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Antihypertensive and *In silico* Docking Studies OF Phytoconstituents Isolated from *Syzygium Alternifolium* Bark

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim of the Study: Pharmacological examination was done for methanolic extract *Syzygium alternifolium* bark (MESA) for its antihypertensive activity, Angiotensin converting enzyme (ACE) inhibition by *in vitro* and *in vivo* studies, antioxidant activity *via* radical scavenging activity.
Methods: Albino Wistar rats were allowed to treat with dexamethasone (30 µg/kg/day *s.c*) or saline for about 14 days. Methanolic extract of *Syzygium alternifolium* (300 mg/kg, b.w., *p.o.*) is administered from day 8 to 14 day of study to the treatment group. Chronic fructose treatment in rats showed to increase blood pressure in relationship with insulin blockade. MESA (300 mg/kg b.w, *p.o*) was capable of preventing the origin of hypertension by diminishing the raised blood levels i.e., blood pressure.
Results and Discussion: The decrease in the blood pressure is accredited to the restraint of ACE. The preliminary phytochemical examination suggests that the MESA has alkaloids, flavonoids, glycosides, steroids, sugars, proteins and tannins. MESA exhibited 1, 1-diphenyl-2-picrylhydrazyl interrupting radical chain reactions with IC_{50} value of 12.34 µg/ml just as superoxide radical

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scavenging ability with IC_{50} value of 21 µg/ml. MESA showed antihypertensive activity by limiting angiotensin converting enzyme and interrupted free radical reactions i.e., antioxidant property. These findings reveal the existence of probable active constituents of MESA. Understanding the molecular method of activity of natural product is a vital stage for creating drugs from them. The docking studies of the number of compounds were performed using mCule software. The constituents have good binding ability with ACE 1 inhibitor, Calcium channel blocker, and Renin inhibitor proteins and Ramachandran plot is analyzed.

Conclusion: The compounds from *Syzygium alternifolium* bark have shown antihypertensive activity when compared with standard drug Amlodipine.

Keywords: Syzygium alternifolium Bark; dexamethasone; hypertension; fructose; docking studies.

1. INTRODUCTION

Hypertension is cardiovascular illness; some place in raised blood vessel stress causes obsessive differences in the hypertrophy and vasculature of left heart ventricle. Hypertension is viewed as a condition of oxidative pressure that can add to increase of atherosclerosis and other hypertension prompted organ mutilation. Angiotensin changing over protein (Angiotensin converting enzyme), results angiotensin II from angiotensin I and bradykinin (a hypotensive peptide) to inactive components. High ACE action prompts expanded conversion of the angiotensin II and hypertension. Consequently, improvement of agents that obstruct the change of angiotensin I - angiotensin II, and bradykinin to latent parts started as a remedial methodology to treat hypertension. Inhibitors of ACE are important in the treatment towards hypertension & the maintenance of electrolyte balance. Captopril, besides is known to have negative effects when used for a long time. Because Reactive Oxygen Species (ROS) induced free radical stress in cardiac and vascular myocytes has proved to produce cardiovascular tissue injury and maintain vascular wall homeostasis, an equilibrium between ROS, endogenous transmitters angiotensin II & nitric oxide is critical. Hypertension aroused by chronically elevated levels of angiotensin II is interceded by superoxide anions partly, as has been well documented [1].

In our investigation, dexamethasone and fructose were used to induce hypertension, while amlodipine applied as reference medication. Chronic dexamethasone usage resulted in very regular manifestation *i.e.,* hypertension. Specific mechanism is still to be discovered. Dexamethasone-induced hypertension was related towards alterations in numerous pathophysiological systems that affect blood pressure, including plasma volume, sympathetic activity, the renin-angiotensin-aldosterone system, vasodepressor & vasopressor systems. [2]. In rats, increased intake of either sucrose or glucose accelerated the incidence of voluntary hypertension or salt hypertension. The exact mechanism for fructose inducing hypertension is unknown. The blood pressure response is related by increased fructose diet and not related to high activity of Renin Angiotensin Aldosterone (RAA) pathway, according to evidence [3]. Insulin low sensitivity & hyperinsulinemia were thought to have part in the etiology of fructose-induced hypertension [4]. *Syzgium alternfolium* (Wight) Walp. (*Myrtaceae*), sometimes known as "Mogi," is a moderate-sized deciduous endemic tree found in abundance in the Seshachalam Hill ranges of the Southern Eastern Ghats, particularly in the Tirumala Hills. Alkaloids, flavonoids, indoles, steroids, carbohydrates, phenols, proteins, lignins, saponins, triterpenoids (friedelane, friedelinol) and stigmasterol can all be identified in bark of stem. The stem & fruits are long been utilized for diabetes treatment in the traditional medical system [5].

Presently research for, molecular docking of ACE, renin and calcium channel inhibitors were performed using diverse computational tools, in perspect to find the optimum inhibitor, which ultimately would provide the basis towards designing drugs against hypertension. The goal of our research is to perform *in vitro, in vivo* antihypertensive activity, antioxidant activity and *In silico* analysis by docking and Ramachandran plot

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Drying

The bark of the *Syzygium alternifolium* was collected in Andhra Pradesh, India. Plant samples were prepared and sent to Dr. K. Madhava Chetty, botanist at S.V. University in Tirupati, for authentication. To remove adherent particles and trash, the entire plant was

thoroughly cleaned with water and dried in the sun. Dried material of plant was allowed to ground with a pulverizer and sieved # 20 before being stored in an airtight container until castoff.

2.2 Preparation of *Syzygium alternifolium* Methanolic Extract (MESA)

Simple distillation was used to extract the powdered plant material from 500 ml of methanol, and the plant material was suspended in a round bottomed flask containing the extraction solvent. After that, a condenser was added, and the flask was heated, allowing active extract components to enter the fluid. Source was filtered at the end of the extraction procedure. The surplus was evaporated using water bath, and the extracts were maintained in desiccators to remove any remaining moisture before being stored in airtight ampoules for future use.

2.3 Preliminary Phytochemical Screening of Plant

The plant is a biosynthetic laboratory, producing a variety of compounds such as glycosides, alkaloids, volatile oils, tannins, and other physiological and medicinal substances in addition to chemical molecules such as carbohydrates, protein, and lipids. MESA was examined for the presence of a number of different chemicals using preliminary phytochemical tests.

