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Evaluation of antihyperglycemic activity and phytochemical screening using bark of *Syzygium cumini*

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ABSTRACT

Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. It is estimated that 25% of the world population is affected. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. Syzygium cumini is one of the most popular plants in the world used frequently in history for its medicinal property. It is need of hour to search for alternative drugs for the most popular plants in the world and has been used frequently in history for its medicinal properties. In the absence of reliable Antidiabetic and antihyperlipidemic drugs in allopathic medical practice, herbs play important role in the management of as Antidiabetic and antihyperlipidemic. So, I have studies the Antidiabetic activity of ethanol and aqueous extracts using bark of Syzygium cumini on Albino Westar rats where as Glibenclamide were used as a standard. Biochemical and histopathological evidences indicated that using treatment with the methanol and aqueous extracts of bark of Syzygium cumini effectively protected rats against alloxan induced diabetes.

Keywords: Diabetes mellitus, *Syzygium cumini*, Bark, ethanol and aqueous extracts etc.

INTRODUCTION

Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. It is estimated that 25% of the world population is affected by this disease. The active principles present in medicinal plants have been reported to possess pancreatic beta cells re-generating, insulin releasing and fighting the problem of insulin résistance. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. *Syzygium cumini* (family-myrtaceae) is one of the most popular plants in the world used frequently in history for its medicinal property. Common names are Java plum, Black plum, Jambul and Indian Blackberry, The original home of jamun is distributed throughout India, in forest upto 1800m usually along the bank and moist localities also cultivated as shade trees along road sides.

Its habitat starts from Myanmar sand extends up to Afghanistan. Seeds contain glycosides, a trace of pale yellow essential oil, fat, resin, albumin, chlorophyll alkaloid- jambo sine Gallic acid, ellagic acid. Corilagin and related tannin,3,6-hexahydroxydiphenoylglucose,1-galloylglucose,3-galloylglucose,quercetin and element such as zinc, chromium, vanadium, potassium and sodium un-saponifiable matter of seed fat contains β-sitosterol. The seeds are sweet, astringent to bowels and good for diabetes.

Fig. 1: Bark of *Syzygium cumini*

Syzygium cumini were reported for antibacterial, anticonvulsant, sedative, hypoglycemic, ant allergic, hepatoprotective and gastro protective activity. The inhibitory effect of *this plant* bark is reported on the inflammation induced by autocoid. So, in present study I perform the Antidiabetic activity of ethanol and aqueous extracts using bark of *Syzygium cumini* Albino Westar rats. Glibenclamide, Fig. 2, were used as a standard.

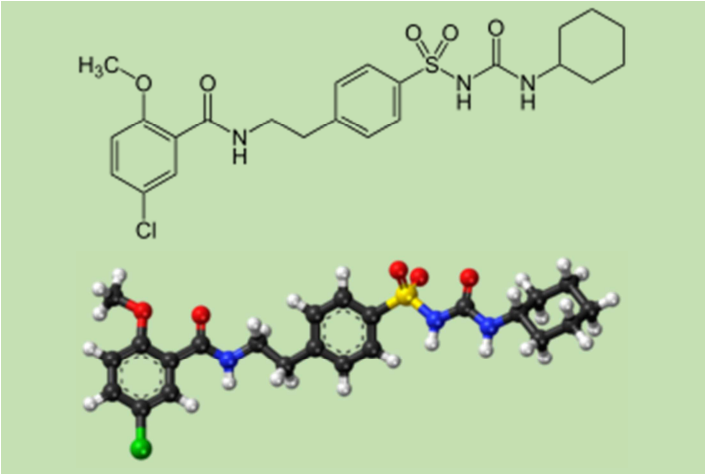


Fig. 2: Structure of Glibenclamide

MATERIALS AND METHODS

The bark of *Syzygium cumini* were collected from the local area of Allahabad District, Uttar Pradesh, India in the month of June-July 2015 and . Healthy, adult Albino Wistar rats (180-200gm) of either sex were purchased from the National Center for Laboratory Animal sciences, Hyderabad used for study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light; and 12 h dark cycle; 23±2^{o} C, 50± 5%, relative humidity), they were fed with standard rat pellet diet(Hindustan Lever Ltd; Mumbai, India) and were ad libitum. The Institutional Animal Ethics Committee approved the study. Remaining reagents were used as supplied by the manufacturer without further purification or investigation.

