Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

The effect of *Rosa damascena* extract activity on angiotensin converting enzyme and renal function in Wistar rats induced high diet of sodium

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Received on: 16/12/2021, Revised on: 20/04/2022, Accepted on: 31/05/2022, Published on: 05/10/2022.

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Keywords: *Rosa damascene,* Extract, ACE, Renal, Sodium

Vol. 9 (4): 07-12, Oct-Dec, 2022.

DOI: http://doi.org/10.56511/JIPBS.2022.9402

Abstract

Background: Hypertension is known as 'the silent killer' because it can appear suddenly and cause various complications which eventually results to death. There are several herbal medicines that can use as antihypertension such as *Rosa damascene* extract. **Objective:** The purpose of this research was to determine the effect of *Rosa damascene* extract (RE) to ACE, renal function (urea, creatinine) and renal histopathology. **Methods:** The subject of research is male Wistar rat with 150-200 gram in weight, divided into five groups, as follow: P0 = control group; P1 = NaCl 8% + RE 500 mg/kgBW; P2 = NaCl 0.35% + RE 500 mg/kgBW; P3 = NaCl 8% and P4 = NaCl 0.35% for 28 days. After 28 days-treatment, rat blood was taken from the heart for analyzing the ACE activity by ELISA method, while uremic, creatinine by spectrophotometer and renal histopatology assessment using hematoxylin eosin stain method. **Results:** It was found the difference of ACE activity, urea, creatinine serum in Wistar rat which was before administration of sodium diet with p value < 0.05. Rose flower extract (Rosa damascene) has been proved to be able to decrease the activity of ACE, urea, creatinine serum in Wistar rat which was before given Sodium diet with value of p < 0.05. Rose flower extract (Rosa damascene) was able to provide a nephroprotection effect. **Conclusion:** Rose flower extract (Rosa damascene) had activity against ACE inhibition, as well as protection of renal function in rats induced by a high sodium diet.

Introduction

Hypertension is still a global problem, because its prevalence continues to increase. Hypertension is an increase in systolic blood pressure of 140 mmHg or diastolic blood pressure of 90 mmHg with two examinations in two different visits. In Western Europe, hypertension cases reach 44% while in North America it is around 28% [1]. According to Kearney et al., the hypertensive population in the world will increase by about 75% by 2025, especially in developing countries. Along with the increase in the adult population, the prevalence of hypertension will continue to increase. In general, hypertension does not have serious symptoms and is often without complaints, this is because it is still getting a steady supply of blood flow [2]. However, hypertension can appear suddenly, causing various complications and causing death. Commonly hypertension is known as the silent killer [3].

According to WHO data, the incidence of hypertension in 2000 was 972 million people or 26.5% worldwide. This figure is expected to increase until 2025 to 29.2%. Africa is the first rank with the most cases of hypertension, which is around 40% and in America, cases of hypertension are around 35%. In Southeast Asia hypertension cases reached 36%, while in Indonesia hypertension cases reached 31.7% or about 63 million people [4]. Cases of hypertension occur in the elderly, but it does not rule out cases of hypertension occurring in adolescents and young adults. Based on the research of Kini (2016), said that the prevalence of hypertension at the age of 20-30 years was around 45.2%.

Where the biggest risk factors are genetics inherited from family history, sodium consumption and obesity [5].
In the RAAS mechanism, hypertension occurs due to increased activity of ACE (angiotensin converting enzyme) through the RAAS. ACE activity can be inhibited through ACE inhibitors (ACE-I) [6]. ACE-I is a treatment for hypertension, such as captopril which is effective in lowering blood pressure in patients with essential hypertension [7]. In addition to pharmacological therapy, several extracts from plants have been proven in vitro as ACE inhibitors. Several studies have also shown that rose flower extract (*Rosa damascena*) can reduce blood pressure and pulse rate in normotensive rats [8]. In another study also showed that rose oil aromatherapy can reduce sympathetic nerve activity by 40% and adrenaline concentrations by 30% [9]. Based on this explanation, the authors are interested in examining the effect of giving rose flower extract (*Rosa damascene*) on ACE activity, renal function (creatinine urea) and kidney histopathology in Wistar rats receiving sodium diet.