2.4 Acute Toxicity Testing

Toxicity tests were carried out in order to determine the safety of the *Syzygium alternifolium* extract. The OECD 425 guidelines were followed when conducting acute toxicity tests. The first test, i.e., sequential test, is a limit test in which a maximum of five animals are used. A 2000 mg/kg test dose or, in rare cases, 5000 mg/kg may be utilized.

2.5 Experimental Protocol

Albino research in Hyderabad provided Wistar albino rats (170-200 gm). The current study was conducted in the Gokaraju Rangaraju College of Pharmacy's CPCSEA-approved animal house in Bachupally, Hyderabad, India. 1175/PO/ERe/S/08/CPCSEA (Reg. No. 1175/PO/ERe/S/08/CPCSEA). The animals were kept in poly acrylic cages with a 12-hour light/12-hour dark cycle, with no more than six animals per cage. Rats have unrestricted access to a conventional food and unlimited water. The albino mice were allowed to spend eight days in the preclinical laboratory environment before the experiment began. The albino mice were cared for and maintained according to the CPCSEA approved guidelines.

2.6 *In Vitro* Antioxidant Activity

2.6.1 DPPH radical scavenging activity

In the presence of DPPH stable radical, the hydrogen-donating capacity of MESA was tested. 2.5 mL of test solution containing various amounts of MESA were added to one mL of 0.3 mM DPPH and allowed to react at normal temperature. Following 30 minutes of development, at the 517 nm absorbance measurement using UV spectrophotometer (Schimadzu) is done. Methanol (1.0 mL) served as the blank, DPPH solution (1.0 mL, 0.3 mM) and methanol (2.5 mL) are the negative control, ascorbic acid is taken as the standard [6].

2.6.2 Superoxide radical scavenging assay

1.4 mL of 50 mM potassium dihydrogen phosphate-potassium hydroxide, pH 7.4, including 1 mM EDTA, 0.5 mL of 100 L hypoxanthine, and 0.5 mL of 100 L NBT were used to make the reaction mixture of 3 mL in each tube. The reaction was initiated by mixing 0.066 units of xanthine oxidase in 100 mL of phosphate buffer, pH 7.4, with 0.5 mL of MESA extract in saline. At 560 nm, NBT reduction was determined using a spectrophotometric technique. Gallic acid is taken as a standard, and the same method was used to evaluate MERB. The data were presented as a percentage of NBT inhibition [7].

2.7 Antihypertensive Activity

Antihypertensive activity was evaluated *in vitro* using angiotensin converting enzyme inhibitor and two *in vivo* models, such as dexamethasone induced and fructose induced hypertension.

2.7.1 *In vitro* evaluation of ACE-inhibitory activity

The spectrophotometric test was used to evaluate ACE inhibitory activity *in vitro*. Rabbit lung provides the substrate, hippuryl histidyl-leucine (HHL), and angiotensin converting enzyme (ACE). Testing solutions (40 µL) were

incubated for 30 min at 37°C with 100 mL of 0.1 M borate buffer (pH 8.3) containing 5 mM HHL and 0.3 M NaCl, as well as 20 mL of ACE (2 mU), before being stopped, added with 150 mL of 1 M HCl. The produced hippuric acid was extracted with 1000 µL of ethyl acetate, centrifuged at 1500 rpm for 10 min, and 750 µL of the organic phase were allowed to evaporate. The residue added to 800 mL of distilled water so that it dissolves and the absorbance was measured at 228 nm. Each trial was carried out in triplicate. Inhibitory activity denotes as protein concentration that requires to low 50% of ACE activity as measured by bicinchoninic acid assay using bovine serum albumin as standard (IC_{50}) [8].

2.7.2 *In vivo* evaluation of antihypertensive activity

2.7.2.1 Dexamethasone induced hypertension

The Wistar rats were feed pellet meal & water, their behavior was monitored daily. On day one, every rat was weighed and their weights were recorded. They were given a positive control (dexamethasone) and a vehicle for about 14 days in a row in order for them to reach hypertensive state. The MESA (300 mg/kg) and regular amlodipine (3 mg/kg, b.w, *p.o*.) medication was started on the 8^{th} and continued until the 14^{th} day.

Study design of dexamethasone induced hypertension method is Group –I serve as Control (Normal saline) whereas Group –II received Dexamethasone (10 µg/rat *s.c.*) Group –III received methanolic extract of *Syzygium alternifolium* (300 mg/kg, *p.o*) and Group –1V received Amlodipine (3 mg/kg/day, *p. o.*). Venous blood pressure, heart beat and arteriolar blood pressure was measured by the tail–cuff method [9].

2.7.2.2 Fructose-induced hypertension

Rats were randomly distributed to groups at 6 weeks of age, and pulse rate and SBP were examined every 3 days. The animals were decapitated at the end of the experiment, and blood samples were taken for biochemical analysis.

The dexamethasone induced hypertension method features a unique study design. Group –I received normal saline as a control, while Group –II received Fructose (10% *p.o*.) induced hypertension. The methanolic extract of *Syzygium alternifolium* (300 mg/kg*, p.o*.) is given to Group III, whereas Amlodipine (3 mg/kg/day, *p. o*.) is given to Group 1V. An automatic plasma analyzer was used to determine the levels of glucose and triglycerides in the blood. The radio-immunologists measured plasma insulin levels [10].

2.8 *In silico* Analysis

Molecular docking: The mechanism of binding of drug with the target protein is called docking. Docking utilization to find inhibitors for specific target proteins and thus to design new stable drugs from docking results. Docking may calculate by binding energy (energy release during protein and ligand interaction). In this study, mCule software was used for docking.

Structure based drug design: Initially, the protein was generated by picking any one of the chains from the PDB. Water molecules are removed from the chains. Attributes were chosen. Protein-ligand docking was performed for proteins 1EVE, 6KZP, and 3OWN using the mCule online software.

mCule docking results: Docking results show that some of our compounds bind well to ACE I inhibitors (PDB ID: 1EVE), Calcium channel blockers (PDB ID: 6KZP), and Renin inhibitors (PDB 3OWN)

Ramachandran plot: The PROCHECK validation server generated a Ramachandran plot, which was used to assess the model's quality by having a look on the allowed & banned parts of the plot [11].

