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Research article

Transportation stress: Impact on behaviour and welfare in meat-type chickens under indian scenario

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ABSTRACT

In order to find standard transport time and its effect on the welfare, 480 marketable commercial broiler chickens (CARIBRO VISHAL; avg. 2.0 kg body weight) were transported for 2, 4, 8h and without transport in crates in three replicates in a completely randomized design. Transport affected welfare as well as behaviour of broiler chickens. Transport stretch impacted the gait score and tonic immobility with maximum aggravation with extended duration. Runaway results shown that 8h group exhibited more run-away time (279.20sec). Number of birds affected by physical injuries increases with the duration. Bodyweight change was significant among transported groups, especially 8h travelled group exhibited more (−8.21%) body weight loss. It is concluded that a transport period of more than 4h in Indian conditions is not recommended as its causing a significant level of stress in the birds leading to stress, production loss and hampering welfare of broilers.

1. Introduction

Poultry production, consumption and its demand is experiencing an increasing trend globally and especially in developing country. In production chain of boilers, transportation is considered as a critical point which involves pre-transport handling, long-term feed and water deprivation, social disruption, noise, over-crowding, motion as well as vibration, and thermal extremes leads to stress and consequent losses of yield parameters [1]. During transportation, the birds could experience acute stress leading to physiological and behavioural changes [2] such as extreme fearfulness and economic losses to the producers [3,4]. Transport stress causes physiological changes in muscles which affects the carcass quality of the birds during slaughter [5]. There will be a depletion in muscle glycogen reserves and thereby affects the muscle pH and subsequent storage to a greater extent. On the other hand, this stressful condition affects the normal intestinal microbiota and leading to excessive growth of opportunistic pathogens [6].

Animal welfare during transport is difficult to measure and interpret. Thus, indirect measurements of animal welfare are needed,

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and one way to proceed with these measurements is using animal-based performance variables such as physical injuries, fluctuations in body temperature, weight loss, tonic immobility, run away test, and gait score following handling and transport. Various behavioral and endocrine reactions to fearful events are widely used as indicators of fear status of the birds. Gait score (GS) assesses the impairment in walking ability of the birds [7] and the birds which exacerbate leg weakness due to less activity implies that the birds are in longer transport time in transport crates suffers leg weakness which results in gait impairment. The runaway (RA) test can be used to examine the sociality of the birds after a period of stress [8].

Lesser runaway time has related to lower stress and anxiety in birds [9]. Similarly, tonic immobility (TI) is related to stress and fearfulness, with birds showing higher duration of immobility period could be related to higher level of stress and fearfulness [10]. During transport, death is not immediate but the bird's welfare is compromised by pain and distress with prolonged suffering which lead to dead on arrival at slaughter house [11].

The use of poor transport crates with high stocking density, dynamic micro-environment in the vehicle, poor road facilities and longer distance of travel to processing plant from farm could compromises their welfare and also decreases the meat quality. Poultry welfare has been recognized as one of the many factors used to show whether a poultry production unit is a reliable one since, consumers will not accept product systems that causes poor welfare [12]. In tropical countries like India, where the meat type birds (broilers) are raised in open sided system and not in environmentally controlled houses. So their response to transport stress will be different from the fully environment controlled automated production system. Further, with deviations on the growth rate of Indian broiler chickens from Western countries, the response to transport stress should vary. Keeping in view the above facts, the present research work was carried out to evaluate the welfare parameters of meat type birds at different transport times in order to optimize suitable transportation time.

2. Materials and methods

2.1. Welfare note

The present welfare study of broiler birds was endorsed by the Institute Animal Ethics Committee (IAEC) (452/01/ab/CPCSEA) of