Materials and Methods

Materials

Materials were used in this study are analytical balance, rat cage, syringe, Microhematocrit Ependorf tube, Micropipette, Microplate reader with filter 450 ± 10 nm, Needle sonde a. 3 months old male Wistar rat, weight 150-200 gram and ELISA kit .

Plant collection

Rosa damascene was collected at flower local market at Karo, Indonesia. The plant has been authenticated by Herbarium Medanense with approval number: 413/MEDA/2021. Flower petals used in this study.

Extraction procedure

The dried *Rosa damascene* flower (450 g) were crushed in a blender, then macerated in ethanol 96% for 3 hours thereafter moved to perlocator tube. Percolation was stopped if the last 500 mg of solvent were evaporated, leaving no residuals. The solvent was evaporated at low pressure with a temperature of not more than 40°C using a Rotary evaporator [10].

Identification of phytochemical contents

The extract was screened for the presence of alkaloids, flavonoids, glycosides, tannins, saponins, triterpenoids, and steroids using standard procedure for qualitative determination. [10, 11]

Animals and blood sample

Animals used in the study were 30 male Wistar rats (10-12 weeks old), with a sample weight of 180- 220g. Rats were housed under a standard room temperature environment with a constant relative humidity under 12-h light/dark cycles. The animals were fed with a standard laboratory of pellet diet with tap water. Acclimatization of tested animals was carried out for 1 week prior to the study. The blood sample was taken from a cardiac puncture. The study has been approved by Research Ethics Committees (AREC) University of Prima Indonesia in 2021.

Experimental design

The division of groups and treatment in this study were:
As many as 30 rats were randomly divided into five groups so that in one group there was eight rats. The group divisions are:

a. Group P0 = consisted of 6 male Wistar rats which were given drinking plain water.
b. Group P1 = 6 male Wistar rats treated with a high sodium diet (8% NaCl solution in distilled water water) and given rose extract orally at a dose of 500 mg/kg body weight.
c. Group P2 = 8 male Wistar rats treated with a high sodium diet (a solution of 0.35% NaCl in distilled water water) and given orally with rose extract at a dose of 500 mg/kg body weight.
d. Group P3 = consisted of 8 male Wistar rats treated with a high sodium diet (8% NaCl solution in distilled water water) and not given rose extract.
e. Group P4 = consisted of 8 male Wistar rats treated with a high sodium diet (0.35% NaCl solution in distilled water water) and not given rose extract.

The administration of saline solution by sonde technique to ensure that nothing is wasted or left. The administration of rose flower extract and sodium diet was carried out by force-feeding and carried out every day. After 28 days of treatment, the rats were anesthetized and blood samples were taken for measurement of ACE, urea, and creatinine and stored in a 1 ml tube containing 1.8 g K3EDTA. ACE activity was measured by ELISA method, while urea creatinine by spectrophotometer. Microscopic examination on tissue section was conducted by slicing with a microtome after the liver and kidney has been embedded in paraffin with haematoxylin and eosin. Observation was performed using a light microscope.

Statistical analysis

Statistical analysis was performed using analysis of One Way Anova. p-value for significance was set at 0.05. Values for all measurements were expressed as the mean ± SD [12].

Result and discussion

Phytochemical constituent

The content in rose flower extract (*Rosa damascene*) consists of Alkaloids, Flavonoids, Glycosides, Saponins, Tannins and Triterpenes/Steroids.

ACE inhibition activity

The result on inhibition activity of ACE (angiotensin converting enzyme) can be seen on table 1.

Table 1. ACE activity of Wistar rats after being given treatment (ng/ml).

Group	n	ACE (mean± SD)
P0	6	566.78 ± 60.52
P1	6	466.09 ± 48.19
P2	6	413.95 ± 68.01
P3	6	644.48 ± 75.92
P4	6	484.17 ± 59.40

P0= Control; P1 = Dietary administration of 8% sodium + rose extract; P2 = Administration of 0.35% sodium diet + rose extract; P3 = Administration of 8% sodium diet without extract
P4 = Dietary administration of 0.35% sodium without extract.