2.9 Statistical Analyses

The Results were expressed as the mean ± S.E.M. The significance of the results was calculated using ANOVA and Dunnett's test and results were deliberated statistically noteworthy when significant $p<0.0001$, $p<0.001$, $p<0.01$, ns-non significant.

3. RESULTS

Methanolic extract of *Syzygium alternifolium* bark was evaluated for its *in vitro* and *in vivo* antihypertensive activity utilizing applicable animal models. Below, the results procured from the study are given.

3.1 Percentage Yield of MERB Obtained by Simple Distillation

The MESA bark was prepared by simple distillation method. The % yield of the extract was calculated by utilizing the following formula.

$$\% \text{ Yield of extract} = \frac{amount\ of extract\ obtained}{amount\ of\ powder\ used} * 100$$

= 18 % w/w

3.2 Preliminary Phytochemical Analysis

Flavonoids, Alkaloids, glycosides, terpenoids, steroids, carbohydrates, proteins and tannins were found in the *Syzygium alternifolium,* methanolic extract according to early phytochemical analysis.

3.3 Acute Toxicity Studies

Methanolic extract of *Syzygium alternifolium* was evaluated up to a level of 2000 mg/kg bd. wt. Up to 2000 mg/kg bd. wt., on Swiss albino mice, the animal showed no symptoms of toxicity or fatality. As a result, up to 2000 mg/kg bd. wt. of the extract was proven to be safe.

The antioxidant activity of the plant extracts was determined using a DPPH radical scavenging test and a superoxide radical scavenging assay.

3.4 Anti-oxidant Activity

3.4.1 DPPH radical scavenging activity

Table 1 show that the percent inhibition increases as the dose of plant extract is raised, however the responses are not linear for the three dose levels. Inhibition data is further processed to determine relative inhibition compared to the standard. The greater the DPPH radical scavenging activity, the higher the relative inhibition, and the benchmark utilised is ascorbic acid. At lower doses (1-5 g/mL), ascorbic acid produced inhibition, as expected. At higher dose levels (5-25 g/mL), the Methanolic extract of *Syzygium alternifolium* showed substantial inhibition, with a relative percent inhibition of 30% of the standard.

3.4.2 Superoxide radical scavenging activity

The Methanolic extract of *Syzygium alternifolium* exhibited increased percent inhibition as the dose of plant extract increases, although the responses are not linear for the three dose levels represented in Table 2. The greater the superoxide radical scavenging activity, the higher the relative inhibition. Gallic acid is used as the standard in this investigation. Gallic acid showed inhibition at lower dose levels (0.25 to 0.75 g/mL), as expected. Plant extracts also showed significant inhibition at higher dose levels (5-25 g/mL) and had a relatively low relative percent inhibition compared to Gallic acid. The standard and test IC_{50} values are compared. Gallic acid has a low IC_{50} value (0.64), whereas MESA has a high IC_{50} value. The relative percent inhibition decreases at increasing doses, suggesting that the complete dose is not used to elicit the response and that the dose response for the three dose levels is not linear.

Following the efficiency of free radical scavenging activities in two models, researchers attempted to investigate anti-hypertension activity in *in vitro* models.

3.5 Antihypertensive Properties

Antihypertensive activity was evaluated *in vitro* using an angiotensin converting enzyme inhibitor, as well as *in vivo* utilising dexamethasone and fructose-induced hypertension models.

Table 1. DPPH radical scavenging activity of MESA

Test extract/standard	Dose (µg/mL)	Percentage inhibition AM±SEM (n=3)	% Relative inhibition	IC_{50} (µg/mL)
Methanolic extract of *Syzygium alternifolium*	5	28.97 ± 0.0290	34.36	12.34
	10	44.23 ± 0.0290	30.99	
	25	80.78 ± 0.3844	27.50	
Ascorbic acid (Standard)	1	16.86 ± 0.0788	100	4.08
	2.5	35.68± 0.2747	100	
	5	58.74 ±0.1362	100	

Table 2. Superoxide radical scavenging activity of MESA

Test compound/ Standard	Dose (µg/mL)	Percent Inhibition AM±SEM (n-=3)	% Relative inhibition	IC_{50} (µg/mL)
Methanolic extract	5	27.58±0.0464	4.47	
of *Syzygium*	10	36.60±0.0288	4.16	
alternifolium	25	58.17±0.0577	3.17	21
Gallic acid	0.25	30.80±0.0057	100	
(Standard)	0.5	43.89±0.0081	100	
	0.75	54.99±0.0057	100	0.64

3.5.1 *In vitro* ACE inhibitory activity

According to Table 3, the percent inhibition for *Syzygium alternifolium* extract increases with increasing dose; however, the responses for 100 and 500 mg/kg are not proportional for the two dose levels. The inhibition data was then processed for relative inhibition. The greater the relative inhibition, the greater the inhibitory activity of ACE. Captopril was used as the standard in this study, and it produced inhibition at lower dose levels (50 µg/mL) than MESA. At higher dose levels (100-500 µg/mL), the *Syzygium alternifolium* extract also showed significant inhibition. The methanolic extracts of *Syzygium alternifolium* showed relative percent inhibition to captopril at 30%.

3.5.2 *In vivo* antihypertensive activity

Two *in vivo* models were used to assess antihypertensive activity: dexamethasone-induced hypertension and fructose-induced hypertension.

3.5.2.1 Effect of Syzygium alternifolium extract on dexamethasone induced hypertension

Following the detection that methanolic extract of *Syzygium alternifolium* inhibited ACE, further research was conducted to evaluate *in vivo* antihypertensive activity. Table 4 shows that when rats were given with methanolic extract *Syzygium alternifolium* (300 mg/kg) at one dose level, their systolic hypertension, diastolic hypertension, and heart beats lowered within 13 days when compared to the dexamethasone group. When compared to the MESA group, the standard amlodipine (3 mg/kg) showed lower systolic BP, diastolic BP, and beats per minute. These findings are consistent with the fundamental principle.

3.5.2.2 Effect of Syzygium alternifolium extract on hypertension induced by fructose

Table 5 shows blood pressure data after treatment with *Syzygium alternifolium* extract at one treatment level (300 mg/kg, b.w.). Feeding of fructose elevated blood pressure values in rats. Following 42 days of using the *Syzygium alternifolium* extract blood pressure levels, Systolic blood pressure, Diastolic blood pressure, and heart rate significantly reduced. When compared to the standard, the test sample findings are significant.

