined with 200 μ l of the α -amylase enzyme (Hi median Rm638) and 100 μ l of 2mM phosphate buffer (PH–6.9). After 20 minutes of sitting, 100 μ l of a 1% starch solution was added. The sample was run for the standards after 200 μ l of the enzyme was substituted with buffer. 500 μ l of a dinitrosalicylic acid solution was added to the control and test after it was allowed to sit for five minutes. Keeping them in a hot water bath took five minutes. The absorbance at 540 nm was measured with a spectrometer, and the percentage reduction of the α -amylase enzyme was calculated [26, 27].

The anti-diabetic Haemoglobin Glycosylation Inhibition Assay:

The anti-diabetic properties of *Adansonia digitata* Linn. fruits were examined using colorimetric measurement of the degree of non-enzymatic glycosylation of hemoglobin at 520 nm. In phosphate buffer (0.01M, Ph 7.4.1m), solutions of glucose (2%), hemoglobin (0.06%), and gentamycin (0.02%) were prepared and mixed. Fruit extracts from *Adansonia digitata* Linn. were weighed, diluted in DMSO to make a stock solution, and then diluted further to make solutions ranging from 1 to 5 µg/ml. One milliliter of each concentration was added to the mixture mentioned above. The combination was incubated at room temperature in the dark for a duration of 72 hours. Hemoglobin's level of glycosylation was measured using a colorimetric technique at 520 nm. Plots of percent inhibition against log inhibitor concentration were used to obtain the IC₅₀ values, which were then computed using nonlinear regression analysis based on the mean inhibitory values. The alpha glucosidase inhibitor of choice was acarbose [28, 29].

ACUTE ORAL TOXICITY STUDY:

There are five steps to the acute oral poisoning up and down method, and each step uses one rat of the same gender. This is what OECD rule 425 says. It might take two to four steps to get a good idea of how dangerous the substance is when eaten, based on how many animals die or get sick. With this method, you can use as few animals as possible and still come to a scientific conclusion based on correct facts [30].

Literature review showed that a study was done on the short-term harm of *Adansonia digitata* fruit products. It wasn't until an amount of 5000 mg/kg that the extract turned poisonous. Because of this, the first amount was 200 mg/kg of *Adansonia digitata* apples. After being given the medicine by mouth, the animals were checked on every hour for 24 hours. Then, they were checked on every 24 hours to see how they were acting in general, every 72 hours to see if they were showing any signs of being poisoned, and after 28 days, to see if any of them had died. We got permission from the Institutional Animal Ethical Committee (IAEC) in India to use wistar albino rats for the study [31, 32]. This group is recognized by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

STREPTOZOTOCIN-INDUCED HYPERGLYCEMIC STUDIES: AN EXPERIMENTAL DESIGN

The following treatments were administered to the rats after they were divided into five groups (n = 6) [33, 34]. **Table 1 shows** Anti-diabetic activity Experimental design.

Table 1: Anti-diabetic activity Experimental design

Sr. No	Group	Treatment	Dose	Number of Animal	Number of days
1	Group 1	Control (Normal)	Saline	06	28 days
2	Group 2	0.9% v/v sasline Diabetic control	2 ml p.o	06	28 days
3	Group 3	Glibenclamide	4 mg/kgp.o	06	28 days
4	Group 4	Extract in Low dose	200 mg/kgp.o	06	28 days
5	Group 5	Extract in High dose	400 mg/kgp.o	06	28 days
			TOTAL	30	28 days

Following an 18-hour fast, the test animals received a streptozotocin injection, and a glucose meter was used to measure their blood glucose levels (BGL). To obtain blood samples, tail cutting was employed. Rats considered to be diabetic were selected for the study based on blood sugar levels greater than 250 mg/dl (WHO, 1985). The rats were divided into five groups of six at random for screening. After combining 45 mg/kg body weight of streptozotocin monohydrate with 0.9% v/v cold normal saline, 24 rats (groups II–IV) were given an intraperitoneal injection before they had any food or drink for eighteen hours. Experimental wistar rats (130–180g b/w) were subjected to this procedure in order to induce hyperglycemia, and six control rats (group-I) received an intraperitoneal injection of the same volume of 0.9% v/v cold normal saline [35, 36].

BLOOD AND ORGANS SAMPLE COLLECTION:

Throughout the course of the 28-day treatment, 0.5 ml of blood was drawn from the lateral tail vein using a lance or butterfly needle. On the first, seventh, fourteen, and twenty-first days, the blood glucose level was measured using a Glucometer. After 28 days, blood was drawn in order to analyze its haematological characteristics. The test animals were given a dose of 10 mg/kg of ketamine hydrochloride to induce slumber, after which they were put to death. The pancreas could be separated and used for histological research [37–40].

