



RESEARCH ARTICLE

Effect of Herbal Mixture as Angiotensin Converting Enzyme Inhibitor in Angiotensin-II Dependent Hypertension

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ABSTRACT

The effect of herbal mixture of *Curcuma longa* (CL) and *Rauvolfia serpentina* (RS) as green ACE inhibitors in Angiotensin-II dependent hypertension was evaluated by measuring the changes in ACE level in blood serum of hypertensive dogs. 2K1C Renovascular Hypertension Model (RVHM) was used to introduce hypertension in dogs. Substantial Hypertension was induced after seven days of renal artery ligation (RAL) with significant increase in the level of ACE in blood serum of spontaneously hypertensive dogs (SHD). Experimental dogs were divided into four groups among them RVH hypertension was induced in the dogs of three groups and fourth group was the normotensive dogs. Hypertensive dogs of one group left untreated while the other two groups treated with herbal mixture and standard drug (Captopril) respectively. The herbal mixture showed significant decrease in Systolic blood pressure (SBP) pressure, diastolic blood pressure (DBP), Pulse rate (PR), and serum ACE level of SHD as compared to Captopril. SBP, DBP and PR were also correlated with hematological parameters. Daily oral administration of herbal mixture resulted in a time-dependent blood pressure and ACE level reduction in SHD, with management of hypertension after nine days treatment. White blood cells (WBC), Lymphocyte (LYM), Mid-sized cells (MID), Granulocyte (GRA), platelet count (PLT), mean corpuscular volume (MPV), platelet distribution width (PDW) and platelet crit (PCT) showed highly significant ($P < 0.01$) positive correlation with SBP, DBP and PR while (red blood cells) RBC showed significant ($P < 0.05$) positive correlation only with SBP and PR. This study demonstrated that the combination of CL and RS effectively reduced blood pressure and heart rate of SHD and this antihypertensive effect is probably due to the reduction of ACE level in blood serum.

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INTRODUCTION

Hypertension is considered a well-known progressive disorder that causes a key risk for the development of heart and kidney diseases (Ettehad *et al.*, 2016). Hypertension is responsible to cause 7.5million annual deaths and indeed the main cause of morbidity due to associated with serious perils diseases like stroke, heart and kidney disorders (WHO, 2016). The prevalence of hypertension in Pakistan is very high (Shafi and Tahir, 2017) and also considered as top alarming physiological disorder in Pakistani community. Not to speak of country like ours, BP has become a health menace all over the world. Owing to the high prevalence and increased mortality and morbidity due to hypertension and

associated complications, there is a dire need to address this main health challenge.

Apparently, the synthetic medicines seem to be sufficient potent to combat with this dreaded disorder and also cope with the rising prevalence of hypertension and associated diseases, but their side effects are taking their toll from their efficiency. This research has been planned with the objectives to develop safe, efficacious and viable herbal combination as green ACE (Angiotensin Converting Enzyme) inhibitor to manage hypertension as an alternative of complex synthetic drugs.

Among many others, inhibition of ACE is considered to be an effective and useful therapeutic approach in the management of hypertension. Therefore, in the development of drugs to control high blood pressure, ACE

inhibition has become an important field of research for pharmaceutical scientists. Many synthetic ACE inhibitors are currently in practice for the management of hypertension and cardiovascular disorders (Wijesekara and Kim, 2010). However, these synthetic ACE inhibitors have some considerable side effects like cough, taste disturbances, skin rashes and angioedema (Iwaniak *et al.*, 2014). Owing to adverse side effects of synthetic ACE inhibitors, scientists have turned towards natural sources and Pakistan is bestowed with wealth of medicinal plants widely used by the complementary and alternative medicine practitioners (CAM) for many ailments.

The potential of plant bioactives as green ACE inhibitors is still unexplored and very limited. Traditionally used medicinal plants have been validated scientifically through animal models. Most of the scientific reports about the hypertensive potential of medicinal plants lack systematic studies on their mode of action, efficacy and safety (Yang *et al.*, 2014).