3.5.2.3 Effect of Syzygium alternifolium methanolic extract on fructose-induced biochemical alterations in hypertension

The glucose levels after treatment with methanolic extract of *Syzygium alternifolium* at a concentration of (300 mg/kg, b.w.) were given in Table 6. When compared to the fructose-induced group, glucose, triglycerides, and insulin levels decreased after treatment with *Syzygium alternifolium* extract within 42 days. When compared to the fructose group, amlodipine (3 mg/kg) showed a substantial reduction in glucose, triglycerides, and insulin levels.

Table 3. *In vitro* ACE inhibitory activity of MESA

Test compound/ Standard	Dose (µg/mL)	Percent Inhibition AM±SEM (n=3)	% Relative inhibition	IC_{50} (µg/mL)
Methanolic	100	35±0.05	79.5	450
extract of *Syzygium alternifolium*	500	52±0.21	93.9	
Captopril (Standard)	50	47.51±0.01	100	30.07

Table 4. Effect of MESA on the antihypertensive parameters in dexamethasone induced hypertension in rats

Group	Dexamethasone induced hypertension, AM±SEM (n=6)			
	Blood Pressure	Systolic Blood Pressure	Diastolic Blood Pressure	Beats Per Minute
Control	110.0±0.705	114.9±1.67	92.48±1.70	229.9±3.794
Dexamethasone (0.2 ml *s.c*)	141.1±0.96*	126.0±1.64**	117.9±1.23*	321.8±1.265*
MESA 300 mg/kg, b.w.	114.0±1.45$^{***a\ ns}$	117.1±1.37$^{ns\ b}$	104.6±1.15$^{***a\ ns}$	267.4±1.20^{*aA}
Amlodipine 3 mg/kg, b.w.	111.92±0.830$^{a\ ns}$	116.88±0.86^{nsb}	95.88±0.51$^{ns\ a}$	240.1±5.46$^{a\ ns}$

*Values were expressed as mean ± SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing with control, negative control & standard. Significant values are expressed as control group (*p=0.0001, **=p<0.005, ***=p<0.01) Disease control (a=p=0.0001, b=p<0.005) & Amlodipine (A=p<0.0005), ns=non-significant*

Table 5. Effect of MESA on the antihypertensive parameters in fructose fed albino wistar rats

Group	Antihypertensive parameters AM±SEM, (n=6)			
	Blood pressure	Systolic Blood pressure	Diastolic blood pressure	Heart rate
Control	103.3±3.34	105.0±2.5	72.8±1.78	221±1.31
Fructose	157.0±2.29**	144.2±3.54**	126.0±1.43**	322.5±2.20**
MESA 300 Mg/kg, b.w.	106.4±3.28$^{a\ ns}$	115.5±1.88$^{***\ a\ ns}$	86.2±0.83^{**Aa}	233.8±3.60^{*Ab}
Amlodipine 3 mg/kg, b.w.	105 .0±1.34$^{a\ ns}$	108.1±1.42$^{a\ ns}$	75.3±2.5$^{a\ ns}$	225.3±2.00$^{a\ ns}$

Values were expressed as mean ± SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing with control, negative control & standard. Significant values are expressed as control group (=p<0.0005, **=p=0.0001, ***=p<0.01) Disease control (a=p=0.0001) & Amlodipine (A=p<0.0005, B=P=0.05), ns=non-significant*

Table 6. Effect of MESA on biochemical parameters in fructose induced hypertension

Groups	AM±SEM (n=6)		
	Glucose (mg/dL)	Triglycerides (mg/dL)	Insulin (IU/mL)
Control	86.69±0.068	135.2±0.13	2.843±0.21
Fructose	167.54±0.32*	327.7.±0.41*	4.452±0.16**
MESA 300 mg/kg, b.w.	97.83±0.26^{*aB}	173.7±0.27^{*aA}	4.32±0.13$^{a\ ns}$
Amlodipine 3 mg/kg, b.w.	88.32±0.54^{***a}	166.5±0.28^{*a}	3.235±0.20$^{a\ ns}$

Values were expressed as mean ± SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing with control, negative control & standard. Significant values are expressed as control group (=p=0.0001, **=p=0.001, ***=p<0.05) Disease control (a=p=0.0001) & Amlodipine (A=p=0.0001, B=p<0.005), ns=non-significant*

3.6 Histopathology Report

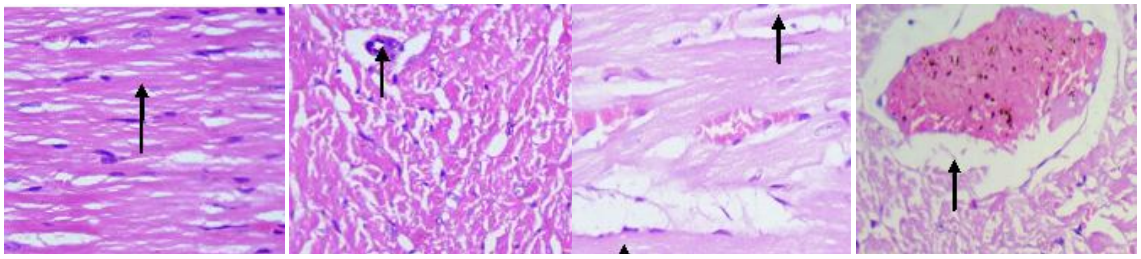
3.6.1 Myocardium histopathology assessment

- The myofibrillar structure with striations and the integrity of the myocardial cell membrane are intact in these cardiac muscle fibres. In the myocardium of control rats, the interstitial space appears to be intact.
- • The myocardium in the induced group has a somewhat random layout and necrosis, myofibrillar structure is enlarged in focal locations, and thrombosed vascular spaces are visible.
- MESA's myocardium displays an intact arrangement of cardiac muscle fibres with a few cardiac muscle fibres displaying necrosis and increased interstitial space at focal regions. Some vascular areas appear to be clogged.
- The heart muscle fibres in amlodipine's myocardium are in good shape. The