Preparation of plant extract

The dried bark was ground into fine powder with an auto-mix blender and kept in a deep freezer until the time use. Powder were further extracted with various solvents such as petroleum ether, chloroform, ethyl acetate, methanol and distilled water by successive cold maceration method and percentage yield in different solvents was calculated.

Physicochemical evaluation

Physicochemical studies was carried out using Ash values (Total ash value, Acid-insoluble ash value, Water soluble ash value), Extractive values (Alcohol soluble extractives, Water-soluble extractives), Loss on drying.

Particle size of the microspheres was determine using optical microscopy. The eye piece micrometer was calibrated with the help of a stage micrometer.

Phytochemical screening was perform using the process of successive solvent cold extraction method of powdered bark of *Syzygium cumini*, obtaining the extracts to use for to confirm the presence of various phytochemical using various identification tests.

The acute oral toxicity study has to be carried out as per the guidelines set by OECD, revised draft guidelines 423, received from CPCSEA, ministry of social justice and empowerment, Govt. of India.

Biological activities was performed in which, the animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The test substance was administered in a single dose using a stomach tube or a suitable intubation cannula. Dose was given in smaller fractions over a period not exceeding 24 hours.

Three animals were used for each step. Dose was selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which was most likely to produce mortality in some of the dosed animals. Extract dose of crude drug were freshly prepared as a fine homogenized suspension in aqueous. The rats were randomized into seven groups comprising of six animals in each groups as reported in Table 1.

Table 1: Dose profile for selected groups

Groups	Dose Profile
I	Normal control rats were given 0.5%Tween 80 for 15 days
II	Diabetic controls have been given 0.5%Tween 80 for 15 days, 5 days after alloxan (150mg/kg, i.p.) treatment.
III	Rats have been given Glibenclamide (10mg/kg/day, p.o.) for 15 days, 5 days after alloxan (150mg/kg, i.p.) treatment.
IV	Test rats have been given methanol extract of *Syzygium cumini* (200mg/kg, p.o.) for 15 days, 5 days after alloxan (150mg/kg, i.p.) treatment.
V	Test rats have been given methanol extract of *Syzygium cumini* (400mg/kg, p.o.) for 15 days, 5 days after alloxan (150mg/kg, i.p.) treatment.
VI	Test rats have been given aqueous extract of *Syzygium cumini* (200mg/kg, p.o.) for 15 days, 5 days after alloxan (150mg/kg, i.p.) treatment.
VII	Test rats have been given aqueous extract of *Syzygium cumini* (400mg/kg, p.o.) for 15 days, 5 days after alloxan (150mg/kg, i.p.) treatment.

Assessment of Oral glucose tolerance test

Five days before the termination of the experiment, the oral glucose tolerance test (OGTT) was performed to assess the glucose tolerance. For this purpose, overnight (18 h) fasted rats were fed glucose (2 gm/kg) orally and blood was collected at 0, 30, 60 and 120 minute interval from orbital sinus for glucose estimation.

Blood samples were collected from tail puncturing of each rat at 0 minute, 30 minute, 60 minute and 120 minute and blood glucose was estimated using glucose estimation kit. Percent reduction in blood glucose was calculated with respect to the initial level.

Assessment of Anti-diabetic activity

Blood samples were collected from tail puncturing of each rat at 0 day, 1stday, 10th day and 15th day and blood glucose was estimated by glucose estimation kit. Percent reduction in blood glucose was calculated with respect to the initial level and performed the Statistical analysis.

RESULTS AND DISCUSSION

The percentage yield in various solvents was reported in Table 2; highest percentage yield was obtained in the aqueous extract 22%w/w than other values. Physicochemical parameter such as ash values, extractive values and loss on drying were determined on the powdered seeds of *Syzygium cumini* and the results were reported in Table 3.The particle diameters of more than 500 microspheres were measured randomly. The size range found to be 324.12µm, Fig. 3. Results of Preliminary study of various phytoconstituents such as alkaloids, glycosides, flavonoids, steroids, Phenolic and tannins were reported in Table 4 & 5.