STATISTICAL ANALYSIS:

The results were shown as Mean $\pm$ S.E.M. One Way of Variable (ANOVA) and Dennett's test were used to look at the data. A p-value of 0.05 is thought to be significant [41, 42].

RESULTS AND DISCUSSION

Table 2: The percentage of yield that was achieved when different extracts of *Adansonia digitata* Linn were extracted using different solvents

Sr. No	Treatment of Extract	Extraction Method	Extract Physical Nature	Color of Extract	Yield Obtained (% W/W)
1	Petroleum ether (Non polar)	Successive solvent extraction method	Semi-solid	Green	2.89
2	Ethyl acetate (Polar)		Sticky	Brownish	3.32
3	Ethanol (Polar)		Solid	Reddish Brown	5.30

Many analytical methods have emerged for the quality assurance of pharmaceuticals derived from plants. Therefore, in order to comprehend the therapeutic efficiency of medicinal plants and to define quality parameters, it is crucial to conduct phytochemical investigations in addition to biological screenings. In this study, several phytoconstituent polarities were separated from the finely ground fruits of *Adansonia digitata* Linn. through the use of solvents of increasing polarity, such as ethyl acetate, ethanol, and petroleum ether, through the process of sequential solvent extraction. The specifics of the metabolites' solubility and polarity in the fruit powder were disclosed by successive solvent measurements. The following are the percentage yields of the different extracts: Ethylacetate (3.32%w/w), Petroleum ether (2.89%w/w), and Ethanol (5.30%w/w). Among the extracts, the ethanolic extract has the highest extractive yield. Using several chemical detection agents, a qualitative preliminary phytochemical analysis was conducted to identify the types of phytoconstituents found in each extract. Proteins, triterpenoids, and steroids were detected in petroleum ether.

There were flavanoids present in ethyl acetate. Alkaloids, steroids, saponins, carbohydrates, glycosides, flavanoids, mucilage, and gums were all detected in ethanolic extract. Quercetin was present in the ethanolic extract, as indicated by the quantitative estimation of the total flavonoid content, which came out to be 5.252 mg QE/gm. Escin was detected in the ethanolic extract, as indicated by the total saponin content of 3.631 mg EE/gm. Using TLC, a single or combination of elements present in each extract was identified and separated as part of a qualitative chromatographic examination of the extracts. The phytoconstituents were separated using a solvent system consisting of methanol and chloroform (96:4). **Table 2 shows** The percentage of yield that was achieved when different extracts of *Adansonia digitata* Linn were extracted using different solvents

INVITRO ANTI-DIABETIC ACTIVITY

α -AMYLASE INHIBITION ASSAY:

Table 3: Values of IC₅₀ for the α -amylase inhibitory test in standard

Sr. No	Concentration (μ g/ml) Standard (Acarbose)	Percentage Inhibition of α -amylase
1	125	79.00 $\pm$ 0.17
2	100	59.40 $\pm$ 0.14
3	75	39.93 $\pm$ 0.43
4	50	30.11 $\pm$ 0.50
5	25	22.96 $\pm$ 1.20
IC ₅₀ Value		370.90

Table 4: Values of IC₅₀ for the α -amylase inhibitory test in extracts

Sr. No	Concentration (μ g/ml) of Extract	Percentage Inhibition α -amylase		
		Petroleum ether	Ethyl acetate	Ethanol
1	125	39.01 $\pm$ 0.02	45.90 $\pm$ 0.02	50.89 $\pm$ 0.01
2	250	40.91 $\pm$ 0.01	66.41 $\pm$ 0.01	71.1 $\pm$ 0.02
3	500	29.71 $\pm$ 0.02	35.99 $\pm$ 0.005	46.91 $\pm$ 0.01
4	1000	14.11 $\pm$ 0.03	21.90 $\pm$ 0.02	36.74 $\pm$ 0.013
5	2000	1.39 $\pm$ 0.02	03.17 $\pm$ 0.02	05.41 $\pm$ 0.02
IC ₅₀ Value		1085.17	555.40	442.89