The results showed that ACE activity was different between the group that received rose extract (P1 and P2) and the group that did not (P3 and P4). When the control group (P0) was compared with the group that received rose extract (P1 and P2), there was also a decrease in ACE activity at P1 and P2. When P0 compared with P3 (rats that received a high-sodium diet) there was a difference. Administration of a low sodium diet at P4, showed a decrease in ACE activity when compared to the control group (P0). The administration of a high sodium diet (P3) showed an increase in ACE activity when compared to the control group (P0), the group of rat that received a low sodium diet (P4), and the group that received rose flower extract (P1 and P2). In the group of rat that received rose flower extract (P1 and P2), the different sodium diets showed that there were differences in ACE activity.

Renal function assessment

The results of the study measuring kidney function, namely with urea, can be seen in the table 2.
The results showed that the urea levels were different between the group that received rose extract (P1 and P2) and the group that did not (P3 and P4). When the control group (P0) was compared with the group that received rose extract (P1 and P2), there was also a decrease in urea levels in P1 and P2. When P0 compared with P3 (rats that received a high-sodium diet) there was a difference. Administration of a low sodium diet at P4, showed a decrease in urea levels when compared to the control group (P0). The administration of a high sodium diet (P3) showed an increase in urea levels when compared to the control group (P0), the group of rats that received a low sodium diet (P4), and the group that received rose flower extract (P1 and P2). In the group of rats that received rose flower extract (P1 and P2), the administration of different sodium diets showed that there were differences in urea levels.

Table 2. Urea and Creatinine levels in rats after being given treatment.

Groups	n	Urea (mean± SD)	Creatinine (mean± SD)
P0	6	40.09 ± 5.56	0.50 ± 0.02
P1	6	38.48 ± 5.36	0.58 ± 0.10
P2	6	29.79 ± 3.32	0.40 ± 0.42
P3	6	56.14 ± 7.61	0.71 ± 0.78
P4	6	39.75 ± 6.99	0.55 ± 0.06

P0= Control; P1 = Dietary administration of 8% sodium + rose extract; P2 = Administration of 0.35% sodium diet + rose extract; P3 = Administration of 8% sodium diet without extract
P4 = Dietary administration of 0.35% sodium without extract.

Meanwhile, the creatinine level was different between the group that received rose extract (P1 and P2) and the group that did not (P3 and P4). When the control group (P0) was compared with the group that received rose extract (P1 and P2), there was also a decrease in creatinine levels at P2. When P0 compared with P3 (rats that received a high-sodium diet) there was a difference. The administration of a high sodium diet (P3) showed an increase in creatinine levels when compared to the control group (P0), the group of rats that received a low sodium diet (P4), and the group that received rose flower extract (P1 and P2). In the group of rats that received rose flower extract (P1 and P2), the administration of different sodium diets showed that there were differences in creatinine levels.

Histological assessment

The results of histological assessment kidney organ were showed in figure 1-5. The result was in agreement with the biochemistry examinations, that the rose extract prevented degradation kidney cells. The polyphenol content in rose extract might play important role in inhibiting activity.
In the results of the observation of the P4 group, necrotic cells appeared with a score of 2 lesions 31-50% of the entire field of view. Hydropic degeneration with a score of 2 lesions 31-50% of the entire field of view. Hydropic degeneration shows the size of the cells look large and white. This occurs due to hydropic changes or the occurrence of vacuolar degeneration which is an initial reversible injury. The administration of rose flower extract with different sodium diets (high sodium diet at P1 and low sodium diet at P2) had differences.