Oral glucose tolerance test (OGTT)

After 120 min of glucose administration the fall observed with the methanol extract of dose of 200mg/kg was found to be 196.1 ± 0.78 and of 400mg/kg was found to be 189.9 ± 1.5, and simultaneously the fall has also been observed in aqueous extract of dose of 200mg/kg was found to be 192 ±0.96, and of 400mg/kg was found to be 180.1 ± 0.7, however the standard drug Glibenclamide produced a fall of 287.4 ± 2.6 in diabetic rats, Table 6 and Fig. 4.

Antidiabetic activity

It has been noted that the effect of treatment of the extracts shows significant reduction on blood glucose levels of diabetic rats, on first day it was found to be 249.04 ±3.89 and on 15th day it was found to be 180.21 ± 8.68, whereas the standard drug Glibenclamide shows the anti-hyperglycemic effect as on 1st day and on 15th day was found to be 249.76 ± 8.85 and 313.28 ± 4.73 respectively, Table 7 and Fig. 5. Histopathological studies reported in Fig. 6.

Table 2:%yield of various extracts of bark of *Syzygium cumini*

S. No.	Extracts	Percentage yield (% w/w)
1.	Petroleum ether	28
2.	Chloroform	36
3.	Ethyl acetate	1.2
4.	Methanol	14
5.	Aqueous	22

Table 3: Results of Physiochemical evaluation

Parameter	Value (%)
Extractive value	
Alcohol soluble extractive	13.7
Water soluble extractive	22.0
Petroleum soluble extractive	8.7
Chloroform soluble extractive	35.0
Loss on drying	4.0
Ash values	
Total ash	4.9
Water soluble ash value	3.0
Acid insoluble ash value	1.8
Swelling factor	16.9

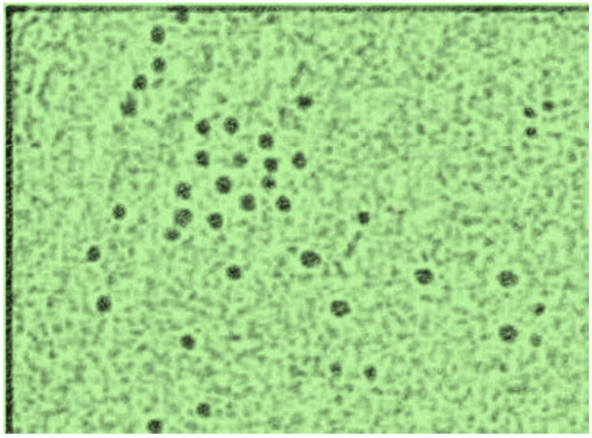


Fig. 3: Powder particles size using projection microscope

Table 4: Phytochemical screening of Bark powder

Chemical constituents	Test	Result
Carbohydrate	Molisch's Reagent	-
Flavonoids	Shinoda Test	+
Phytosterols	Salkowski's test	+
Glycosides	Legal Test	+
Alkaloid	Dragendroff's Test	+
Tannin and Phenolic	Ferric chloride	+
Amino Acid	Ninhydrine Test	+
Saponin	Foam Test	-
Anthraquninone	Born Trager Test	
(+) = Present, (-) = absent		

Table 5: Preliminary phytochemical studies of the extracts of bark of *Syzygium cumini*

Phytoconstituents/ Extracts	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Aqueous
Alkaloids	-	+	-	+	+
Glycosides	-	+	+	+	+
Flavonoids	-	+	+	+	+
Steroids	-	-	-	+	-
Phenolic and Tannins	-	-	-	+	+
Fixed oils	+	-	-	-	-

Table 6: Effect of methanol and aqueous extract of bark of *Syzygium cumini* on OGTT of diabetic rats