Plants have variable phytonutrients with different pharmaceutical activities. When different plants are combined together, the herbal mixtures show altered ability to cure a disease than individual plants (Eric, 2015). It may be due to synergistic, additive or antagonistic interaction between phytochemicals of different plants. The exploration of synergistic mechanisms in different plants not only helps to discover new potent herbal combinations, but also helps to avoid the possible antagonism or side-effects of alternative herbal products (Yang *et al.*, 2014). Therefore, the present research work was planned to find the antihypertensive potential of herbal mixture of *CL* and *RS*.

Like synthetic drugs, traditional plant-based medicines also interact and affect the functionality of biomolecules (Hormones, enzymes etc.) and invading pathogens (Eric, 2015; Abbas *et al.*, 2017a, 2017b, 2017c, 2018; Hussain *et al.*, 2017; Idris *et al.*, 2017; Mahmood *et al.*, 2018). An understanding of the mechanism of action of alternative medicines is mostly unexplored and needs to be made certain. Regarding present studies in depth scientific validation is required to authenticate the traditional medicine as alternative, green ACE inhibitors for the management of hypertension.

Curcuma longa (*CL*) and *Rauvolfia serpentina* (*RS*) have been reported for their individual antihypertensive potential (Ranjini *et al.*, 2015; Soni *et al.*, 2016). *Rauvolfia serpentina* (*RS*) is effectively used by CAM practitioners for management of hypertension (Lobay, 2015). In this study *CL* was mixed with *RS* to evaluate the synergistic effect on angiotensin-II dependent hypertension induced through 2 kidney 1 clip (2K1C) renovascular hypertension model (RVHM), in dogs. This will not only confirm the synergism but also untap the mode of action of the studied herbal combination.

MATERIALS AND METHODS

Plant material: *Curcuma longa* (*CL*) rhizomes and *Rauvolfia serpentina* (*RS*) roots were purchased from the market and verified by a taxonomist from the Department of Botany, University of Agriculture, Faisalabad through voucher No. 227-2-2016 and 227-6-2016.

Antihypertensive potential of combination of *CL* and *RS*: Stray dogs were selected with an average weight of 14-

16 kg. Animals were housed in separate cages at room temperature under standard laboratory conditions (12 hours light and 12 hours dark cycle), with free access to water and food. The whole trial was conducted under the supervision of veterinary doctors in the laboratory of the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, and all the ethical guidelines were followed during the whole study period.

The dogs under study were divided into four groups with five dogs in each. These groups were categorized as under:

Absolute control: All the five dogs of this group received water and food during the analysis period.

Positive control: Hypertension was induced in normotensive dogs by using 2K1C RVHM (Bhadran and Ahmed, 2014). The dogs were fasted overnight, administered atropine and after 10 minutes anesthesia, Pentothal Na 20mg/kg, was injected. The left kidney was visualized by lateral abdominal incision and renal artery was ligated. The muscle and skin layer (incision site) were sutured with highly sterile suture needles. Postoperative medication, injection of Meloxin, Oxidil (i.v.) and ASD, was given to prevent any bacterial infection for three days. Pyodine was used for daily dressing of surgical wounds. Blood pressure was monitored at 24 hours interval by tightening an inflatable cuff around the dog's paw for measuring the systolic, diastolic blood pressure and pulse rate by using a digital sphygmomanometer. After surgery, the dogs of the positive control group received only food and water during the analysis period.

Plant combination treatment: The herbal mixture was prepared by mixing equal quantity (1:1) of roots of *Rauvolfia serpentina* and rhizomes of *Curcuma longa* powder and the mixture (5mg/kg bw) was given orally to the dogs. High blood pressure was induced in spontaneously hypertensive dogs (SHD) of this group to a considerable level after 7 days of renal artery ligation. From the 8th day, the treatment was started with the herbal mixture up to the 16th day.

Standard treatment: Captopril (5mg/kg) was given to SHD of this group as standard treatment for 9 days after induction of hypertension.

Measurement of BP: BP was measured daily after 1 hour of oral administration of medicine. All measurements were repeated 5 times and averaged.