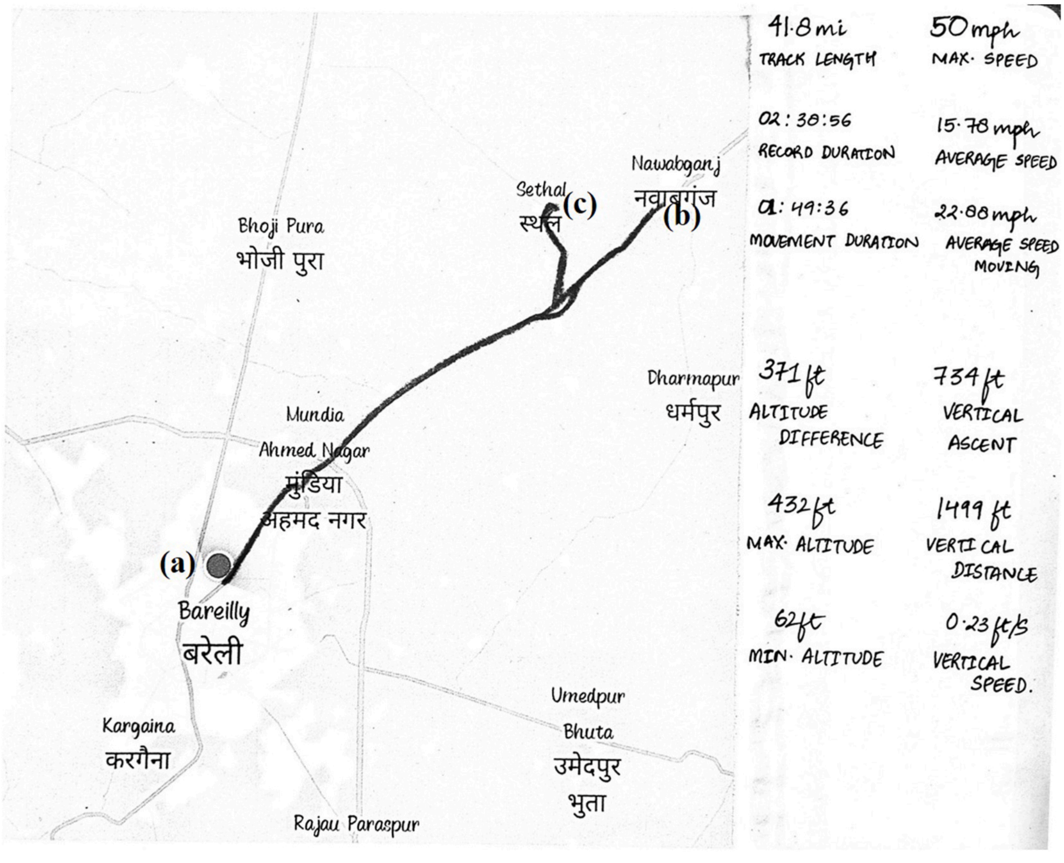

Fig. 1. Map indicating various location points including the loading, unloading and welfare assessment of broiler chickens. a) the farm location and loading point; b) point to the broiler birds were subjected to straight transportation; c) point were unloading of birds and welfare assessment was performed.

the ICAR-Central Avian Research Institute, Izatnagar, Uttar Pradesh, India.

2.2. Experimental birds and design

In order to simulate the impact of transport stress at different duration of movement on broiler welfare and stress, the study was carried out in a completely randomized design with 480 mixed male and female commercial broilers (CARIBRO VISHAL) of 42 days of age with the body weight of approximately 2.1 ± 0.03 kg/bird. These birds were portioned into two sets; the first set (120 birds) were maintained at farm premises without subjecting to transportation to serve as control representative while remaining 360 birds were subjected to transportation for different time intervals. The birds were transported using transportation truck which were driven for different periods by road and detailed route map, speed of vehicle, altitude were represented in Fig. 1. Following, 2h of transport, 120 birds were removed from the vehicle to assess the welfare parameters, while same number of birds were added to the transport vehicle in order to maintain the bird density. Similarly, other two group's birds were removed following 4 and 8h, respectively. The transport time not included the initial loading and unloading durations. The birds were assessed for their welfare parameters in replicates based on different regions of the transport vehicle. Different colour markers were applied on the bird's leg to correspond to the initial (pre-transport) body eight. The birds were transported in a medium-sized transport truck with integrated plastic crates having dimension 30" × 20" × 10" (L × W × H) and transportation was between 05:30 to 15:30hrs at an average speed of 36.4 km/h for transport stress. The load density of 39 kg per m^2 as per European Union (EU) broiler welfare directive was maintained throughoutthe study by filling the empty cages with non-coloured marked birds having similar age and bodyweight. The experiment was repeated for three times (CRD) and the data were presented as average of three trials. They were performed on three days - alternate days in week to minimal day-to-day environmental variations. The micro-climatic conditions on the day of transportation was recorded between 12 and 20 °C. After the completion of the stipulated transport time for each group, the birds were unloaded accordingly and weighed immediately to determine the body weight changes following transport stress. After body weight assessment, the birds were subjected to supervision of welfare parameters like core body temperature, gait score, run away test, tonic immobility (TI) and physical injuries.