Groups (N=6)	Treatment / mg/kg	Blood Glucose Levels (mg/dl)			
		0 min	30 min	60 min	120 min
I	Normal control	90 ± 1.3	126 ± 1.2 (↑40.0%)	117 ± 1.4 (↑30.0%)	93 ± 0.9 (↑3.33%)
II	Glibenclamide(10 mg/kg)	234 ± 1.9	295 ± 2.26 (↑26.06%)	291 ± 1.5 (↑ 24.35%)	287.4 ± 2.6 (↑ 22.64%
III	Control 0.5% Tween 80	179.2 ± 0.8	208.9 ± 1.6 (↑16.57%)	222 ± 1.02 (↑23.88%)	186.1 ± 0.8 (↑3.85%)
IV	Methanol extract (200 mg/kg)	166.3 ± 0.9	212.6 ± 1.09 (↑27.84%)a	239.9 ± 0.9 (↑44.25%)	196.1 ± 0.78 (↑17.91%)
V	methanol extract (400 mg/kg)	183.3 ± 0.6	212.6 ± 1.05 (↑15.98%)a	248.3 ± 0.76 (↑35.46%)	189.9 ± 1.5 (↓3.6%)a
VI	Aqueous extract (200 mg/kg)	209.1 ± 1.4	241.5 ± 0.6 (↑15.49%)	261.6 ± 0.56 (↑25.10%)	192 ± 0.96 (↓8.18%)
VII	Aqueous extract (400 mg/kg)	195.5 ± 2.1	227.7 ± 0.76 (↑6.47%)a	236 ± 0.11 (↑20.71%)	180.1 ± 0.7 (↓12.77%)

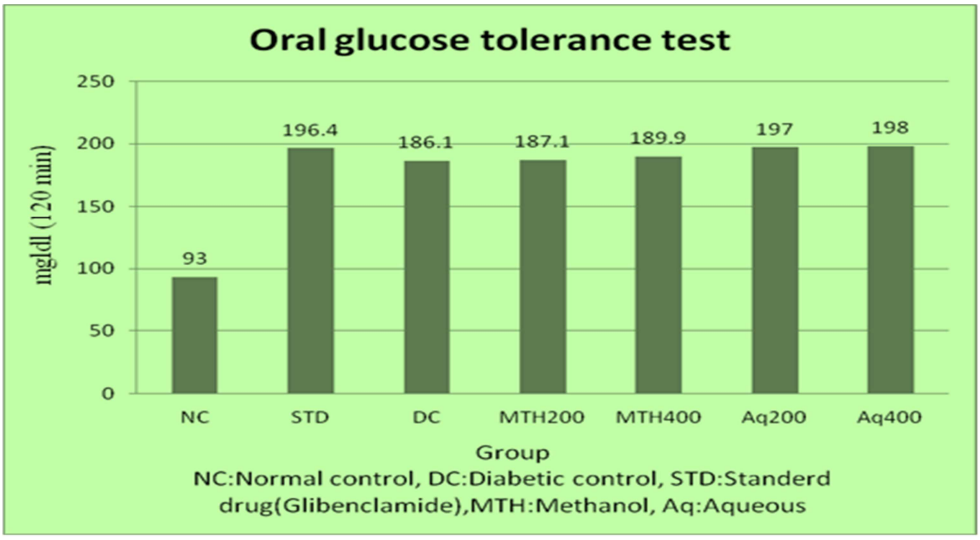


Fig. 4: Effect of bark extract *Syzygium cumini* on OGTT of diabetic rats

Table 7: Effect of methanol and aqueous extracts of bark of *Syzygium cumini* on blood glucose

Groups (N=6)	level Blood sugar in Group (15 days) mg/dL (mean ± SD)				
	Initial	Day 1	Day 5	Day 10	Day 15
Normal control	70.78 ± 7.03	65.05 ± 9.33	66.70 ± 9.85	67.00 ± 7.41	65.48 ± 5.88
Glibenclamide,10 mg/kg	249.76± 8.85	262.28 ±14.75	285.85 ± 4.78	309.20 ± 8.09	313.28 ± 4.73
Control, 0.5% Tween 80	250.85±8.40	252.49 ± 5.57	239.23 ± 8.42	204.38 ± 5.84	192.03 ±5.80
Methanol extract 200 mg/kg	248.04 ±3.89	249.65 ± 7.85	221.24 ± 5.41	189.10 ± 8.22	178.14 ± 9.30
methanol extract 400 mg/kg	249.70 ± 8.85	256.08 ± 4.98	239.88 ± 8.84	214.23 ± 3.33	182.85 ± 4.58
Aqueous extract 200 mg/kg	251.84 ± 4.90	256.57 ± 5.57	233.45 ± 6.30	192.77 ± 4.89	154.85 ± 10.24
Aqueous extract 400 mg/kg	248.38 ± 3.50	251.17 ± 8.14	217.97 ± 4.52	190.10 ± 7.91	180.21 ± 8.68