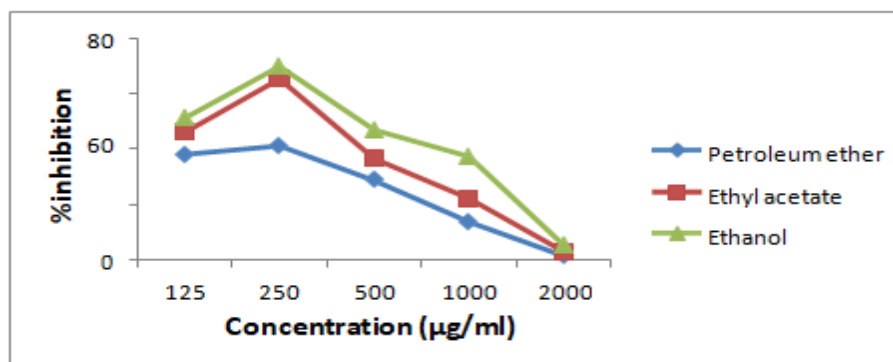


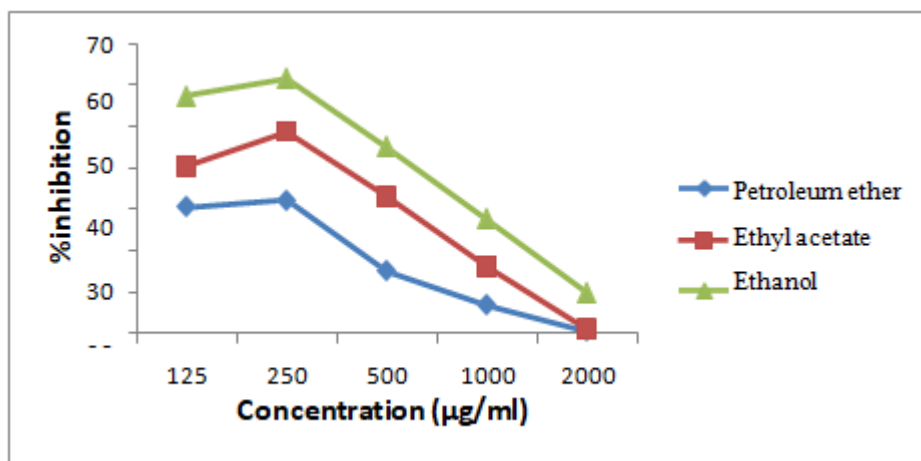
Figure 1: Diagrammatic depiction of the α -amylase inhibition test

HAEMOGLOBIN GLYCOSYLATION INHIBITION ASSAY:**Table 5: The standard haemoglobin glycosylation inhibitory assay's IC₅₀ values**

Sr. No	Concentration (µg/ml) of Standard (Acarbose)	Percentage haemoglobin glycosylation inhibition
1	125	71.09±0.12
2	100	56.11±0.11
3	75	39.70±0.90
4	50	29.17±0.56
5	25	16.99±1.98
IC ₅₀		475.40

Table 6: The haemoglobin glycosylation inhibitory assay's IC₅₀ values in extracts

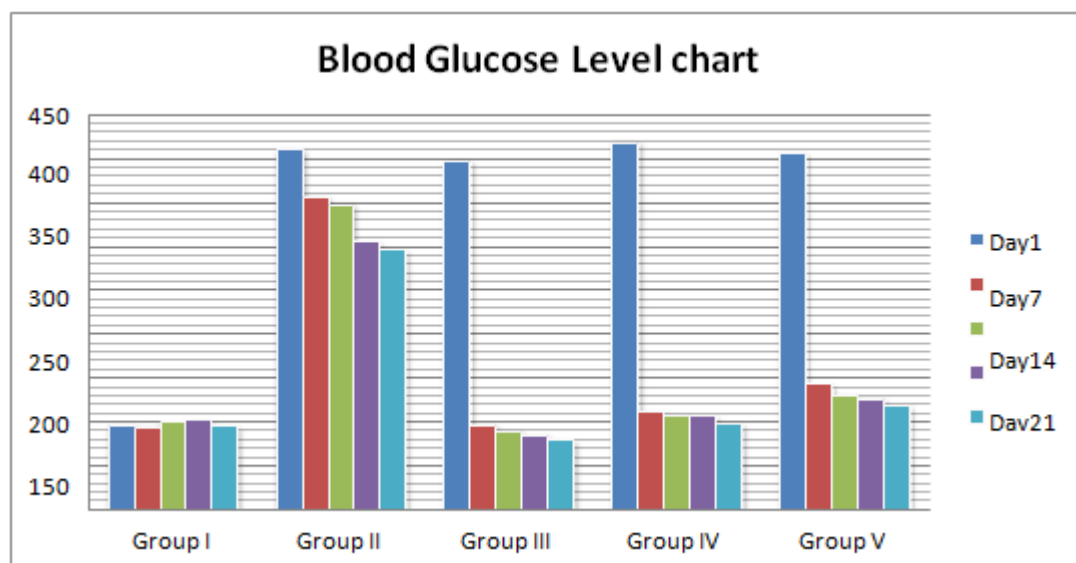
Sr. No	Concentration (µg/ml) of extracts	%Inhibition haemoglobin glycosylation in extracts		
		Petroleum ether	Ethyl acetate	Ethanol
1	125	31.11±0.01	41.17±0.02	56.81±0.01
2	250	32.60±0.01	49.90±0.01	62.12±0.01
3	500	14.90±0.04	32.09±0.01	46.12±0.01
4	1000	06.69±0.01	15.90±0.03	26.76±0.02
5	2000	0.49±0.02	01.09±0.02	09.96±0.02
IC ₅₀		1566.30	1138.99	567.90