2.3. Parameters evaluated

2.3.1. Gait score

A three-point gait scoring system was used to determine the walking fitness of broilers following transportation [7]. The score as to the scale of zero, one and two based on the effect on motor gain functions. Briefly the birds will be allowed to walk for minimum of 5' for their gait and simultaneously assessed the shanks, hocks and wings for any deformities.

Fig. 2. Setup for the run-away test along with entry and exit for birds and not influenced bythe surrounding environment.

2.4. Body temperature

One of the symptoms that may accompany anxiety conditions is a rapid change in body temperature [13]. So, in our experiment, immediately after transport the core temperature of the bird's were recorded. The cloacal membrane temperature was measured using clinical thermometry. The temperature was recorded in degree Fahrenheit using Omran digital thermometer.

2.5. Runaway (RA) test

Run away test was conducted to check the brisk walk/locomotion gait ability of the bird's promptly after transport [8]. To accomplish this procedure, an iron corridor covered with gunny bags on the sides of the corridor was divided into three zones. The corridor opens with a start box and then was the stimulus bird zone. The other end of the corridor through which the bird's runaway were littered with feed materials. The birds were introduced at the start box and the time spent by the birds to the feed stimulus was measured by an unobtrusive observer from behind during the 10 min test. The length, width and height of the corridor were 180 × 40 × 40 cm, respectively (Fig. 2). Twenty birds from each treatment were tested individually and the times taken for the birds to move out of the corridor from the start box were noted.

2.6. Tonic immobility (TI)

The immobility response by the birds following transportation was assessed by measuring the tonic immobility response time produced [10]. The response time was calculated by physical restraint of the birds on its sternum region for duration of 45sec. The reaction exhibited by the birds after 45 s was monitored and subsequently noted. For this procedure, the chicken was kept on its back with gentle restraint from the sternum and neck of the chicken for a period of 45sec. After this period, the hand was removed gently and time was noted in stop watch till the bird regains to show mobility to revert back to its normal position. The duration of TI was defined as the interval between the moments when the bird entered into TI until it righted itself. If TI was not induced after 5 trials, the bird was given a score of 0 for TI. A maximum duration of 10 min was imposed for the test [14] (Fig. 3).

2.7. Physical injuries and body weight change

The birds' well-being was determined by observing and clinically examining their physical condition. Bruises, fractures, muscle injuries, and hematoma were among the physical injuries seen after the transport. In each treatment group, the total number of birds with and without physical injuries after transportation was recorded. Body weight change was observed in terms of change in absolute body weight. The birds belong to each treatment groups were weighed both in before and after transport.

2.8. Statistical analysis

Data generated from the above experiments were subjected to analysis of variance by one-way ANOVA $X_{ij} = \mu_i + \varepsilon_{ij}$ where, μ_i – mean and ε_{ij} are normally distributed independent random errors. The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 16.0 and the means were compared by Tukey's multiple range test for statistical significance at $P \leq 0.05$.

3. Results

The data pertaining to the welfare and allied traits as influenced by the travelling time in broilers chickens are elucidated here under. Results of impact of transport duration on welfare parameters such as gait score, body temperature, run away test, tonic

Fig. 3. Procedure of tonic immobility of the experimental birds assess their welfareness following transportation.

immobility test and physical injuries were presented in Table 1.

3.1. Gait score

The walking/locomotion ability of the transported birds at different durations were analysed immediately after transport. The results of the gait score (Table 1) clearly indicates that an increase in transport duration had significantly ($P = 0.001$) affected the gait score condition of the birds and the birds transported for extended duration (8h) exhibited poorer score (1.60). The movement of the birds which transported for 8h duration period was extensively impaired. The control group birds showed good gait score with no impairment (0.00) compared to all other group of birds. But, the birds transported for 4h duration evinced a lower level of gait impairment (0.20) compared to other transported groups 2 and 8h.

3.2. Tonic immobility

Transport stretch had a significant ($P = 0.001$) impact on the tonic immobility activity of broilers between the groups. In the present assessment study, an increase in transport duration directly correlated with aggravation of tonic immobility agitation of the birds. The groups transported for extended duration (8h), whose legs became rigid due to prolonged standing stress exhibited more time (50.70 s) to retrieve its normal comfortable position than the control group (2.8sec).