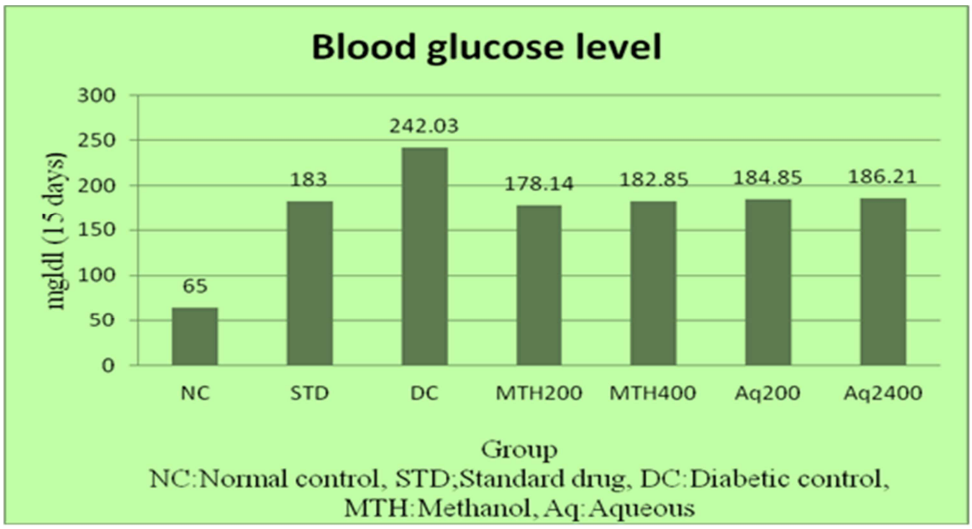
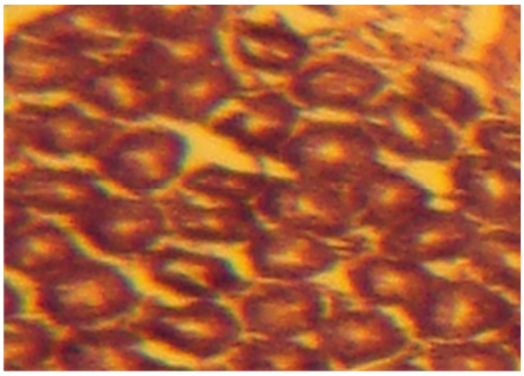
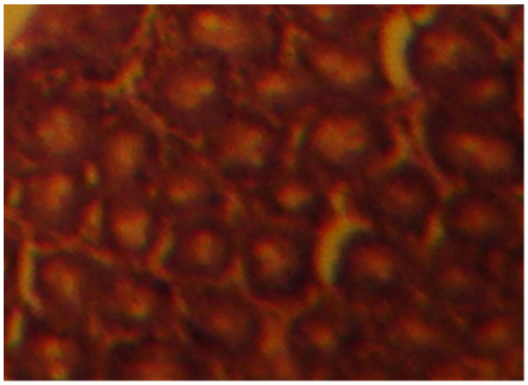


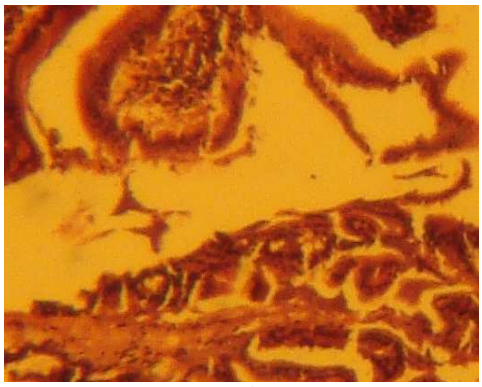
Fig. 5: Effect of bark extract on blood glucose level in alloxan induced diabetic rats



