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Nephroprotective Effect of Herbal Extracts of *Bryophyllum calycium* and *Solanum xanthocarpum* on Induced Urolithiaisis in Wistar Rats: Haemato-Biochemical Evaluation

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Abstract

The experiment was conducted on 90 adult healthy Wistar rats. Rats were randomly divided in to 15 equal groups, each of 6 rats, and were kept in separate cages. Group I served as normal healthy control without any treatment, while Group II & III served as vehicle (bicarbonate) control and lithiatic control, respectively. In rats of Group III to IX urolithiasis was induced using 0.75 % (v/v) ethylene glycol and 2% (w/v) ammonium chloride in drinking water for 28 days. The rats of Group I, II, and X to XV were given pure wholesome water till 28 days. After 28th day, the rats of urolithiatic treatment Groups IV, V, VI, VII, VIII and IX were given aqueous and alcoholic extracts of *Bryophyllum calycium* and *Solanum xanthocarpum* @ 300 mg/kg bwt orally as either single extract or combination as biherbal extracts in 0.5 % sodium bicarbonate using syringe and rat lavage needle, and so also was done for rats of Group X to XV as extract control groups. Blood samples were collected twice: i.e. on day 28 of induction of urolithiasis and then on day 56 of experiment from all rats. No significant difference was observed in any of the haematological parameters and even in serum albumin and globulin levels before and after treatment in different groups. However, increased levels of serum BUN, uric acid and creatinine were observed in the urolithiatic groups as compared to the normal control group on 28th day. While serum total protein levels were decreased in the calculi induced groups as compared to the normal control group. However, co-treatment of aqueous or alcoholic extract of *Bryophyllum calycinum and Solanum xanthocarpum* significantly restored these changes by 56th day. The effect of biherbal alcoholic extract of the plants was much better in restoring the values and the levels came nearer to normal by 56th day of oral treatment proving nephroprotective effect of these extracts.

Key words: Biherbal extract, *Bryophyllum calycium, Solanum xanthocarpum,* Ethylene glycol, Urolithiasis, Wistar rat.

Introduction

Urolithiasis (stone formation in the kidney) is one of the oldest and most wide spread diseases known to man. It is considered as the third most common affliction of the urinary tract with high recurrence (Agarwal and Varma 2014). Urolithiasis can be produced in rats by induction of acute or chronic hyperoxaluria by using a variety of agents such as ethylene glycol (EG), sodium oxalate, ammonium

oxalate, hydroxyproline and glycolic acid. Administration of ethylene glycol to the experimental animals for 28 days resulted in substantial excretion of oxalate and deposition of micro crystals in kidney (Sandhyarani *et al.,* 2014). A number of plants have been used to cure and correct urinary stones with minimal or no side effects, such as *Tribulus terrestris* (fruits), *Bryophyllum calycinum* (leaves), *Solanum xanthocarpum* (fruits), *Didymocarpus pedicellata* (leaves), *Dolichos biflorus* (seeds), fruits and leaves of *Solanum nigrum,* and seeds of *Cichorium intybus* (Sharma *et al*., 2017). *In vivo* and *in vitro* models have been successfully used to evaluate the antiurolithiatic potential of these medicinal plants. *In vitro* models are significantly and effectively used to evaluate prophylactic management and *in vivo* model gives the direction towards urolithiasis treatment (Ahmed *et al*., 2016). This study was aimed to evaluate haemato-biochemical alterations of aqueous and alcoholic extracts of *Bryophyllum calycium* and *Solanum xanthocarpum* either single or in combination on ethylene glycol and ammonium chloride induced urolithiaisis in adult Wistar rats.

Materials and Methods

The work was carried out from January to April 2017 on 90 healthy mature (8-12 weeks old) female Wistar rats. The project was approved by the Institutional Animal Ethics Committee (IAEC) of the College (AAU/GVC/CPCSEA-IAEC/231/2016, dated: 16/06/2016). For induction of urolithaisis ethylene glycol (EG) and ammonium chloride (AC) were used. Rats were randomly divided in to 15 equal groups, each of 6 rats and were kept in separate cage. Group I served as normal healthy control without any treatment. Group II & III served as vehicle (bicarbonate) and lithiatic (EG+AC) controls, while Groups X to XV served as herbal extract controls. In rats of Group III, IV, V, VI, VII, VIII and IX urolithiasis was induced using 0.75 % (v/v) ethylene glycol and 2% (w/v) ammonium chloride in drinking water for 28 days. Rats of Group I, II, X, XI, XII, XIII, XIV and XV were given pure wholesome water for first 28 days. After 28 days of induction of urolithiasis in Groups IV to IX, different herbal extracts, either single or in combinations, were administered orally for another 28 days using syringe and rat lavage needle in 0.5% sodium bicarbonate as vehicle.