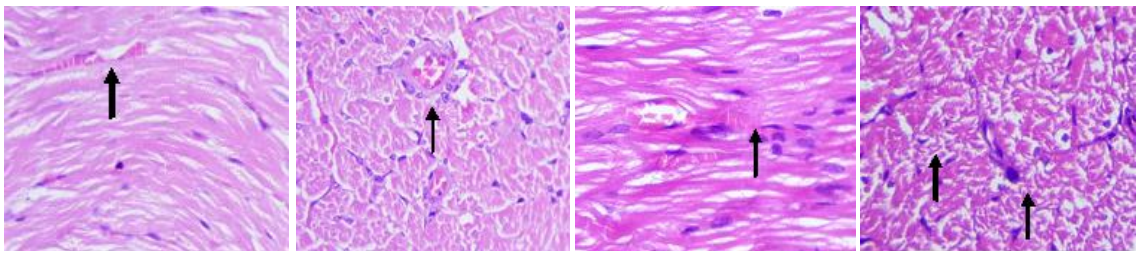
myocardial cell membrane is intact, the myofibrillar structure with striations is intact, and the interstitial gap appears to be intact in these cardiac muscle fibres shown in Fig. 1.

3.6.2 Arteries Histopathology Reports

- The arterial layers seem to be intact. The tunica intima is made up of sub-endothelial connective tissue that lines the endothelium. Myocytes cells are present in the tunica media. In the control group, the tunica adventitia is a loose meshwork of connective tissue with blood vessels.
- The layers of the artery appear somewhat disturbed in the stimulated group. The tunica intima is made up of lining endothelium, with some foamy macrophages visible in the sub-endothelial connective tissue. Smooth muscle cells are present in the tunica media. The tunica adventitia is made up of hyalinized blood vessels and regions of haemorrhage.
- MESA's arterial layers seemed to be intact. The tunica intima is made up of lining endothelium, while the vascular endothelium connective tissue contains foamy macrophage clumps. Smooth muscle cells are present in the tunica media. The tunica adventitia is made up of mononuclear inflammatory cells that are dispersed throughout the tunica.
- Amlodipine's arterial layers appear to be intact. The tunica intima is made up of lining endothelium, subendothelial connective tissue, and foamy macrophages in small numbers. Smooth muscle cells are present in the tunica media. The tunica adventitia is a loose connective tissue meshwork with blood vessels as represented in Fig. 2.

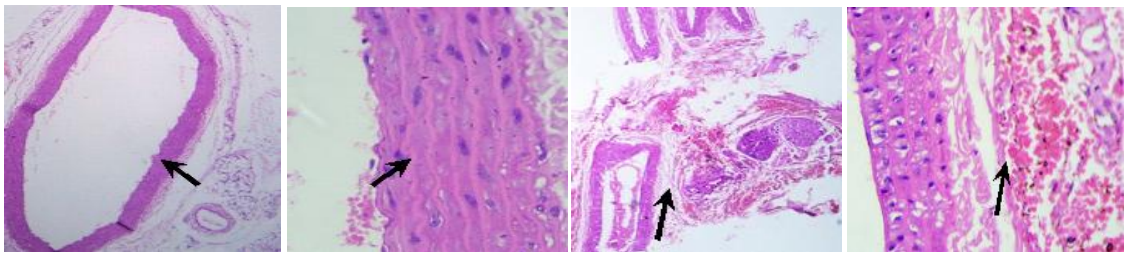


CONTROL **INDUCED GROUP**



MESA TREATED GROUP **AMLODIPINE TREATED GROUP**

Fig. 1. Histopathology of myocardium of various groups in fructose induced hypertension model



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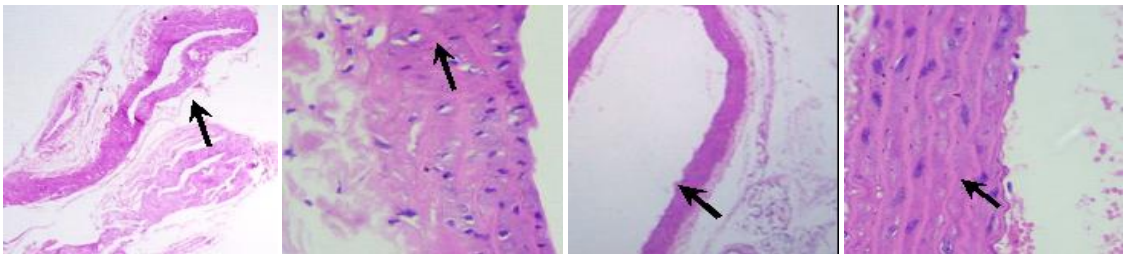


MESA TREATED **AMLODIPINE TREATED**

Fig. 2. Histopathology of arteries of various groups in fructose induced hypertension model

3.7 *In silico* Analysis

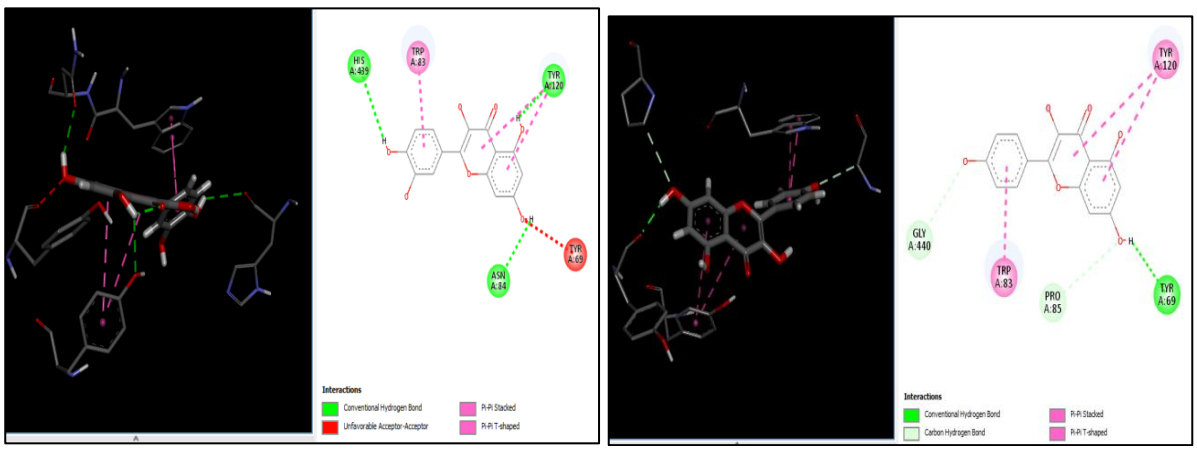
Table 7. Docking score of chemical constituents and amlodipine with protein 1EVE, 6KZP, 3OWN