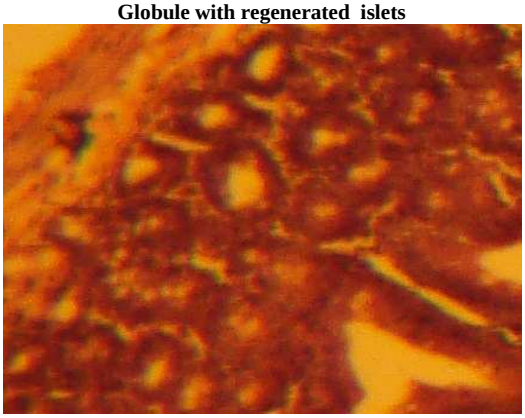
Normal Langerhans cells



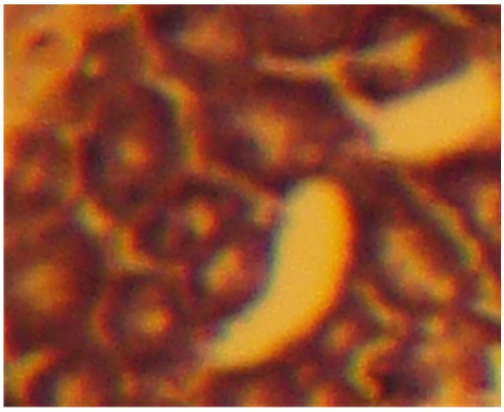
Globule with regenerated islets



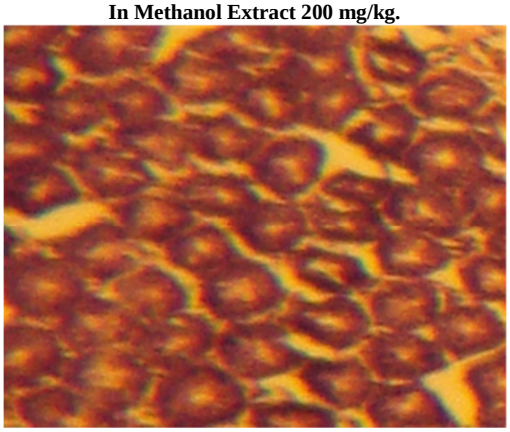
Damage islet of Langerhans



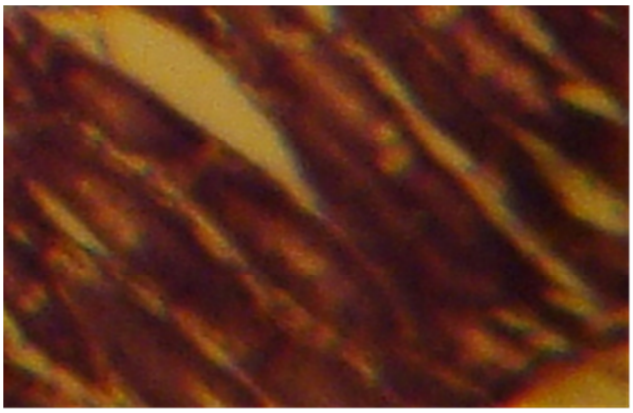
In Methanol Extract 200 mg/kg.



In methanol extract 400 mg/kg.



Aqueous extract 200 mg/kg.



Aqueous extract 400 mg/kg.
Fig. 6:Diagrammatic representation of histopathological studies

CONCLUSION

Studies demonstrated that the methanol and aqueous extracts of bark of *Syzygium cumini* (200 and 400 mg/kg) showed significant antidiabetic activity, in which the level of 400 mg/kg showed potent antidiabetic activity than the dose level of 200 mg/kg. The biochemical and histopathological evidences showed that the treatment with the methanol and aqueous extracts using bark of *Syzygium cumini* effectively protected rats against alloxan induced diabetes. It provides a support for the traditional use in alloxan induced diabetes mellitus. Further studies should be conducted to determine the active compounds or principle that is responsible for the antidiabetic effects and the mechanism of action involved in this study.

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