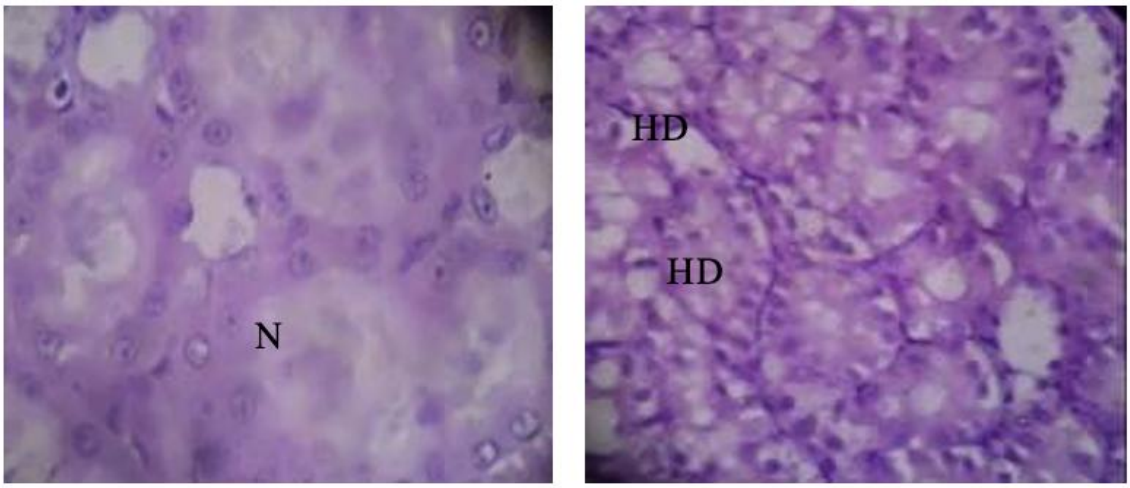

Figure 1. Kidney histopathology of P0 group (Control group).
NC = Necrotic cast, HD = Hydropic degeneration

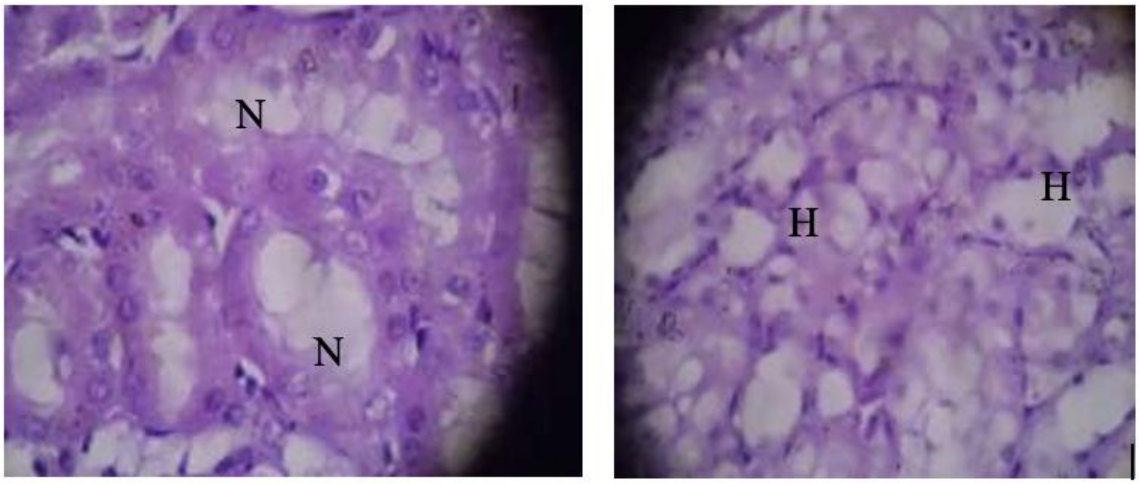

Figure 2. Kidney histopathology of P1 group rats.
NC = Necrotic cell, HD = Hydropic degeneration

