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Effect of Arjun (*Terminalia arjuna*) bark powder supplementation on haemato-biochemical parameters in Uttara layers

Kalicharan Nayal, Anil Kumar, Gururaj VK and Shrishtipriya Prasad

Abstract
In the present investigation, a feeding trial of 12 weeks of duration was conducted on 96 Uttara layers of 20-week age to study the effect of dietary supplementation of Arjun (*Terminalia arjuna*) bark powder on haemato-biochemical parameters. The laying hens were randomly divided in to four treatment groups (T_0, T_1, T_2 and T_3) four replicates of 6 birds each. The different groups were supplemented with Arjun bark powder at 0, 1g, 2g, and 4g/100gm respectively to standard feed. At the end of Phase I and Phase II blood was collected for haematobiochemical parameter analysis. The blood picture revealed that Hb, PCV, TEC and TLC values in Arjun bark-supplemented groups are significantly improved. Serum glucose, cholesterol, LDL, SGOT and SGPT values in Arjun bark-supplemented groups decreases significantly and HDL value increases significantly however no significant difference observed in other biochemical parameters. From the results of present study it can be concluded that Arjun bark can be used as herbal feed additive/ phytobiotic in poultry diet by incorporating 2 percent of Arjun (*Terminalia arjuna*) bark powder in the basal diet of Uttara chicken.

Keywords: Arjun bark powder, haemato-biochemical, phytobiotic, Uttara layer

1. Introduction
Total poultry population in India is 851.81 million and total egg production of India is 103.32 billion numbers.
India stands 6th in world's poultry meat production with meat production of 4.06 MT which is almost 50% of the total meat production of India. The share of agriculture in GDP is 19.9% and overall dairy, poultry and aqua industries contribute 4.4% to the nations GDP which itself symbolise the importance of poultry sector (20th Livestock Census Report, DADF, GOI). Poultry sector generate income for the nation as well as provide employment in urban and semi-urban areas. The per capita availability of eggs in India is 79 eggs/annum/person and per capita availability of meat in India is 5.87 kg/annum while the ICMR recommendation is 10.5 kg meat/annum/person & 180 egg/annum/person. So there is huge scope for poultry industry expansion in India (BAHS, 2020). Tamil Nadu is leading in poultry production followed by Andhra Pradesh & Telangana. The industry pockets are in various parts of country like Assam, West Bengal and Orissa in east, Haryana in north, Maharashtra in south-west, Karnataka in south-east and in Kerala in extreme south with major production seen in southern area of India. The total backyard poultry is 317.07 million & total commercial poultry is 534.74 million with increase of 45.79 percent and 4.5 percent respectively from previous census. The organised sector of the poultry industry accounts for around 70 percent of overall output, with the unorganised sector accounting for the remaining 30 percent (CARI, 2013) [4].
Uttara is an indigenous chicken breed found in hilly areas of Uttarakhand. Uttara are generally raised in a backyard system in different areas of Uttarakhand. It provides livelihood and nutritional security to the families that raise birds in those areas (Rana *et al*., 2016) [19]. The Uttara has a feathered shank and a deep black plumage.
Since 2006, when antibiotic growth promoters were completely banned or severely restricted due to their negative residual effects, most producers have supplemented poultry rations with one or more natural additives as a safe and inexpensive alternative source on acceptable way to increase production performance, maintain animal health, and prevent harmful residues in food (Castanon, 2007) [5]. *Terminalia arjuna* has been found to have potent hydrolipidemic characteristics. The saponin glycosides in *Terminalia arjuna* are thought to be responsible for its inotropic effects, while the flavonoids/phenolics may provide antioxidant and vascular

amplification action, thereby validating the plant's various activities for its cardio-protective role (Dwivedi 2007; Maulik and Talwar, 2012; Kapoor *et al*., 2014) [8, 14, 12]. The bark of Arjun is astringent, sweet, acrid, cooling, aphrodisiac and expectorant, but, chiefly used as cardio tonic as it improves blood supply to heart. It is also useful in ischaemic heart disease, and hypertension. The bark has been used to treat fractures, diabetes, anaemia, cardiopathy, ulcers, leukorrhoea, and cirrhosis and has been described as an expectorant, cardiotonic, styptic, astringent, demulcent, anti-dysenteric, urinary astringent (Warrier *et al*., 2014) [26]. The bark has been used as an ulcer wash, and the ashes of the bark have been prescribed for snakebite and scorpion stings (Jain *et al*., 2009) [11].