Fresh leaves of *Bryophyllum calycinum* were procured from herbal garden of Department of Pharmacology & Toxicology and fruits of *Solanum xanthocarpum* were procured from the river bank area of Kapadvanj, Gujarat state. The above leaves and fruits were cut in small pieces, dried under shade, powdered by mechanical grinder and stored in air tight containers. Exactly 100 g of coarse powdered material of both the plants were successfully extracted in Soxhlet extractor with water and also with alcohol. Extracts so obtained were decanted in beaker and then concentrated to fullest extent in water bath. The aqueous and alcoholic extracts were preserved in refrigerator at 4°C for further use.

Group IV and V were given aqueous and alcoholic extract of *Bryophyllum calycinum @* 300 mg/kg BW, respectively; Group VI and VII were given aqueous and alcoholic extract of *Solanum xanthocarpum* @ 300 mg/kg bw, respectively, and Group VIII and IX were given aqueous and alcoholic extract of *Bryophyllum calycinum + Solanum xanthocarpum* (1:1) @ 300 mg/kg, respectively. Similarly, Group X and XI were given aqueous and alcoholic extract of *Bryophyllum calycinum;* Group XII and XIII aqueous and alcoholic extract of *Solanum xanthocarpum*, and Group XIV and XV were given aqueous and alcoholic extract of *Bryophyllum calycinum+Solanum xanthocarpum,* (1:1) @300mg/kg, bw respectively. Blood samples were collected twice from all the animals with the help of capillary tube by puncturing the retro-orbital plexuses: first after 28 days of induction of urolithiasis and then on 56th day (after treatment) of experiment. Blood samples collected in K3EDTA test tubes (2 ml) were evaluated for various haematological parameters using automatic blood cell counter, while blood (2 ml) collected in centrifuge tubes without anticoagulant were allowed to clot at room temperature (26±2^{o}C), and serum harvested by centrifugation at 3000 rpm for 15 minutes at 10^{o}C (Eppendorf 5804 R, Germany) was stored at -40^{o}C, and used for biochemical and mineral analysis using standard procedures and assay kits on BS-120 chemistry analyser. The data were analyzed statistically using ANOVA and NMRT or 't' test (Snedecor and Cochran, 1986).

Table 1: Changes in haematological parameters before and after herbal treatment in induced urolithiatic rats