Compounds	1EVE	6KZP	3OWN
Squalene	-9.4	-7.2	-7.5
Acarbose	-8.8	-7.4	-6.6
Quercetin	-10.4	-8.1	-7.2
Kaempferol	-10.2	-7.4	-7.4
Lutidine	-5.5	-4.5	-4.8
Apigenine	-10.2	-7.7	-7.4
2,5 monomethylene-1-rhamnitol	-6.3	-5.4	-5.3
4 oxo 5 phenyl pentanoic acid	-7.3	-5.5	-6.7
Caffeic acid	-7.5	-5.8	-6.5
Gentistic acid	-6.6	-5.2	-6.1
m hydroxyl bezoic acid	-6.3	-4.9	-5.3
Amlodepin	-8.1	-7.52	-5.6

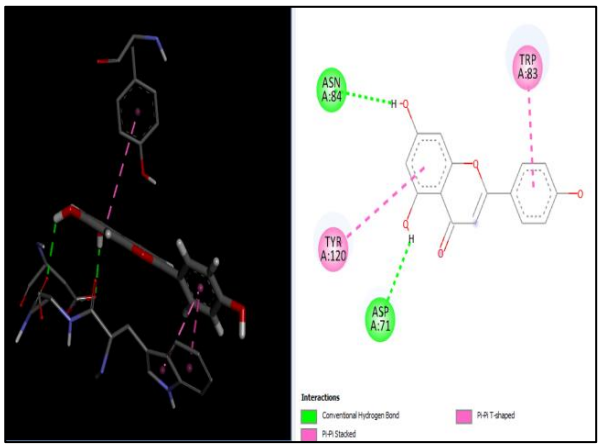
G score = glide score, Higher the negativity, the more favourable the binding.