2. Materials and Methods

The Instructional Poultry Farm, Nagla, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar, is where the current experiment was conducted (Uttarakhand). The IAEC-approved feeding study was conducted from December 20, 2021, to March 20, 2022 in a completely randomized design. *Terminalia arjuna* bark was collected from Vendor. The bark was shade dried to avoid nutrient loss and grounded to powder by an electric grinder of Agronomy Department, College of Agriculture, Pantnagar. Proximate analysis was performed in Department of Animal Nutrition College of Veterinary and Animal Sciences Pantnagar to calculate the composition of basal diet and diet with 1%, 2% and 4% Arjun bark powder supplementation.

At the conclusion of Phase I and Phase II blood samples were taken. A sample of blood (approximately 3.0 ml) was taken and placed in test container with clear labels for blood collection. The vials contain EDTA as an anticoagulant for haematological analysis received one portion on (1.0 ml). 2 ml of blood was simultaneously injected in to a different set of vials containing clot activator (without anticoagulant) and left to stand at room temperature in a slanting position for 3 to 4 hours in order to promote the clot formation. Following blood clotting, serum samples were separated by centrifugation at 3 000 rpm for 4-5 minutes. The separated serum was then collected into an eppendorf tube and stored at -20 °C in a deep freezer for later analysis. Haemoglobin was observed by Drabkins method. Hemocytometer method was used for TEC and TLC values. PCV values were observed by Microhematocrit method. MCV, MCH and MCHC were calculated by formulas manually. AccuSure Glucometer Test Strips were used for glucose values. Erba Cholesterol *DES* Test Kit was used for total cholesterol and HDL Cholesterol direct reagent Kit was used for HDL values. LiquiMAX LDL Cholesterol direct reagent Kit was used for LDL values. Erba Total Protein Test Kit was used for Total Protein and Erba Albumin Test Kit was used for albumin concentration. AUTOSPAN SGOT Kit for SGOT and AUTOSPAN SGPT Kit was used for SGPT values. Globulin values were calculated by subtracting albumin concentration from total protein concentration. Statistical analysis was done with help of one-way ANOVA (for more than two groups of data) using SPSS software package version 22.0. The significant mean differences were separated by Tukey post-hoc analysis with significance level defined at $p<0.05$.

3. Results and Discussion

Table 1 show the composition of basal diet and composition of basal diet when incorporated with 1%, 2% and 4% Arjun bark powder. The Crude Protein value decreases from control towards the treatment. The Crude Fibre and Ether Extract values increases from Control towards the treatment.

Table 1: Chemical composition of diet (on % dry matter basis) of different treatment group fed to laying Uttara fowl during experimental period.

Particulars	Treatment/Groups			
	T_0	T_1	T_2	T_3
	Basal diet	Basal diet + 1% Arjun bark Powder	Basal diet + 2% Arjun bark Powder	Basal diet + 4% Arjun bark Powder
Moisture	8.30	8.26	7.59	7.20
Crude Protein	22.54	22.50	20.25	18.86
Crude Fibre	7.37	7.5	7.62	7.87
Ether Extract	2.85	2.9	2.94	3.03
Ash	6.97	7.28	8.2	8.3
Nitrogen Free Extract	60.27	59.82	60.99	61.94

Table 2, Table 3 and Table 4 show values of haematological parameter in Phase I (20-26 week), Phase II (26-32 week) and overall period. It was observed that the value of haemoglobin in T_1, T_2 and T_3 was significantly higher ($p<0.05$) than T_0. The significant increase in haemoglobin values in treatment groups probably due to presence of minerals *viz*. Calcium, Magnesium, Aluminium, Zinc, Copper which are important elements for synthesis of haemoglobin and due to haematinic property of Arjun bark. Additionally to, the compounds found within the bark stimulate and promote the formation of red blood cells and increasing the number of red blood cells would contribute to increase the concentration of hemoglobin (Hb).