3.3. Run-away test

Similarly, run-away test was conducted to check the physical soundness of the birds after every time frame of transport. Run-away test results exhibits a linear dependency with transportation schedule. The group transported for 8h, expressed a sluggishness in its movement when birds were placed in the run-away area to examine its walking ability. The 8h transport group exhibited significantly ($P = 0.001$) more run-away time (279.20sec) when compared with the other groups. Considering both transport duration and welfare, birds transported for 4h duration recorded lesser run-away time (75.50sec) compared to other transported groups (2 and 8h).

3.4. Physical injuries

Physical injuries of the birds during transport states that welfare of the birds was highly compromised. In our experiment, the physical injuries between the experimental groups were substantially higher (70%) in the 8h transport duration than the other groups. Increase in transport duration significantly ($P = 0.001$) increased the number of birds affected with physical injuries. Broilers in non-transported group showed no physical injuries which could be mainly due to lack of transport.

3.5. Body weight change and core temperature

The findings of transport duration effect on absolute and percentage body weight in broiler chickens were presented in Table 2. The outcome of the body weight due to transport were severely manifested in the form of loss in weight. Body weight change observed in the present study showed significant ($P = 0.01$) changes among the treatment groups, especially 8h travelled group exhibited more loss (−8.17%) in body weight after transport due to stress of longer duration. No mortality of birds was observed in any treatment groups due to transport and its duration in this experiment. In the present study, the body temperature was not affected by time span of transport. The regression analysis on per cent change in body with respect to live body weight and duration of transportation, revealed that there exists a negative correlation between the duration of transport (% change in body weight (Y) $= -13.38 + 1.22 \times$ duration (h of transport) $+ 6.72 \times$ live body weight (Kg); $R^2 = 0.959$) (Fig. 4).

4. Discussion

4.1. Gait score

Gait scoring system used to appraise the presence or absence of leg weakness or walking difficulties in birds due to transit [15]. The

Table 1
Effect of duration of transportation on gait score, body temperature and other welfare parameters in broiler chickens.

Group[a]	GS	BT (°F)	RA (Seconds)	TI (Seconds)	PI (%)
0 h	$0.00^{c} \pm 0.00$	106.83 ± 0.21	$62.90^{c} \pm 10.34$	$2.80^{c} \pm 0.66$	$0.00^{b} \pm 0.00$
2 h	$0.60^{b} \pm 0.22$	106.42 ± 0.16	$168.00^{b} \pm 10.03$	$5.10^{c} \pm 1.31$	$10.00^{b} \pm 10$
4 h	$0.20^{bc} \pm 0.13$	106.51 ± 0.19	$75.50^{c} \pm 12.24$	$19.60^{b} \pm 4.84$	$30.00^{ab} \pm 15$
8 h	$1.60^{a} \pm 0.16$	106.76 ± 0.14	$279.20^{a} \pm 31.35$	$50.70^{a} \pm 3.97$	$70.00^{a} \pm 15$
P value	0.001	0.221	0.001	0.001	0.001

n = 20; Mean bearing different small letter (a-c) superscript column-wise differ significantly ($P \leq 0.001$).
[a] GS: gait score; BT: body temperature; RA: run-away test; TI: tonic immobility; PI: physical injury.

Table 2
Effect of duration of transportation on body weight (absolute and percentage) in broiler chickens.

Group	Body weight (Kg)		% Change in body weight[a]
	Before transport	After transport	
0 h	2.14 ± 0.03	2.14 ± 0.03	0.00[b] ± 0 0.00
2 h	2.22 ± 0.05	2.11 ± 0.08	−4.78[a] ± 1.55
4 h	2.19 ± 0.09	2.06 ± 0.07	−5.94[a] ± 1.11
8 h	2.23 ± 0.03	2.05 ± 0.02	−8.21[a] ± 0.36
P value			0.001

Mean bearing different superscripts (a-b) superscript column-wise differ significantly ($P \leq 0.001$).
[†]Percentage change in body weight is mean change in total of thirty birds in three replicates.
[a] The percent change in body weight were Arcsine transformed before statistical analysis.