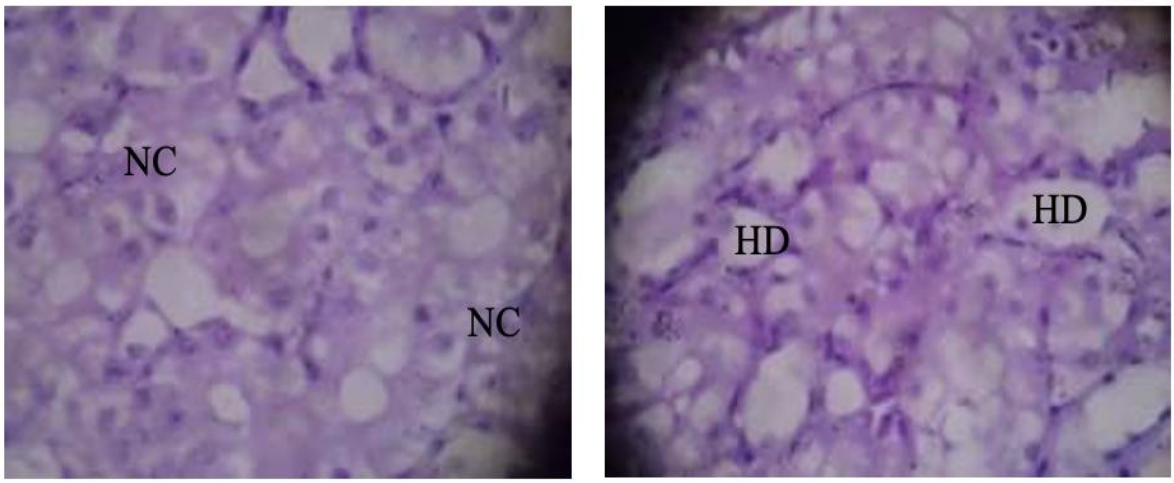

Figure 3. Kidney histopathology of P2 group rats.
NC = Necrotic cell, HD = Hydropic degeneration

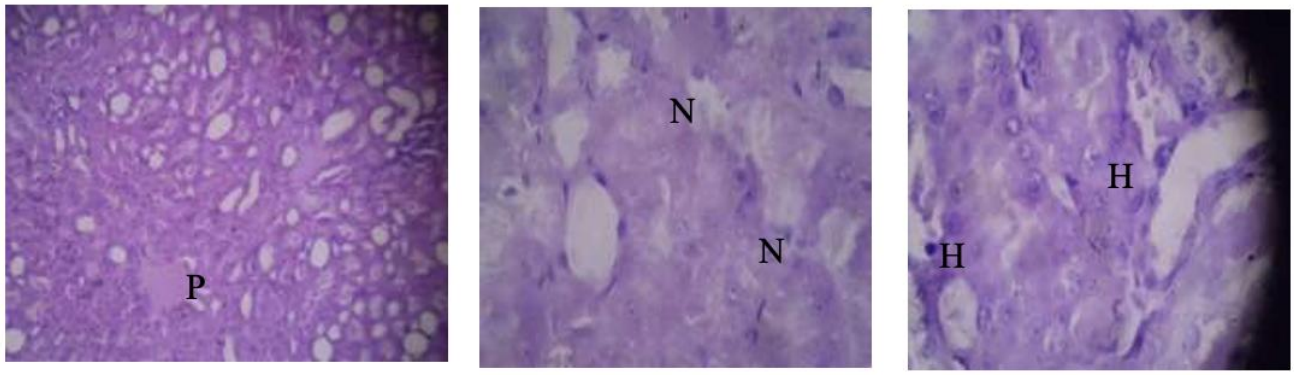

Figure 4. Kidney histopathology of P3 group rats.
PC = Protein cast, NC = Necrotic cell, HD = Hydropic degeneration