The finding of present research study were similar with the findings of Ansari *et al.* (2016) [1] those find that treatment receiving 1% arjun bark powder in basal diet for 5 weeks show significantly higher ($p<0.05$) haemoglobin value than T_0. These finding were in contrast with the finding of Suely *et al.* (2016) [22], those found exposure to a concentration of the Arjun bark extract had caused substantial changes in the hematological parameters of fishes. In general, Hb level decrease in treatment fishes as compared to those of the T_0. Hamood and Abdalhussain (2018) [10] also found that the treatment of *Terminalia chebula* powder resulted in significant increase ($p<0.05$) in Hb in treated group as compared to control group.

The values of PCV continuously increase from T_0 to T_3. The value of PCV in T_1, T_2 and T_3 were found significantly higher ($p<0.05$) than T_0. As a result of the liver being stimulated and activated by the assembly of activated enzymes present in

bark, which in turn stimulates the kidneys to secrete the endocrine glycoprotein, Blood composition, the compounds found in the plant also stimulate and promote the formation of red blood cells within the biological process. This may be the cause of the rise in cell size values (PCV). The finding of present research study are similar with the findings of Ansari *et al.* (2016) [1] those find that treatment receiving 1% arjun bark powder in basal diet for 5 weeks showed higher PCV value than T_0. Hamood and Abdalhussain (2018) [10], also found that the treatment of *Terminalia chebula* powder results in significant increase (p<0.05) in PCV in treated group as compared to control group.

The values of TEC continuously increased from T_0 to T_3. The value of TEC in T_1, T_2 and T_3 were found significantly higher (p<0.05) than T_0. The finding of present research study were in agreement with the findings of Ansari *et al.* (2016) [1] those find that treatment receiving 1% arjun bark powder in basal diet for 5 weeks show higher TEC value than T_0. The values of TLC continuously increased from T_1 to T_3. The values of TLC in T_1, T_2 and T_3 were found significantly higher (p<0.05) than T_0. The finding of present research study were found similar with the findings of Ansari *et al.* (2016) [1] those find that treatment receiving 1% arjun bark powder in basal diet for 5 weeks show higher TLC value than T_0. TLC value continuously increases from T_0 to T_3.

There were non-significant differences observed among MCV value of different treatment groups. In Contrast to above finding Suely *et al.* (2016) [22] found that exposure to a concentration of Arjun bark extract cause increase in MCV values in treated fishes. MCH value among different treatment groups were found non-significant. In Contrast to above finding Suely *et al.* (2016) [22] found that exposure to a concentration of Arjun bark extract cause increase in MCH values in treated fishes. In Phase I, the values of MCHC continuously increase from T_0 to T_3. The value of MCHC in T_2 and T_3 were found significantly higher (p<0.05) than T_0. There was no significant difference or pattern observed between MCHC value in Phase II and overall period among the groups. In Contrast to above finding Suely *et al.* (2015) [22] found that exposure to a concentration of Arjun bark extract cause decrease in MCHC values in treated fishes.

Table 2: Least square Means ± S.E. of haematological parameters of Uttara layers during Phase I. (20 weeks to 26 weeks of age)

Parameters	T_0	T_1	T_2	T_3
Hb (g/dl)	7.60^{a}±0.12	8.57^{b}±0.22	9.25^{c}±0.17	9.80^{c}±0.13
PCV (%)	27.25^{a}±0.53	30.50^{b}±0.27	31.37^{b}±1.08	31.87^{b}±0.89
TEC(×10^6/µl)	2.81^{a}±0.11	3.00^{ab}±0.11	3.26^{b}±0.11	3.39^{b}±0.12
TLC(×10^3/µl)	2.01^{a}±0.11	2.54^{b}±0.15	2.70^{b}±0.13	3.00^{b}±0.13
MCV (fl)	97.99±4.34	102.77±5.01	96.45±2.75	95.28±5.38
MCH (pg)	27.34±1.18	28.85±1.32	28.59±1.14	29.22±1.22
MCHC (%)	27.06^{a}±0.54	28.15^{ab}±0.51	29.65^{bc}±0.83	30.87^{c}±0.70

Mean bearing different superscript column-wise differ significantly (p≤0.05)

Table 3: Least square Means ± S.E. of haematological parameters of Uttara layers during Phase II. (26 weeks to 32 weeks of age)