score estimates in present study signifies that transport duration substantially affected the gait of the birds. Birds transported for longer duration witnessed high ($P = 0.001$) level of weakness with walking difficulties. The control non-transported birds recorded no gait issues, whereas, 8h transported bird's evinced extreme impairment with a gait score of 1.60. But 4h transport group has significantly less gait score compare to 2 and 8h transport, which indicate that short duration transport stress may get acclimatized but continuous transport for longer period have worsen the condition. This observation could be better described by the fact that the birds would attempt to change their behavioral activity to relieve the transport stress during initial phase (2h) and will cope up with the stress till the tipping point (up to 4h). However, during the chronic stress period beyond the broilers' self-regulating ability, the welfare has been compromised [16]. The behavioral change during long transport period could be due to increase energy expenditure of standing and fatigue and negatively correlated with the bird's behaviour.

Birds exacerbate leg weakness due to less activity, trauma and vibration, which implies that birds were, remain confined in the transport crates for longer duration without any movements and this could predispose birds to leg weakness and gait impairment. This might be due to the fact that, when the broilers were placed in crate for more than 4h will lead to sternal recumbence [17]. The broilers with extended duration of transport (8h) were vulnerable to lameness. This could be nailed down as a reason for persistent locomotion problem in poultry market due to longer, stressful transport between farms to slaughter house. Apart from lameness and slaughter house loss, it also compromises the welfare and behaviour of the birds which will be normal exhibited when birds reared in deep litter or cages. Withdrawal of feed and water accompanied with continuous journey for longer duration will abruptly break the welfare of broilers leads to severe stress during transit and compromise meat quality [18].

4.2. Body temperature

The body temperature of the birds/food animals prior to slaughter has major impact in the post-mortem muscle metabolism [19]. The temperature directly or indirectly influences the ultimate pH in meat. When birds exposed to higher amount of stress due to transport causes early post-mortem glycolysis resulting in pale, soft and exudative (PSE) condition [20]. No significant ($P \geq 0.05$) difference in body temperature was recorded between birds with and without transport. The vibrations due to vehicle movement during transport was expected to increase body temperature of the birds [21], however no such change was observed in the present study. The numerically lower temperature in transported birds might be due to longer exposure to wind during transport and dissipate body heat to environment. Conducting transportation studies at early hours of the day having less environmental temperature, could be the reason for non-significance [22]. However, the transportation during different season (summer) or period (late afternoon) could have resulted in significant changes in body temperature.

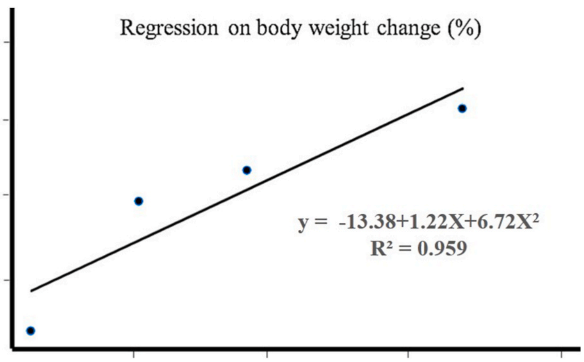

Fig. 4. Regression between per cent change in body weight (%) with duration oftransportation (h) and live body weight (Kg). X: duration of transportation (h); X^2: live body weight (Kg).

4.3. Run-away time

Leg disorders have several consequences for the welfare of the birds. Run-away tests conducted to explore the walking behavior and moods of broiler birds after fixed duration of 0, 2, 4 and 8h of transport. In the present study, lapse of transport time had a significant ($P = 0.001$) impact on the run-away test. Earlier observations indicated that due to stress the birds will be more frightened and tried to move out of the runaway corridor faster than the unstressed one [8,9]. While in our experiment, birds in stress free group (control) covered the runaway corridor in the shortest duration (62.9 s) when compared to other groups. But, 4h transport group has significantly less run away time compared to 2 and 8h transport, which indicate that birds after 4h transport may got acclimatized but further continuous transport may lead to more stress. The response to run-away test at different transport could be due to similar physiological changes during transportation as discussed earlier in the gait score. Together with exhaustion, anxiety, leg disorders or weakness due to transport and stress the birds were reluctant to move [9]. In the present study, the 8h transported birds took significantly more run-away time (279.2sec) and control (stress free) group which stepped the runaway corridor in shorter span (62.9sec). The results clearly depict that welfare and comfort of the broiler birds got deteriorated with increase in the duration of transport.