Measuring hematological parameters and ACE activity: Blood samples (5mL) were collected from dogs of all studied groups before and after surgery at every 48 hours interval until the treatment was given to dogs. Hematological parameters were studied from fresh blood samples and separated serum to analyze ACE activity. ACE activity was measured with the method described by Belovic *et al.* (2013), blood serum (50µL of 100mU/ml) was incubated with borate buffer (50µL) at 37°C for 10 minutes. A substrate solution (150µL), prepared by mixing 8.3mM, Hip-His-Leu in borate buffer, was added into the reaction mixture and incubated for 80 minutes at 37°C. The reaction was stopped by the addition

of HCl (250 μ L of 1M). The resulting hippuric acid was extracted with ethyl acetate (1500 μ L) and centrifuged at 4000 rpm for 15 minutes. After centrifugation, 750 μ L solution from upper layer was pipette out and placed in a test tube. The solution was dried under air flow at 7°C. Dried material was mixed with 1ml of distilled water in a test tube and used to measure absorption at 228nm by using UV/Visible spectrophotometer. The reaction blank was prepared by the addition of HCl before the addition of substrate with the same procedure as discussed above. ACE activity was calculated with following formula.

$$\% \text{ ACEI} = \frac{100[(A-B)-(C-D)]}{(A-B)}$$

Where, A represent absorbance in the presence of ACE, B absorbance of reaction blank, C absorbance in the presence of ACE and inhibitors, D absorbance of reaction blank.

Statistical analysis: The results were presented as mean \pm SE of five parallel determinations and means were analyzed by using one way and two way analysis of variance (ANOVA) followed by Tukey's test. Correlation was calculated by using pearson's correlation method (correlating single value of cellular parameters with BP determinants) with the formula given below:

$$r = \frac{N\sum xy - (\sum x)(\sum y)}{\sqrt{[N\sum x^2 - (\sum x)^2][N\sum y^2 - (\sum y)^2]}}$$

Where:

- N = number of pairs of scores
- $\sum xy$ = sum of the products of paired scores
- $\sum x$ = sum of x scores
- $\sum y$ = sum of y scores
- $\sum x^2$ = sum of squared x scores
- $\sum y^2$ = sum of squared y scores

RESULTS

The mixture of CL and RS(CL+RS) was evaluated as green ACE inhibitor to manage the angiotensin-II dependent hypertension in dogs, induced through Goldblatt 2 kidney 1 clip Reno vascular hypertension model (2K1C RVHM). Twenty dogs were divided into four groups, including absolute control, positive control, treatment (plant combination) and standard (Captopril) groups with five dogs in each group. The results of Systolic blood pressure (SBP), Diastolic blood pressure (DBP) and pulse rate (PR) are presented in (Fig. 1, Fig. 2 and Table 1). Absolute

control group showed normal values of SBP (125.00 \pm 0.74 mmHg), DBP (89.03 \pm 0.99 mmHg) and PR (103.37 \pm 0.99 beats per min) during the experiment.

In the positive control group the increase in SBP was observed from fourth day after renal artery ligation (RAL) and considerably increased upto 7th day in the dogs of this group. The dogs of positive control group were kept untreated after kidney ligation. SBP, DBP and PR increased continuously after RAL and remain high throughout the experiment.

The increased levels of SBP, DBP and PR in the experimental dogs of the group treated with plant combination (RS+CL) were subsequently decreased, at the dose of 5mg kg⁻¹day⁻¹ BW from the 8th day of RAL. After nine days of treatment, the hypertensive dogs were completely managed, which shows the significance of herbal combination possessing antihypertensive potential that was comparable to the standard drug.

ACE activity in blood serum of different experimental groups: Serum of all experimental animals was studied for measuring the level of ACE in blood. The values of serum ACE level have been presented in Table 2. The average serum ACE activity was 0.665 \pm 0.026 U/mL in the absolute control group. The serum ACE activity increased in the dogs which were operated through RAL. The serum ACE level increased continuously from 0.664 to 2.288 \pm 0.010 U/mL in the hypertensive dogs of positive control group throughout the experimental period.

Correlation of hematological parameters with SBP, DBP and PR: The correlation of various hematological parameters with SBP, DBP and PR has been presented in Table 3. WBC, LYM, MID, GRA, PLT, MPV, PDW and PCT showed highly significant (P<0.01) positive correlation with SBP, DBP and PR while RBC showed significant (P<0.05) positive correlation only with SBP and PR. MCV and RDW-SD depicted non-significant positive correlation with SBP, DBP and PR. MCHC exhibited non-significant positive correlation with SBP and PR while negative correlation with DBP. The MCH was negatively correlated with SBP, DBP and positively correlated with PR, but these correlations were statistically non-significant. The RDW-CV showed non-significant positive correlation with SBP and DBP, but showed significant negative correlation with PR. The HCT showed non-significant positive correlation with SBP, DBP and negative correlation with PR. The P-LCR depicted highly significant positive correlation with SBP and DBP, but non-significant negative correlation with PR. The hemoglobin (HGB) was negatively correlated with SBP, DBP and PR, but this correlation was statistically non-significant.