Parameters	T_0	T_1	T_2	T_3
Hb (g/dl)	8.11^{a}±0.24	$9.26^{b\pm}$0.19	9.57^{c}±0.33	9.85^{c}±0.38
PCV (%)	28.12^{a}±0.44	32.87^{b}±0.77	34.00^{b}±0.42	35.00^{b}±0.42
TEC (×10^6/µl)	2.47^{a}±0.15	3.07^{ab}±0.13	3.21^{b}±0.10	3.40^{b}±0.11
TLC (×10^3/µl)	2.11^{a}±0.10	2.41^{b}±0.14	2.70^{b}±0.11	3.07^{b}±0.16
MCV (fl)	100.83±5.45	108.16±4.67	100.95±4.33	109.81±4.00
MCH (pg)	34.34±1.61	32.90±1.60	29.64±1.15	33.20±1.01
MCHC (%)	34.26^{a}±0.85	30.50^{ab}±1.03	29.43^{bc}±0.61	30.31^{c}±0.48

Mean bearing different superscript column-wise differ significantly (p≤0.05)

Table 4: Least square Means ± S.E. of haematological parameters of Uttara layers during overall period. (20 weeks to 32 weeks of age)

Parameters	T_0	T_1	T_2	T_3
Hb (g/dl)	7.86^{a}±0.15	$8.92^{b\pm}$0.16	9.41^{bc}±0.18	9.82^{c}±0.19
PCV (%)	27.69^{a}±0.35	31.69^{b}±0.50	32.69^{b}±0.66	33.44^{b}±0.62
TEC (×10^6/µl)	2.64^{a}±0.10	3.04^{b}±0.08	3.24^{bc}±0.07	3.39^{c}±0.08
TLC (×10^3/µl)	2.06^{a}±0.07	2.47^{b}±0.10	2.70^{b}±0.08	3.04^{c}±0.10
MCV (fl)	107.39±4.94	105.38±2.99	98.70±2.54	102.54±3.74
MCH (pg)	30.53±1.54	29.64±0.89	28.54±0.97	30.10±1.07
MCHC (%)	28.43±0.59	28.19±0.51	28.91±0.66	29.53±0.75

Mean bearing different superscript column wise differ significantly (p≤0.05)

Table 5, Table 6 and Table 7 show values of biochemical parameter in Phase I (20-26 week). Phase II (26-32 week) and overall period.

The values of serum Glucose continuously decreases from T0 to T3. The value of serum glucose in T1, T2 and T3 is significantly lower (p<0.05) than T0. The Arjuna bark stimulate B cell and elevate insulin that increase the glucose utilization in tissues thereby decreasing glucose level. In diabetic rats the value of hexokinase and phosphoglucoisomerase decrease and aldolase increase, *Terminalia arjuna* reverse these values to normal. Present findings were found similar with findings of Ragavan and Kumari (2006) [17] those found that oral administration of *Terminalia arjuna* in rats for 30 days significantly reduce the glucose level. Similar results were observed by Thomson *et al*. (2014) [25], those found that supplementation of Arjun bark increase the insulin level and reduce glucose levels. The antidiabetic activity of Arjun bark is observed *in vitro*. Shengule *et al.* (2018) [20], found that oral treatment with Arjun bark powder significantly reduce the area under curve of glucose level by 33.1% (p<0.01).

The values of serum Cholesterol continuously decreases from T_0 to T_3. The values of serum cholesterol in T_1, T_2 and T_3 were significantly lower (p<0.05) than T_0. In agreement to the above findings Gupta *et al.* (2001) [9] found a significant lowering in total cholesterol level in patient fed with Arjun bark powder. Priya *et al.* (2019) [16] also found a significant lowering in cholesterol value in patient having Arjun bark supplementation in comparison to T_0 values. Patil *et al.* (2010) [15] found a significant lowering in cholesterol level in experimentally induced hypercholesteremic rat when fed with Arjun bark extract. Tiwari *et al.* (2008) [24] found that when high cholesterol diet is administered in rabbit the cholesterol level rise but when it is supplemented with Arjun bark also the rise in cholesterol is considerably very less. When high cholesterol diet is removed the cholesterol level decrease in

Arjun fed diet. Subramaniam *et al.* (2011) [21] found a significant reduction in total cholesterol level in rabbits fed with extract of Arjun bark. Ansari *et al.* (2016) [1] found that treatment receiving 1% Arjun *(Terminalia arjuna)* bark in basal diet show lower cholesterol level as compare to control.