Group No.	Group Name	Haemoglobin (g/dl)		Total erythrocyte count (x10^6/µl)		Total leucocyte count (x10^3/µl)		Granulocyte (%)		Lymphocyte (%)		Monocyte (%)	
		day 28	day 56	day 28	day 56	day 28	day 56	day 28	day 56	day 28	day 56	day 28	day 56
I	Normal Control	15.46 ±0.37	15.38 ±0.34	8.47 ±0.35	8.20 ±0.27	7.22 ±0.63	7.48 ±0.64	32.68 ±0.22	33.04 ±0.34	82.30 ±0.51	81.90 ±0.97	3.18 ±0.24	3.33 ±0.20
II	Vehicle Control	15.46 ±0.71	15.28 ±0.29	7.81 ±0.78	8.08 ±7.63	7.63 ±0.22	8.05 ±0.22	32.65 ±0.34	32.67 ±0.31	82.72 ±0.84	81.61 ±0.58	2.83 ±0.09	2.97 ±0.09
III	Lithiatic Control	14.56 ±0.26	14.98 ±0.20	8.37 ±0.28	6.71 ±1.37	8.38 ±0.23	8.53 ±0.27	31.89 ±0.38	32.12 ±0.95	82.60 ±0.38	83.56 ±0.23	2.47 ±0.19	2.57 ±0.20
IV	AQ. EX. B.C. 300 mg/kg	13.70 ±0.33	14.15 ±0.11	7.96 ±0.25	7.80 ±0.22	8.17 ±0.19	8.02 ±0.18	30.87 ±0.37	31.30 ±0.31	80.54 ±0.45	81.30 ±0.66	2.59 ±0.16	2.68 ±0.17
V	AL. EX. B.C. 300 mg/kg	13.36 ±0.29	13.28 ±0.28	8.04 ±0.38	8.33 ±0.28	7.95 ±0.22	8.01 ±0.18	29.81 ±0.38	29.35 ±0.50	80.18 ±0.50	81.26 ±0.50	3.05 ±0.08	2.98 ±0.17
VI	AQ.EX.S.X. 300 mg/kg	13.96 ±0.24	14.15 ±0.27	8.11 ±0.30	8.33 ±0.22	8.09 ±0.24	8.20 ±0.19	29.42 ±0.41	28.94 ±0.22	81.15 ±0.31	80.96 ±0.37	2.26 ±0.26	2.57 ±0.16
VII	AL.EX.S.X. 300 mg/kg	14.31 ±0.39	14.58 ±0.27	8.25 ±0.23	8.63 ±0.32	8.31 ±0.30	8.20 ±0.26	30.44 ±0.39	30.67 ±0.23	81.42 ±0.48	81.68 ±0.29	2.09 ±0.17	2.56 ±0.27
VIII	BH.AQ.EX. (BC+ SX) 300 mg/kg	14.06 ±0.37	13.80 ±0.26	8.10 ±0.30	8.47 ±0.28	8.24 ±0.20	7.89 ±0.26	30.56 ±0.25	30.71 ±0.29	81.67 ±0.35	82.54 ±0.35	2.10 ±0.13	2.46 ±0.19
IX	BH.AL.EX. (BC+ SX) 300 mg/kg	13.71 ±0.41	14.00 ±0.26	7.81 ±0.25	7.86 ±0.26	8.64 ±0.26	8.19 ±0.22	29.00 ±0.40	28.94 ±0.20	81.91 ±0.34	82.23 ±0.22	2.13 ±0.20	2.67 ±0.85
X	AQ.EX. Control B.C.	14.48 ±0.43	15.05 ±0.42	7.95 ±0.19	8.38 ±0.13	7.93 ±0.24	8.06 ±0.23	28.92 ±0.45	29.49 ±0.41	80.92 ±0.21	81.86 ±0.24	3.09 ±0.13	3.04 ±0.17
XI	AL. EX. Control B.C.	15.26 ±0.31	15.43 ±0.25	8.21 ±0.23	8.36 ±0.27	7.69 ±0.34	8.16 ±0.35	33.11 ±0.47	33.10 ±0.27	81.37 ±0.32	81.77 ±0.30	2.83 ±0.20	2.87 ±0.11
XII	AQ.EX. Control S.X.	15.13 ±0.41	15.08 ±0.49	8.70 ±0.19	8.69 ±0.17	7.65 ±0.20	7.59 ±0.18	32.82 ±0.65	33.60 ±0.32	82.41 ±0.28	82.26 ±0.23	2.81 ±0.18	3.09 ±0.23
XIII	AL. EX. Control S.X.	14.75 ±0.17	14.95 ±0.15	7.34 ±1.47	8.90 ±0.35	7.80 ±0.14	7.88 ±0.16	33.27 ±0.67	33.50 ±0.46	82.49 ±0.43	82.09 ±0.18	2.79 ±0.17	3.36 ±0.16
XIV	BH.AQ.EX. Control (BC+SX)	14.88 ±0.46	14.66 ±0.43	8.61 ±0.21	7.87 ±.24	7.87 ±0.24	8.15 ±0.27	32.84 ±0.80	33.07 ±0.55	81.07 ±0.33	81.64 ±0.14	2.94 ±0.24	2.82 ±0.15
XV	BH.AL.EX. Control (BC+SX)	15.50 ±0.24	15.43 ±0.28	8.05 ±0.22	8.06 ±0.09	8.11 ±0.26	7.98 ±0.17	32.67 ±0.19	32.73 ±0.38	81.90 ±0.33	82.35 ±0.43	3.05 ±0.15	3.14 ±0.12