Table 1: Changes in PR (heart beat/min) in different experimental groups

Days	Treatments			
	Absolute control	Positive control	Plant combination	Captopril
1st	105.80 \pm 3.06d-h	101.20 \pm 1.50e-k	97.60 \pm 1.94h-l	89.80 \pm 1.28lmn
4th	102.80 \pm 1.24e-k	94.40 \pm 2.29klm	97.80 \pm 1.98h-l	98.00 \pm 1.76h-l
7th	99.40 \pm 2.20g-l	109.80 \pm 1.43cde	104.20 \pm 1.77d-j	80.00 \pm 2.51op
10th	100.00 \pm 1.14f-k	112.80 \pm 1.39bcd	104.80 \pm 1.59d-i	85.00 \pm 1.58mno
13th	109.60 \pm 2.36c-f	130.60 \pm 1.50a	100.60 \pm 1.36e-k	82.00 \pm 2.35nop
16th	102.60 \pm 1.44e-k	131.20 \pm 1.50a	95.60 \pm 2.38i-l	75.20 \pm 1.28p

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means.

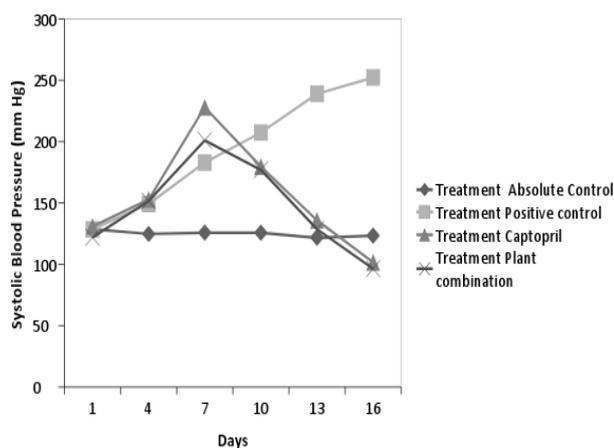


Fig. 1: Changes in SBP in different experimental groups.

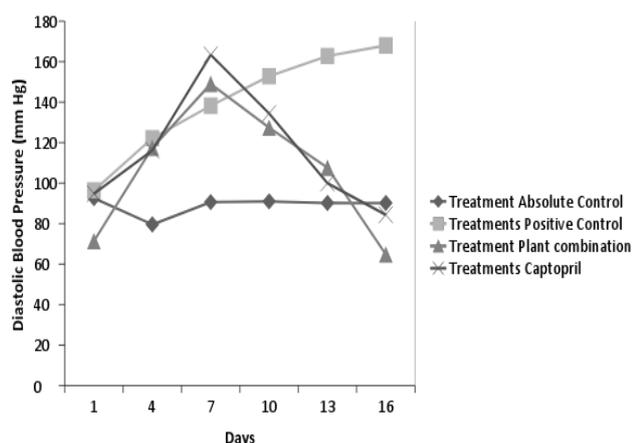


Fig. 2: Changes in DBP in different experimental groups.

Table 2: Changes in ACE activity (U/mL) in blood serum of different experimental groups

Days	Treatment Groups			
	Normal control	Positive control	Captopril	Plants combination
1 st	0.666±0.010 ^{ij}	0.664±0.016 ^{ij}	0.654±0.017 ⁱ	0.735±0.020 ⁱ
4 th	0.667±0.008 ^{ij}	0.986±0.009 ^g	0.986±0.016 ^g	1.106±0.026 ^g
7 th	0.660±0.015 ^{ij}	1.475±0.016 ^d	1.459±0.011 ^d	1.682±0.010 ^c
10 th	0.665±0.012 ^{ij}	1.706±0.015 ^c	1.283±0.013 ^e	1.114±0.019 ^f
13 th	0.663±0.012 ^{ij}	2.071±0.014 ^b	0.846±0.011 ^h	0.873±0.017 ^h
16 th	0.671±0.010 ^{ij}	2.288±0.010 ^a	0.819±0.009 ^h	0.712±0.017 ^{ij}