The values of serum HDL continuously increase from T_0 to T_3. The HDL values in T_1, T_2 and T_3 were significantly higher (p<0.05) than T_0. Priya *et al.* (2019) [16] found that the HDL value in patient having Arjun bark supplementation increase significantly in comparison to T_0 values. Patil *et al.* (2010) [15] also found a significant increase in HDL level in experimentally induced hypercholesteremic rat when fed with Arjun bark extract. Tiwari *et al.* (2008) [24] found a significant increase in HDL level in Arjun bark supplemented diet in rabbits. The HDL value is slightly above normal values. Subramaniam *et al.* (2011) [21] found a significant increase in HDL level in rabbits fed with extract of Arjun bark. Tahsin *et al.* (2021) [23] found that *Terminalia arjuna* extract successfully increase the HDL level in a dose-dependent manner in rats which was lowered by induction of diabetes. Ansari *et al.* (2016) [1] also found that treatment receiving 1% Arjun *(Terminalia arjuna)* bark in basal diet show higher HDL level as compare to control.

The values of serum LDL continuously decrease from T_0 to T_3. The LDL value in T_1, T_2 and T_3 were significantly lower (P<0.05) than T_0. Gupta *et al.* (2001) [9] found a significant lowering in LDL level in patient fed with Arjun bark powder which is similar to present findings. Priya *et al.* (2019) [16] also found that the LDL value in patient having Arjun bark supplementation decrease significantly in comparison to T_0 values. Patil *et al.* (2010) [15] also found a significant lowering in LDL level in experimentally induced hypercholesteremic rat when fed with Arjun bark extract. Subramaniam *et al.* (2011) [21] found a significant reduction in LDL level in rabbits fed with extract of Arjun bark. Tahsin *et al.* (2021) [23] found that treating diabetic rat with *Terminalia arjuna* extract reverse the alloxan mediated rise in LDL level to normal. Ansari *et al.* (2016) [1] found that treatment receiving 1% Arjun (*Terminalia arjuna*) bark in basal diet show lower LDL level as compare to control.

The values of SGOT continuously decrease from T_0 to T_3. The value of SGOT in T_1, T_2 and T_3 were significantly lower (p<0.05) than T_0. Present study was in agreement with the findings of Desai *et al.* (2015) [6] those found that the SGOT value in the group supplemented with ethanolic extract of Arjun bark significantly decreases in albino rats those fed with hyperlipimedic diet. Doorika and Ananthi (2012) [7] found that groups supplemented with aqueous extract of Arjun bark show significant decrease (p<0.05) in SGOT values as compare to T_0.

The values of SGPT continuously decrease from T_0 to T_3. The value of SGPT in T_1, T_2 and T_3 were significantly lower (p<0.05) than T_0. The results are in agreement with the findings of Desai *et al.* (2015) [6] those found that the SGPT value in the group supplemented with ethanolic extract of Arjun bark significantly decreases in albino rats those fed with hyperlipimedic diet. Doorika and Ananthi (2012) [7] found that groups supplemented with aqueous extract of Arjun bark show significant decrease (p<0.05) in SGPT values as compare to T_0.

Manan *et al.* (2012) [13] found that inclusion of *Terminalia arjuna* bark extract in diet of broiler chicken results in decrease in the mean serum ALT values the values are in agreement with the above findings.

Table 5: Least square Means ± S.E. of serum-biochemical parameters of Uttara layers during phase I. (20 weeks to 26 weeks of age)