AQ.EX./AL.EX. = aqueous/alcoholic extract, B.C./S.X. = *Bryophyllum calycinum / Solanum xanthocarpum;* BH = Biberabal

Table 2: Changes in serum albumin, globulin and total protein concentrations before and after herbal treatment in induced urolithiatic rats

Group No.	Group Name	Albumin (g/dl)		Globulin (g/dl)		Total Protein (g/dl)	
		day 28	day 56	day 28	day 56	day 28	day 56
I	Normal Control	4.94±0.27	5.18±0.39	3.20±0.53	3.05±0.31	8.14±0.38	8.33±0.36
II	Vehicle Control	5.16±0.19	5.36±0.34	2.23±0.47	2.70±0.28	7.39±0.36	7.70±0.37
III	Lithiatic Control	4.36± 0.28	4.47±0.09	2.38± 0.30	1.98±0.19	6.74±0.28	6.66±0.18
IV	AQ. EX. B.C.300 mg/kg	5.08±0.36	5.34±0.41	2.55±0.64	2.62±0.36	6.64±0.60	7.20±0.42
V	AL .EX. B.C.300 mg/kg	4.30±0.23	4.31±0.36	2.08±0.61	2.35±0.27	6.39± 0.57	7.77±0.48
VI	AQ. EX.S.X.300 mg/kg	4.87± 0.40	4.91±0.32	2.10±0.74	2.14±0.31	6.98±0.58	7.88±0.67*
VII	AL.EX.S.X.300 mg/kg	4.55±0.23	4.86±0.25	1.91±0.57	2.64±0.57	6.47±0.50	7.84±0.55
VIII	BH.AQ.EX. (BC+SX) 300 mg /kg	4.44±0.19	4.77±0.33	1.27±0.57	2.00±0.29	6.71±0.44	7.57±0.45*
IX	BH.AL.EX. (BC+SX) 300 mg/kg	4.38±0.08	4.49±0.10	1.91±0.42	2.65±0.31	6.70±0.42	7.90±0.44*
X	AQ.EX. Control B.C.	4.12±0.17	4.58±0.20	3.56±0.30	3.15 ±0.60	7.98±0.30	8.95±0.27
XI	AL. EX. Control B.C.	4.17±0.27	4.60±0.29	3.56±0.68	3.18±0.18	7.73±0.63	7.67±0.41
XII	AQ.EX. Control S.X.	4.28±0.14	3.87±0.28	3.29±0.56	2.78±0.44	8.17±0.47	8.70±0.64
XIII	AL. EX. Control S.X.	4.48±0.41	4.35±0.16	3.19±0.21	2.52±0.49	8.28±0.71	8.08±0.54
XIV	BH.AQ.EX. Control (BC+SX)	4.39±0.19	4.82±0.33	3.81±0.50	3.06±0.33	8.20± 0.56	8.27±0.44
XV	BH.ALCH.EX. Control (BC+SX)	4.47±0.19	4.90±0.30	3.64± 0.60	2.70±0.27	8.11±0.49	8.26±0.65

AQ.EX./AL.EX. = aqueous/alcoholic extract, B.C./S.X. = *Bryophyllum calycinum / Solanum xanthocarpum;* BH = Biberabal, *P<0.05 between days.

Table 3: Changes in serum calcium, magnesium and phosphorus concentrations before and after herbal treatment in induced urolithiatic rats