Hydrophobic bond interactions of ligands with 1EVE, 6KZP and 3OWN protein

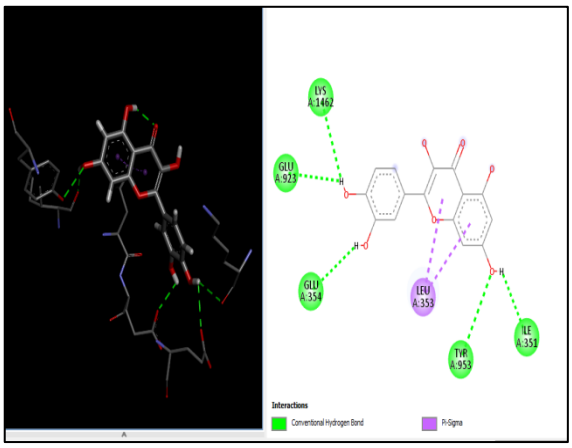
PDB ID: 1EVE



A) Quercetin -10.4 B) Kaempferol -10.2

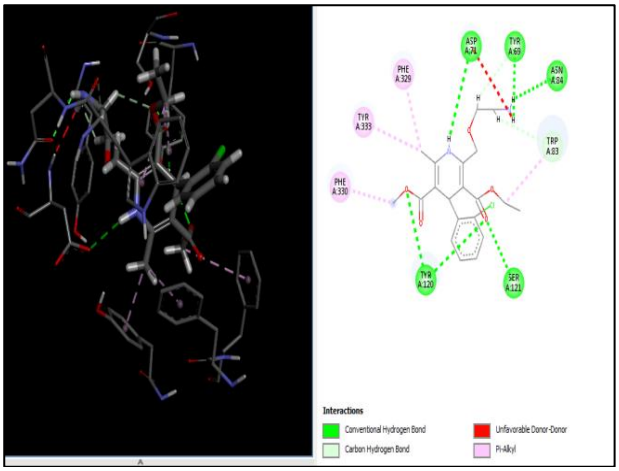


C) Apigenin -10.2

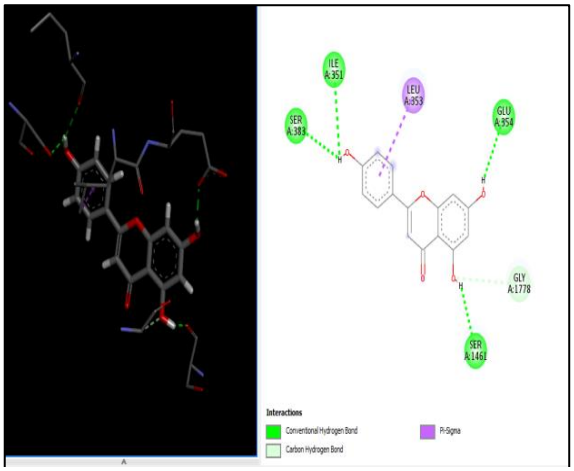


D) Amlodipine -8.1

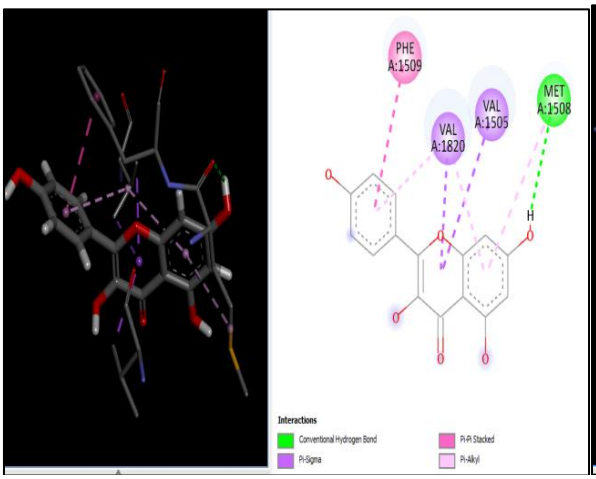
PDB ID: 6KZP



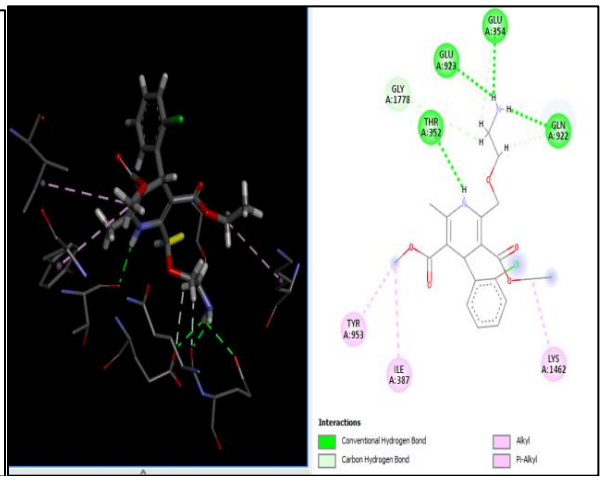
A) Quercetin -8.1



B) Apigenin -7.7

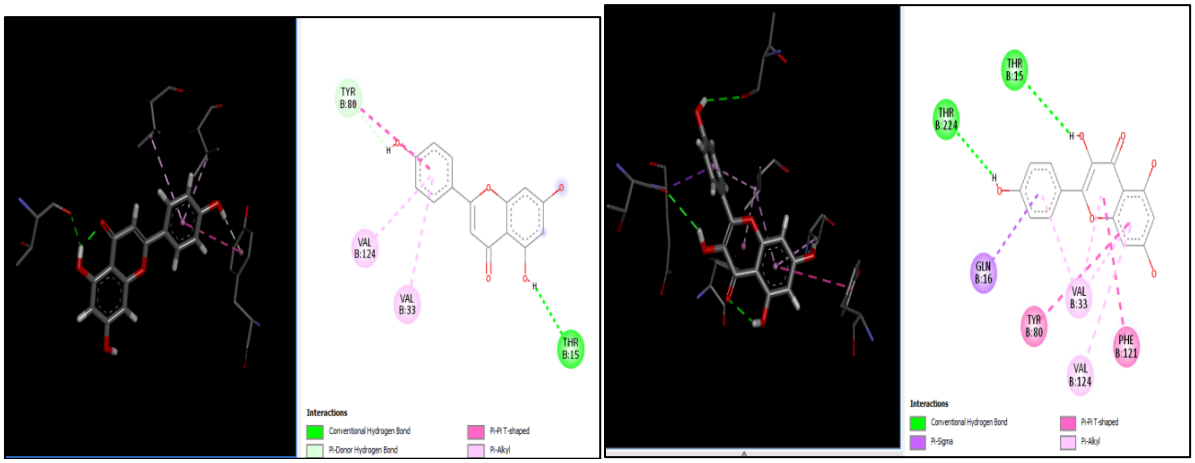


C) Kaempferol -7.4

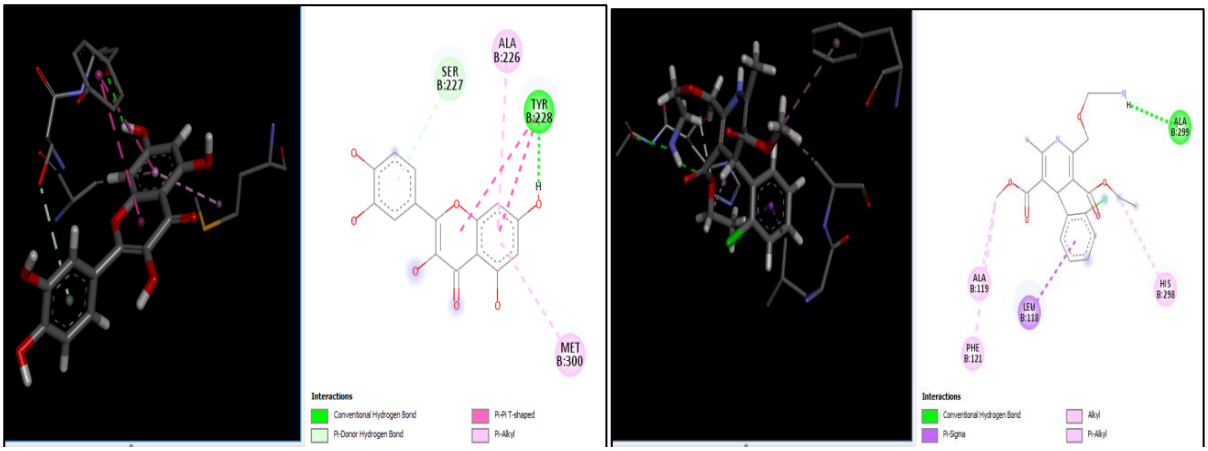


D) Amlodipine -7.52

PDB ID: 3OWN

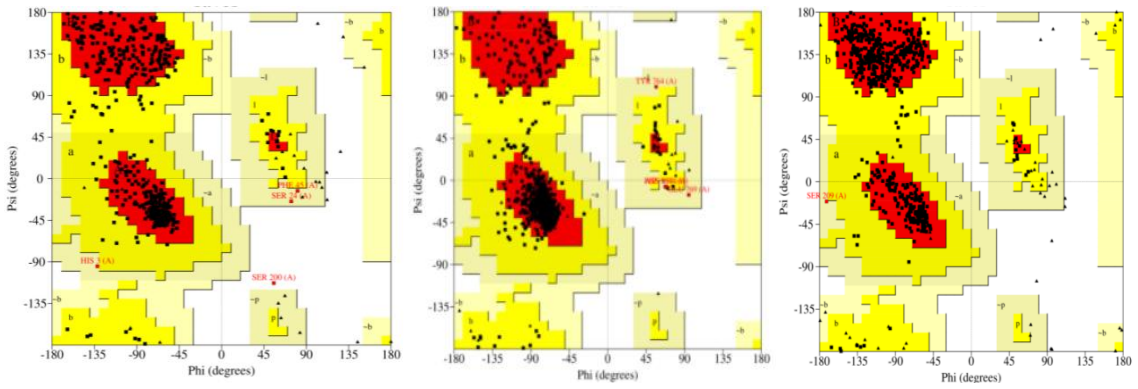


A) Apigenin -7.4 B) Kaempferol -7.4



C) Quercetin -7.2 D) Amlodipine -5.6

Fig. 3: Hydrophobic interactions of constituents and amlodipine with the 1EVE, 6KZP and 3OWN protein



a) 1EVE **b) 6KZP** **c) 3OWN**

Fig. 4. Ramachandran plot of protein 1EVE, 6KZP and 3OWN protein

Table 8. Ramachandran plot status with 1EVE, 6KZP, and 3OWN proteins

Residues	1EVE	6KZP	3OWN
Most favourable region (%)	87.3	92.1	92.1
Additional allowed regions (%)	11.8	7.4	7.7
Generously allowed regions (%)	0.7	0.4	0.2
Disallowed regions (%)	0.2	0.0	0.0

Analysis of the Ramachandran plot: 1EVE, 6KZP, and 3OWN are proteins analysed for Ramachandran plots to determine amino acid presence in various areas of each protein, as shown in Table 6 and the figure.