4.4. Tonic immobility (TI)

Tonic immobility is relatively a robust measure of underlying fearfulness, particularly in poultry. Stress and fearfulness significantly prolongs the tonic immobility [8,9]. An increase in transport time significantly ($P = 0.001$) affected the TI, 50.7sec *vs*. 2.8sec in 8h transport and control birds, respectively. This increase in duration of immobility might be due to over physical exertion and fear of motion during the transport. Another study with birds indicated that transport of 1.5h (90min) leads to severe stress and anxiety [10] and transport along with holding of birds has substantial impact on the TI duration [23]. Therefore, it can be portrayed that this significant difference could be by virtue of straight-line relationship between travel time and apprehension/anxiety of birds. The transport duration is directly proportional to TI; because increasing transport time will enhance exposure to vibrations, shocks and food as well water withdrawal [14].

4.5. Physical injuries

Results of percentage of birds with physical injuries after transport reveal's those birds from longer transport duration (8h) suffered more from physical injuries significantly ($P = 0.001$) than other treatments. This could be mainly due to higher stocking density, crates size, vibration and shocks during transport and transits stretch [11]. The increase in injury to the birds during transport might be due to fright which they tried to compensate by spreading their wings or resting too long against the crates floor. The flapping of with during dropping of crates leads to increased incidence of physical injury (bruises, fractures, dislocations, and muscle rupture and head trauma) to the transported birds [15]. The injuries may not have been entirely attributable to handling but during the journey, following impacts during vehicle movement, muscle strain from balancing in response to vehicle movement, or from hyperthermia. The observation from the present study shown that birds are injured due to longer transport time which might also be due to poor condition of the roads, confinement for longer time and hardness of cages. It also clearly shows that birds are more predisposed to injuries during the process of transport. No birds were injured in the treatment group without transport.

4.6. Body weight change

Percent body weight loss increased with increase in duration of transport with significant ($P = 0.005$) difference between treatments. Body weight loss in broiler chickens directly related to the distance and duration of transport [24]. An average of 4.20 and 1.80% loss in live body weight and excreta was reported following the transportation of broilers [25]. The increase in transport distance leads to increased muscle shrinkage especially in pectoral muscle due to occurrence of glycolysis at a faster rate [26]. So, it can be confirmed from our study that increase in transport distance and withdrawal of feed prior to transport increase the body weight loss in broiler chickens in a significant level.

4.7. Mortality percentage

No mortality has been observed among the transported and non-transported groups in our experiment. Mortality in broilers transported to processing plants from farm varied according to the transport distance and season [4]. No mortality during the study might be attributed to the health status, slow growing bird variety and the winter time of transport. The effect of season on mortality rate is the highest in summer and winter, and that mortality rate increases at very high and very low temperatures [27]. In addition to geographical, climatic and seasonal aspects, factors like production, slaughter and transportation conditions, transportation infrastructure, and transportation vehicles affect mortality and production losses [24].

5. Conclusions

As the transport time increases the gait impairment, tonic immobility period and physical injuries increases which indicates higher level of stress and fearfulness. The birds exacerbated leg weakness due to less activity in longer transport. The birds with 8h of transportation period took more run-away time and which is related to sociality of the birds. From all these results, it can be concluded that, in Indian conditions the continuous transport for 8h is not welfare friendly and alters the behavioural response and hence, transportation for more than 4h is not recommended.

Ethical permission

The welfare study on broiler birds was endorsed by the Institute Animal Ethics Committee (IAEC) (452/01/ab/CPCSEA) of the ICAR-Central Avian Research Institute, Izatnagar, Uttar Pradesh, India.

Data availability statement

The data has not been deposited in a publicly accessible repository, but will be made available upon reasonable request.

CRediT authorship contribution statement

M. Siddharth: Project administration, Methodology. **Jaydip Jaywant Rokade:** Funding acquisition, Conceptualization. **Subrat Kumar Bhanja:** Funding acquisition, Conceptualization. **Jagbir Singh Tyagi:** Funding acquisition, Conceptualization. **Madheswaran Monika:** Investigation, Data curation. **Beulah V. Pearlin:** Software, Investigation, Data curation. **Akhilesh Kumar:** Supervision, Software, Methodology. **Marappan Gopi:** Writing – review & editing, Writing – original draft, Validation, Formal analysis, Conceptualization.

Declaration of competing interest

All the authors declare no competing interest.

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