Group No.	Group Name	Serum Calcium (mg/dl)		Serum Magnesium (mg/dl)		Serum phosphorus (mg/dl)	
		day 28	day 56	day 28	day 56	day 28	day 56
I	Normal Control	8.10±0.59	8.33±0.36	2.12±0.29	2.32±0.38	6.66±0.35	6.57±0.35
II	Vehicle Control	7.93± 0.80	7.79±0.80	2.16±0.30	1.96±0.10	6.87±0.17	6.77±0.22
III	Lithiatic Control	10.30±0.70	10.36±0.81	2.74±0.27	1.83±0.20	9.47±0.20	9.77±0.19
IV	AQ. EX. B.C.300 mg/kg	9.83±0.36	8.96±0.29*	2.32±0.30	2.34±0.18	8.72±0.34	7.85±0.50**
V	AL .EX. B.C.300 mg/kg	9.26±0.44	9.08±0.38	2.47±0.33	2.52±0.17	9.20±0.31	6.80±0.33**
VI	AQ. EX.S.X.300 mg/kg	9.73±0.80	8.80±0.38*	2.13±0.32	2.56±0.34*	9.43±0.22	7.18±0.27*
VII	AL.EX.S.X.300 mg/kg	9.28±0.57	9.21±0.69	2.29±0.41	2.70±0.40*	8.44±0.21	8.01±0.24*
VIII	BH.AQ.EX. (BC+SX) 300 mg /kg	10.54±0.33	8.93±0.50*	2.30±0.32	2.82±0.19*	8.95±0.34	8.14±0.39*
IX	BH.AL.EX. (BC+SX) 300 mg/kg	10.50±1.72	8.36±0.34**	2.51± 0.28	3.21±0.21**	9.60±0.23	7.40±0.22**
X	AQ.EX. Control B.C.	8.00±0.40	8.09±0.28	2.11±0.39	2.30±0.17	6.80±0.34	6.94±0.28
XI	AL. EX. Control B.C.	7.49±0.50	7.80±0.49	1.90±0.34	2.31±0.20	6.98±0.24	6.74±0.13
XII	AQ.EX. Control S.X.	7.72± 0.67	7.78±0.64	1.86±1.20	1.39±0.27	6.76±0.16	6.93±0.15
XIII	AL. EX. Control S.X.	8.42±0.45	8.46±0.44	1.86± 0.25	2.41±0.15	6.72±0.19	6.55±0.16
XIV	BH.AQ.EX. Control (BC+SX)	8.25±0.80	8.14±0.71	1.73±0.21	1.83±0.20	6.96±0.21	6.62±0.18
XV	BH.ALCH.EX. Control (BC+SX)	8.05±0.79	7.90±0.57	2.05±0.15	1.91±0.12	6.83±0.25	6.70±0.15

AQ.EX./AL.EX. = aqueous/alcoholic extract, B.C./S.X. = *Bryophyllum calycinum / Solanum xanthocarpum;* BH = Biberabal, *P<0.05, **P<0.01 between days.

Results and Discussion

The haematological findings recorded before (28th day) and after (56th day) herbal treatment did not reveal significant variations in any of the haematological parameters evaluated in different treatment and control groups. Although the haemoglobin concentration and RBCs count were found to be reduced non-significantly in urolithiatic groups by day 28 compared to control groups (Table 1). These observations indicate that the ethylene glycol and ammonium chloride though induced renal calculi as evident by Ultrasonography, did not alter the haematological parameters. Akila and Manickavasakam (2012) and Mashiyava *et al.* (2015) also reported non significant effect of herbal extract in ethylene glycol and ammonium chloride induced urolithiasis in rats.

Further, no significant difference was observed in serum albumin and globulin levels before and after herbal treatment in different groups in the present study. In the urolithiasis induced groups, increased levels of serum calcium, phosphorus and decreased levels of serum magnesium and total protein were observed as compared to the normal control group by day 28 of treatment (Tables 2 and 3). However, the single extract or the co-treatment with aqueous and alcoholic extracts of *Bryophyllum calycinum and Solanum xanthocarpum* for 28 days significantly restored these changes, i.e., by 56th day of experiment in most of these groups. Biherbal alcoholic extract compared to mono-herbal extract of the said plants was much better in restoring the values of serum proteins and minerals, which came down nearer to normal by 56th day. Patel *et al.* (2012) reported non-significant decrease in serum calcium level in treatment group compared to model control. Earlier study (Mashiyava *et al.,* 2016) has shown significant increase in mean values of serum magnesium following administration of ethylene glycol, while significant decrease / restoration following administration of extracts of *Bryophyllum calcynium* and *Tribulus terretis*. Jagannath *et al.* (2012) found significant increase in mean serum phosphorus concentration following administration of

Table 4: Changes in serum BUN, uric acid and creatinine concentrations before and after herbal treatment in induced urolithiatic rats