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

DISCUSSION

Both plants *Curcuma longa* (CL) and *Rauwolfia serpentina* (RS), were previously reported as antihypertensive herbs (Soni *et al.*, 2016) and possess ACE inhibition potential (Lekshmi *et al.*, 2014; Ranjini *et al.*, 2015). Therefore, in the present study the mixture of CL and RS (CL+RS) was evaluated as green ACE inhibitor to manage the angiotensin-II dependent hypertension in dogs, induced through Goldblatt 2 kidney 1 clip Reno vascular hypertension model (2K1C RVHM). SBP, DBP and PR were increased in dogs after kidney ligation and completely managed after treatment with herbal combination (CL+RS) which possessing antihypertensive potential that was equivalent to the standard drug (Captopril). RVHM model is frequently

used for assessment of the antihypertensive effects of different extracts of plants and pharmaceutical preparations (Bhadran and Ahmed, 2014). In this model renal artery ligation caused continuous increase in blood pressure due to increased plasma renin activity, which in turn increases circulating angiotensin-II. The increased angiotensin-II releases aldosterone from adrenal cortex leading to a gradual retention of salt and water. Retention of salt and water leads to decreased renin production in kidneys that induces hypertension. The hypertension induced in this way is dependent on blood volume. Hence, salt and water balance is critically involved in the pathogenesis of Reno vascular hypertension (RVH) (Zhou *et al.*, 2014).

The herbal mixture of CL and RS showed effective reduction in SBP, DBP and PR in hypertensive dogs as compared to standard drug and this decrease might be due to interaction of bioactive compounds in mixture of plants. The use of combination therapy in hypertensive dogs may simultaneously address multiple pharmacological targets and provide better clinical efficacy that is beyond the reach of single compound based synthetic drugs. Some of the researchers have reported antihypertensive potential of the studied plants (Soni *et al.*, 2016) but yet no or very less literature about in vivo trials, surgically induced hypertension or synergism in herbal combination is available. Therefore, it is very novel to mention that combination of CL and RS can manage BP as good as the synthetic drug (Captopril).

The hypertension induced through RVHM also leads to an elevated ACE level in blood serum of hypertensive dogs. The treatment with plant combination (CL+RS) depicted significant recovery in serum ACE level. This recovery might be due to the presence of phytochemicals like flavonoid, alkaloids and tannins etc. These phytochemicals may possess ACE inhibition effect (Balasuriya and Rupasinghe, 2011; Sharifi *et al.*, 2013; Saputri *et al.*, 2015) which can effectively reduce the increased BP. RVH particularly leads to increase in the level of angiotensin II in blood serum and flavonoids, tannins and alkaloids (Harisaranraj *et al.*, 2009) present in medicinal plants may restrict the formation of angiotensin II and reduce the level of active ACE to manage the BP through their synergic effect if these medicinal plants are used in combinations. The results of present study were also found in accordance with the previous study conducted by Sharifi *et al.* (2004) who have examined the alteration of angiotensin converting enzyme (ACE) activity in the aortae, heart, kidney and lungs as well as plasma during the development of hypertension in one-kidney, one-clip (1K1C) RVHM in rats. They also reported that ACE activity in aortae and heart was gradually increased with the development of hypertension. In another study, Akinyemi *et al.* (2015) tried to correlate the ACE inhibition with phenolic contents present in rhizome extract of CL. The BP management through ACE inhibition potential is either owing to any one potent phenolic or due to synergism of multiple phytochemicals (Kang *et al.*, 2015; Pang *et al.*, 2015). The same mechanism of action of herbal mixture (CL+ RS) i.e. through managing the level of ACE in blood seems to be involved in the present study.