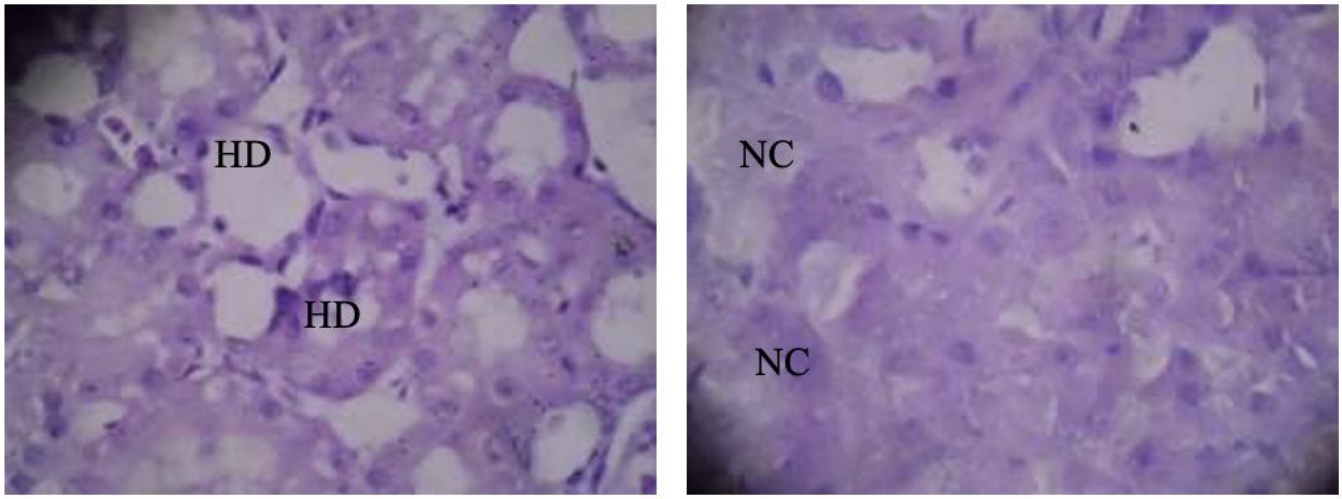

Figure 5. Histopathology of the kidney of P4 group rats.
NC = Necrotic cell, HD = Hydropic degeneration

This is in line with the research of Filippini, *et al.* (2021) reported that decreasing sodium intake was proven to be able to reduce blood pressure levels, where sodium intake of 100 mmol/day was able to reduce the average systolic and diastolic pressures, namely 4.47 mmHg and 1.90 mmHg in diastolic [13]. In the study of Kwakernaaka, *et al* (2013) found that sodium has a significant relationship with ACE. Research conducted by Kwakernaaka, *et al* (2013) found systolic blood pressure (mmHg) in the high sodium diet group was 133 ± 20 while in the low sodium diet group it was 122 ± 17. In the diastolic blood pressure (DBP) (mmHg) group, it was found that in the low sodium diet group. The high sodium diet was 80 ± 13 while in the low sodium diet group it was 73 ± 11. In the mean aerial pressure (mmHg) it was found that the high sodium diet group was 97 ± 15 while in the low sodium diet group it was 89 ± 12 [14].

Result in this study, the groups of P1 and P2 compared to P0 showed that rose flower extract still worked to inhibit ACE, both in high-sodium and low-sodium conditions In addition to reducing sodium intake, antihypertensive administration is also able to reduce blood pressure levels. One of the main therapies for antihypertensives is the ACE-inhibitor class, where ACE-I is able to inhibit the conversion of angiotensin I (Ang I) to angiotensin II (Ang II). Where angiotensin II is a strong vasoconstrictor that can increase blood pressure. Angiotensin II also stimulates the proximal tubule of the nephron to reabsorb sodium and water, thereby increasing sodium reabsorption in the kidneys which ultimately increases blood pressure volume [15].

ACE inhibitor therapy showed an extraordinary effect over other antihypertensive drugs, its cause that ACE inhibitors could minimize the CNS side effects. However, ACE inhibitors have some side effects such as cough, rash on the body. According to Akram *et al* (2020), the use of natural ACE is believed to be safer and more economical to reduce blood pressure levels. One of the plants that can reduce ACE levels is rose (*Rosa damascena*) [16].

The amount of ACE activation that was inhibited by the ethanolic extract of rose flower could be seen from the difference in the mean ACE at P3 and P1, and the difference in the mean at ACE at P4 and P2. On high sodium diet, rose flower extract reduced ACE to 178.39 ng/mL. Thus, giving rose flower extract to rats that received a high-sodium diet,

making ACE activity greater than that of rats that received a low-sodium diet. Giving rose flower extract as an ACE inhibitor is better when combined with a low sodium diet. This proves that a low sodium diet can be used as an additional non-pharmacological therapy for patients with hypertension. Research by Crestani (2014) stated that the high NaCl 4% diet group showed increased expression of angiotensin II type 1 receptors and decreased expression of angiotensin II type 2 receptors in the aorta. Giving a low sodium diet can also increase the elasticity of blood vessels, so that it can reduce increased blood pressure [17].

The increase in ACE activity was found in P3 (rats that received a high sodium diet), both when compared to the control group (P0), the group of rats receiving rose flower extract (P1 and P2), or the group on a low sodium diet (P4). A decrease in sodium levels in the blood triggers the RAAS mechanism. Decreased sodium levels will continue to make ACE activated until blood pressure rises to normal levels [18].

Conclusion

Based on the results and discussion of the study, it was concluded that rose flower extract (*Rosa damascene*) had activity against ACE inhibition, as well as protection of renal function in rats induced by a high sodium diet.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this review article.

Authors Contribution

All the authors have contributed equally in designing, drafting the manuscript as per the journal submission format. All authors read and approved the final manuscript.

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