Group No.	Group Name	Serum BUN (mg/dl)		Serum Uric acid (mg/dl)		Serum creatinine (mg/dl)	
		day 28	day 56	day 28	day 56	day 28	day 56
I	Normal Control	12.80±0.38	12.87±0.26	2.00±0.17	2.30±0.20	0.49±0.04	0.57±0.01
II	Vehicle Control	12.20±0.58	12.32±0.38	2.42±0.22	2.39±0.19	0.41±0.03	0.47±0.04
III	Lithiatic Control	36.82±0.53	37.01±0.40	4.01± 0.48	4.33±0.55	1.95±0.18	1.85 ±0.18
IV	AQ. EX. B.C.300 mg/kg	34.70±0.41	23.93±0.31*	4.57±0.44	4.19±0.40*	1.84±0.30	1.21±0.23*
V	AL .EX. B.C.300 mg/kg	33.47±0.80	21.89±0.50**	3.79±0.19	3.40±0.14*	1.91±0.02	1.11±0.20*
VI	AQ. EX.S.X.300 mg/kg	31.99±0.42	17.99±0.85**	4.70±0.28	4.28±0.30*	1.73±0.17	1.18±0.31*
VII	AL.EX.S.X.300 mg/kg	32.37±0.39	20.63±0.28**	3.99±0.41	3.03±0.34*	1.62±0.16	1.10±0.18*
VIII	BH.AQ.EX. (BC+SX) 300 mg /kg	32.62±0.32	19.58±0.54**	4.29±0.35	3.59±0.29**	1.99±0.23	0.98±0.20**
IX	BH.AL.EX. (BC+SX) 300 mg/kg	35.79±0.40	20.29±0.83**	3.65±0.20	2.60±0.31**	1.98±0.09	0.86±0.12**
X	AQ.EX. Control B.C.	12.60±0.53	12.47 ±0.47	2.20±0.20	2.39 ±0.23	0.52±0.07	0.54±0.07
XI	AL. EX. Control B.C.	12.27±0.38	12.88±0.55	2.23±0.26	2.01±0.30	0.50±0.07	0.51±0.07
XII	AQ.EX. Control S.X.	11.66 ±0.38	11.81±0.37	2.00±0.24	1.89±0.21	0.56±0.06	0.58±0.06
XIII	AL. EX. Control S.X.	10.90±2.20	14.02±0.46	2.22±0.21	2.34±0.17	0.48±0.04	0.52±0.10
XIV	BH.AQ.EX. Control (BC+SX)	12.07±0.58	14.14±0.49	2.31±0.26	2.38±0.18	0.58±0.07	0.60±0.07
XV	BH.ALCH.EX. Control (BC+SX)	11.14±0.25	11.37±0.36	2.11±0.26	2.19±0.18	0.48±0.05	0.50±0.03

AQ.EX./AL.EX. = aqueous/alcoholic extract, B.C./S.X. = *Bryophyllum calycinum / Solanum xanthocarpum;* BH = Biberabal, *P<0.05, **P<0.01 between days.

ethylene glycol (0.75%) & ammonium chloride (2%), which decreased significantly following administration of extract of *Asparagus racemosus.*

In the present study, increased levels of serum BUN, uric acid and creatinine were also observed in the calculi induced groups as compared to the normal control groups on 28th day (Table 4). However, co-treatment with aqueous and alcoholic extract of *Bryophyllum calycinum and Solanum xanthocarpum* significantly restored these changes by 56th day of treatment in these groups. Biherbal alcoholic extract of the plants was much better in restoring these parameters also within 28 days of oral treatment. Rathva (2016) reported significant increase in mean values of BUN following administration of ethylene glycol, which decreased significantly to normal levels following administration of extracts of *Solanum xanthocarpum* and *Acaraynthus aspera*. The markedly elevated serum levels of creatinine in urolithiatic rats were indicative of prominent necrosis of renal epithelia. Damage was observed at the end of nephron and collecting tubules. Retention of oxalate in the kidney is one of the causative factors for peroxidative degeneration of renal epithelia.

It is note worthy that no haemato-biochemical changes were observed on administration of herbal extracts, the values were at par with normal in groups.

From the results of this study, it is concluded that alcoholic extract of *Bryophyllum calycinum* and *Solanum xanthocarpum* compared to aqueous extracts and single herbal aqueous or alcoholic extract of the said plants was much better in restoring the altered biochemical profile in ethylene glycol and ammonium chloride induced urolithiasis in Wistar rats without altering the haematological parameters.

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Conflict of Interest: Authors declare no conflict of interest for this research work.

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