4. DISCUSSION

In Dexamethasone and fructose induced hypertension models the methanolic extract of *Syzygium alternifolium bark* was tested for anti-hypertensive activity *in vitro* and *in vivo.* MESA after preliminary phytochemical investigation was testing and shown to be safe in acute toxicity studies.

Chronic use of the glucocorticoid dexamethasone caused hypertension, which is accompanied by increased endothelin, renin-angiotensin, sympathetic system, and hemodynamic changes [12]. Mineralocorticoid receptors have a low affinity for dexamethasone. Dexamethasone raises blood pressure in men without having any mineralocorticoid effects, as seen with the absence of hypovolemia in the urination and an increase in physical weight [13].

Fructose consumption has risen in recent decades, and it is suspected to be contributing to the expanding epidemic of metabolic diseases. Several animal experiments have shown that eating fructose promotes sodium and chloride absorption, resulting in a salt overload that raises blood pressure [14]. Hypertension has risen in relation to the increase in fructose consumption. This is in line with earlier research that has found that increasing dietary NaCl reduces renin expression and activity [15]. Finally, following 24 hours of fructose feeding, rats' urine sodium chloride excretion was found to be less than half that of the control group. Hypertension is thought to be exacerbated by increased salt absorption by the gut and decreased salt excretion by the kidney in fructose-fed mice. In fructose-fed hypertensive rats, angiotensin II (Ang II), a vasoconstrictor, is up regulated. Ang II works by connecting to the angiotensin type I and II receptors (AT1 and AT2), while the majority of its well-known effects are accomplished through interactions with AT1 [16,17].

DPPH scavenging and superoxide radical scavenging activities were used to evaluate antioxidant activity *in vitro*. The plant contained triterpenoids, which are responsible for its antioxidant activity. Antioxidant activity is thought to be mediated by phenolic and flavonoids. The phenolic hydroxyl group is responsible for antioxidant activity in flavonoids, which are the most naturally occurring chemicals. These prevent the chain reaction from producing free radicals. Synergistic effects in DPPH scavenging activity and superoxide radical scavenging activity were discovered in herbal formulations, as opposed to separate components.

ACE inhibitors block an angiotensin-converting enzyme, which converts angiotensin I to angiotensin II, according to *in vitro* ACE inhibitory action. Reduced angiotensin II production improves natriuretic, decreases blood pressure, and inhibits smooth muscle and cardiac myocyte remodelling [18].

When given at a dose of 300 mg/kg, MESA reduced the systolic blood pressure, diastolic, elicited significance response with standard amlodipine. MESA decreased the Glucose, triglycerides and insulin levels in serum compared to disease group and showed significance response with control group. Compounds present in *Syzygium alternifolium* might be responsible for antihypertensive action majorly flavonoids than others That compounds are selected for docking are Quercetin, Kaempferol, Lutedine Apigenin, Acarbose and polyphenols and standard amlodepine are docked with ACE 1 inhibitor (PDB ID: 1EVE), Calcium channel blocker (PDB ID: 6KZP) and Renin inhibitor (PDB 3OWN) and they are given in Fig. 3. Ramachandran plot analysis is done and represented in Fig. 4. Angiotensin converting enzyme inhibitor prevents the conversion of Ang I to Ang II so antihypertension activity is exhibited. Renin Inhibitors stops conversion of angiotensinogen to ang I and antihypertensive action is achieved. Amlodipine is a peripheral

vascular dilator that acts directly upon vsmcs to reduce systemic vascular resistance, lowering blood pressure through calcium channel blocker activity. Histopathology of myocardium and arteries showed less necrosis with few vascular spasms and few macrophages compared to Disease group. Amlodipine showed antihypertensive results.

Flavonoids are a type of secondary metabolite found in plants that has a number of useful pharmacological effects. Scientists investigated the possible use of flavonoids including flavonoid-rich extracts as biological ACE inhibitors after learning about their effectiveness as biomolecules. ACE activity has been recognised as a vital factor in regulating excessive blood pressure [19]. Compounds like Quercetin, Kaempferol and apigenin known to exhibit good docking score compared to others including standard amlodipine. Ramachadran plot resulted the presence of amino acid in most favourable region is greater than 87%. In the present study the superposition of Quercetin, Kaempferol and apigenin and other compounds docking found with ACE 1 inhibitor (PDB ID: 1EVE), Calcium channel blocker (PDB ID: 6KZP) and Renin inhibitor (PDB 3OWN) protein have certified the precision of present docking study and Ramachandran plot that resulted anti-hypertensive activity.

5. CONCLUSION

The methanolic extract reduced the systolic blood pressure, diastolic blood pressure and number of beats in minute significantly in dexamethasone induced group. The BP was also reduced (systolic, diastolic and heart rate) in rats with fructose induced hypertension. Histopathological studies revealed antihypertensive activity in response to preliminary constituents identified as Quercetin, Kaempferol, Apigenin, Acarbose and Squalene apart from other compounds as they elicited good docking score and Ramachandran plot validated the selected proteins is in favourable region. Further isolated compounds mechanism of action and *in vivo* activities of these compounds are to be established.

ETHICAL APPROVAL

The ethical clearance for the research entitled “Antihypertensive and *In silico* Docking studies of phytoconstituents isolated from *Syzygium alternifolium* Bark” was approved by the Institutional Animal Ethics Committee of GRCP bearing Regd no. 1175/PO/Re/S/08/CPCSEA. All the animal experimentation was performed as per the guidelines of CPCSEA.

CONSENT

It is not applicable.

ACKNOWLEDGEMENT

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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