Parameters	T_0	T_1	T_2	T_3
Glucose (mg/dl)	239.46^{c}±1.31	234.01^{b}±1.14	232.46^{b}±1.31	225.47^{a}±1.50
Cholesterol (mg/dl)	140.43^{c}±1.96	129.16^{b}±2.10	121.91^{ab}±2.47	117.28^{a}±3.56
HDL (mg/dl)	63.43^{a}±0.41	72.93^{b}±0.62	72.32^{b}±0.81	82.11^{c}±0.55
LDL (mg/dl)	73.18^{c}±2.03	52.54^{b}±1.96	44.80^{b}±2.97	31.50^{a}±4.04
Total Protein (g/dl)	4.25±0.14	4.33±0.17	4.11±0.17	4.25±0.14
Albumin (g/dl)	1.96±0.18	1.73±0.13	1.82±0.06	1.69±0.11
Globulin (g/dl)	2.30±0.25	2.73±0.13	2.28±0.18	2.55±0.18
SGOT/AST (IU/l)	125.12^{d}±0.33	112.38^{c}±0.37	110.31^{b}±0.36	106.76^{a}±0.32
SGPT/ALT (IU/l)	15.46^{c}±0.21	13.03^{b}±0.39	11.66^{a}±0.23	12.91^{b}±0.36

Mean bearing different superscript column-wise differ significantly (p≤0.05)

Table 6: Least square Means ± S.E. of serum-biochemical parameters of Uttara layers during phase II. (26 weeks to 32 weeks of age)

Parameters	T_0	T_1	T_2	T_3
Glucose (mg/dl)	240.15^{c}±1.83	232.06^{b}±1.16	229.42^{b}±1.30	223.46^{a}±1.72
Cholesterol (mg/dl)	140.07^{c}±2.03	127.30^{b}±2.14	123.13^{ab}±0.97	116.27^{a}±2.55
HDL (mg/dl)	66.62^{a}±0.43	76.82^{b}±0.54	76.95^{b}±0.52	81.70^{c}±0.32
LDL (mg/dl)	70.12^{c}±2.02	46.67^{b}±2.00	42.11^{b}±0.91	30.77^{a}±2.68
Total Protein (g/dl)	4.30±0.13	4.21±0.18	4.22±0.23	4.47±0.13
Albumin (g/dl)	1.86±0.03	1.76±0.10	1.68±0.11	1.96±0.12
Globulin (g/dl)	2.44±0.13	2.45±0.18	2.53±0.27	2.50±0.19
SGOT/AST (IU/l)	125.71^{c}±0.32	113.09^{b}±0.28	108.07^{a}±0.33	112.30^{b}±0.39
SGPT/ALT (IU/l)	15.58^{b}±0.38	13.47^{a}±0.26	12.79^{a}±0.22	13.59^{a}±0.27

Mean bearing different superscript column wise differ significantly (p≤0.05)

Table 7: Least square Means ± S.E. of serum-biochemical parameters of Uttara layers in overall period. (20 weeks to 32 weeks of age)

Parameters	T_0	T_1	T_2	T_3
Glucose (mg/dl)	239.81^{c}±1.09	233.04^{b}±0.83	230.94^{b}±0.97	224.47^{a}±1.14
Cholesterol (mg/dl)	140.26^{c}±1.37	128.23^{b}±1.47	122.52^{ab}±1.29	116.78^{a}±2.12
HDL (mg/dl)	65.03^{a}±0.50	74.88^{b}±0.64	74.64^{b}±0.76	81.91^{c}±0.31
LDL (mg/dl)	71.66^{c}±1.44	49.61^{b}±1.55	43.46^{b}±1.54	31.14^{a}±2.35
Total Protein (g/dl)	4.27±0.09	4.27±0.12	4.17±0.14	4.36±0.10
Albumin (g/dl)	1.91±0.09	1.75±0.08	1.76±0.06	1.83±0.09
Globulin (g/dl)	2.37±0.14	2.59±0.12	2.41±0.16	2.53±0.13
SGOT/AST (IU/l)	125.42^{c}±0.24	112.74^{b}±0.24	109.19^{a}±0.37	109.54^{a}±0.76
SGPT/ALT (IU/l)	15.53^{c}±0.21	13.26^{b}±0.23	12.23^{a}±0.21	13.26^{b}±0.24

Mean bearing different superscript column wise differ significantly ($p \leq 0.05$)

4. Conclusion

On the basis of above findings it is concluded that supplementation of Arjun bark powder in the basal diet of laying hens increased the average Haemoglobin, PCV, TEC and TLC values and it decreases average glucose, cholesterol, LDL, SGOT and SGPT values. Arjun (*Terminalia arjuna*) bark powder in the basal diet of Uttara chicken can be used as herbal feed additive/ phytobiotic in poultry diet.

5. Acknowledgement

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6. Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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