Table 3: Correlation of hematological parameters with SBP, DBP and PR

	SBP	DBP	PR
1 WBC (Cells×10 ³ /uL)	0.676** 0.000	0.606** 0.000	0.470** 0.000
2 LYM (cells ×10 ³ /uL)	0.666** 0.000	0.600** 0.000	0.444** 0.000
3 MID (Cells×10 ³ /uL)	0.674** 0.000	0.593** 0.000	0.526** 0.000
4 GRA (Cells×10 ³ /uL)	0.690** 0.000	0.612** 0.000	0.499** 0.000
5 RBC (Cells×10 ¹² /L)	0.173* 0.034	0.139 0.089	0.164* 0.045
6 HGB (g/dL)	-0.093 0.259	-0.075 0.362	-0.157 0.055
7 MCHC (g/dL)	0.005 0.950	-0.006 0.941	0.007 0.936
8 MCH (pg)	-0.103 0.211	-0.119 0.146	0.010 0.908
9 MCV (fL)	0.075 0.362	0.065 0.427	0.107 0.191
10 RDW-CV %	0.022 0.790	0.043 0.604	-0.172* 0.036
11 RDW-SD (fL)	0.025 0.761	0.017 0.838	0.046 0.574
12 HCT %	0.051 0.539	0.076 0.354	-0.087 0.289
13 PLT Cells×10 ³ /uL	0.696** 0.000	0.658** 0.000	0.326** 0.000
14 MPV (fL)	0.507** 0.000	0.467** 0.000	0.322** 0.000
15 PDW %	0.518** 0.000	0.442** 0.000	0.461** 0.000
16 PCT %	0.454** 0.000	0.428** 0.000	0.210** 0.010
27 P-LCR %	0.502** 0.000	0.504** 0.000	-0.110 0.181

Upper values indicated Pearson's correlation coefficient; Lower values indicated level of significance at 5% probability. *=Significant (P<0.05); **=highly significant (P<0.01).

Correlation is a measure of the extent to which two variables are related. The correlation of various hematological parameters with SBP, DBP and PR has been presented in Table 3. In this study WBC showed significant positive correlation with SBP, DBP and PR. The increase in WBC count may enhance the level of catecholamine and activation of sympathetic nervous system, which can cause an increase in blood pressure and ultimately results in persistent hypertension (Babu *et al.*, 2015). This increased level of WBC can be reduced by using medicinal plants rich in antioxidants including flavonoids, tannins, and polyphenols.

The mean levels of platelet count were also found to be positively correlated with SBP, DBP and PR in hypertensive subjects. These results are in accordance with the findings of Al-Muhana *et al.* (2006) and Babu *et al.* (2015) because they also observed increased level of platelet count in hypertensive animal groups. The increase in platelet consumption at the site of the coronary atherosclerotic plaque causes larger platelets to be released from the bone marrow. Treatment of hypertensive dogs with herbal mixture (RS+CL) may lead to maintain platelets count that was disturbed due to induced hypertension.

In contrast to WBC, HGB showed negative correlation with SBP, DBP and PR in studied hypertensive subjects that might be due to the activation of sympathetic nervous system that leads to stimulate

heart function, enhance cardiac output, trigger vasoconstriction, and elevated renin level (Ashishkumar *et al.*, 2015). The increase in renin level helps to recollect sodium and water from the renal tubule, which leads to increase in body fluid volume (Ashishkumar *et al.*, 2015).

The correlation study revealed that altered Hb level, WBC and platelet count might contribute to the complications of hypertension including heart failure. Divya and Ashok, (2016) also observed lower level of HGB in hypertensive group as compared to normotensive group of male patients. The studied combination (RS+CL) not only normalized the blood pressure but also successfully restored the level of HGB in dogs. Thus it seems logical that normalization of BP and reduction in the complications of hypertension may be strongly associated with the hematological parameters. Both the studied plants have been proved as a good hypotensive plants which can minimize the complication of hypertension being a rich combination of antioxidant phytochemicals. Therefore, these medicinal plants in combination may also be used to keep the levels of hematological parameters normalized and prevent further complications.

Conclusions: The combination of CL and RS being rich in phytochemicals antioxidants is a natural ACE inhibitor that can manage BP and normalize the hematological parameters.

Authors contribution: NJ managed the laboratory facilities and funds to conduct the research and approved the final manuscript. F performed research work, wrote the the manuscript. KR provided the research facilities regarding clinical analysis. SA provided guidance and laboratory facilities regarding spectrophotometric analysis. All authors read and approved the final manuscript.

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