**Figure 2: An illustration of the assay for hemoglobin glycosylation inhibition**

STREPTOZOTOCIN CAUSED DIABETES MELLITUS IN RATS:**Table 7: Adansonia digitata Linn. ethanolic extract's effects on blood glucose levels in streptozotocin-induced diabetic rats (mg/dl)**

Treatment of Extract	Number of days					
	00	01	07	14	21	28
GROUP I (Normal)	102±1.89	97±1.30	95±1.50	99±1.00	102±1.11	96±1.91
GROUP II (Diabetic)	109±2.30	411±1.99	355±1.15	347±1.09	303±2.61	295±2.20
GROUP III (Standard)	112±1.11	396±3.40	95±2.80	89±1.11	83±2.12	78±3.13
GROUP IV (Low dose)	104±2.21	419±2.21	112±1.50	105±3.99	105±1.99	96±1.16
GROUP V (High dose)	116±1.15	406±3.30	143±1.11	128±3.11	125±1.20	119±2.21

The numbers are given as mean $\pm$ SD; n=6; P<0.05 compared to the diabetic control group.

**Figure 3: Blood glucose levels in the study groups shown as a graph (mg/dl)****Table 8: Effects of Adansonia digitata Linn. ethanolic extract on streptozotocin-induced diabetic rats' body weight (mg/dl)**

Treatment of extract	Number of days					
	00	01	07	14	21	28
GROUP I (Normal)	136±4.60	139±8.4	142±4.42	145±5.40	132±4.32	145±3.20

GROUP II (Diabetic)	148±4.71	141±7.21	130±5.31	102±5.40	96±4.55	94±1.56
GROUP III (Standard)	156±8.06	153±5.44	136±5.71	145±6.57	153±8.77	160±5.89
GROUPIV (Low dose)	148±8.71	146±9.61	106±5.90	122±8.49	144±5.56	150±3.39
GROUP V (High dose)	150±2.35	143±8.70	100±4.11	120±4.24	123±8.14	135±4.56

The data are displayed as mean $\pm$ SD; n = 6; P<0.05.in contrast to diabetes management. These studies were done to find out what Adansonia digitata Linn's anti-diabetic effects are. Following the OECD 425 guidelines, which were used in the acute toxicity tests, the fruit extracts did not cause any changes in behavior or death up to a dose of 5000 mg/kg, as the literature study shows. The Globally Harmonized Classification System (GHS) says that these products belong to group 5. The tests that were done on living things used 200 mg/kg and 400 mg/kg of the medicine. In vitro studies were done with fruit extracts from Adansonia digitata Linn. The alpha-amylase inhibitory activity of the ethanolic extract showed a lot of promise for blocking. So, the ethanolic extract was used for study that took place in living things. Streptozotocin-induced diabetes in rats was used to test the extract's ability to treat diabetes. This method looked at things like body weight and blood sugar levels, among other things. Glubenclamide (4 mg/kg p.o.) and ethanolic extracts (200 mg/kg and 400 mg/kg) were given on the first, seventh, fourteenth, twenty-first, and twenty-eighth days. Blood glucose levels were checked after giving the animals ethanolic extracts of Adansonia digitata Linn. and they were clearly lower. The body weight of the extract-treated and normal groups both went up a lot compared to the disease control group.

CONCLUSION

Plant extracts and powders underwent an initial phytochemical investigation, which indicated the existence of phytoconstituents in the samples. In vitro experiments called the -Amylase Inhibition Assay and the Haemoglobin Glycosylation Inhibition Assay were used to narrow down the pool of candidates for the most bioactive extract. As a consequence of this, the anti-diabetic effects of the ethanolic extract were the most potent. As a direct consequence of this, it was selected for investigation in vivo. According to the findings of studies on acute toxicity, the extract might be safely administered at doses of up to 5000 mg/kg. Evaluation of anti-diabetic activity was performed by using a model of diabetes mellitus generated by streptozotocin. Alterations to the body weight as well as the levels of glucose in the blood were the factors that were examined. According to the findings of the current research, ethanolic extract substantially cuts down on the likelihood of developing diabetes in both in vitro and in vivo models. More research is required in order to discover the anti-diabetic activity's underlying mechanism of action (mechanism of action).

DECLARATIONS:

Consent for publication:

All the authors approved the manuscript for publication.

Availability of data and material:

All required data is available.

Competing interests:

All authors declare no competing interests.

Funding:

Not applicable.

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