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# ARTICLE

## DIFFERENCES IN CORPSE STIFFNESS (RIGOR MORTIS) OF ORGANOPHOSPHATE-INDUCED AND ORDINARY (DECEREBRATED) DEAD WISTAR RATS : A RANDOMIZED EXPERIMENTAL STUDY

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## ABSTRAK

Corpse Stiffness (rigor mortis) is a secondary sign of death that can be used to estimate the time and cause of death. Organophosphates are the most toxic pesticides and often cause poisoning until death in humans. This study aims to determine the differences in corpse stiffness (rigor mortis) due to organophosphate poisoning and ordinary death (decerebration) using Wistar rats. The parameters of corpse stiffness (rigor mortis) used include aspects: Corpse Stiffness Appearance, Perfect Formation of Corpse Stiffness, Persistent Corpse Stiffness, and Relaxation of Corpse Stiffness. Data were processed with univariate analysis and then tested with the Independent Sample T-test. There was a significant difference ($p<0.05$) in the duration of the appearance, perfect formation, persistence, and relaxation of corpse stiffness between the control group and the treatment group. Meanwhile, there was no significant difference ($p>0.05$) in persistent corpse stiffness duration between the control and treatment groups, although there was a slight difference in the mean. Between ordinary and deceased rats due to organophosphate induction, there was a significant difference in the duration of appearance, perfect formation, and relaxation of corpse stiffness. However, there was no significant difference in the duration of persistent stiffness.

**Keywords:** Organophosphates; Poisoning; Corpse stiffness; Rigor mortis

## АБСТРАКТ

Трупное окоченение (rigor mortis) - это вторичный признак смерти, по которому можно определить время и причину смерти. Фосфорорганические соединения являются наиболее токсичными пестицидами и часто вызывают отравление вплоть до летального исхода у людей. Цель данного исследования - определить различия в ригидности трупа (трупном окоченении) при отравлении фосфорорганическими соединениями и обычной смерти (децеребрации) на примере крыс Вистар. Используемые параметры ригидности трупа (трупного окоченения) включают следующие аспекты: Внешний вид трупного окоченения, идеальное формирование трупного окоченения, стойкое трупное окоченение и расслабление трупного окоченения. Данные были обработаны с помощью одномерного анализа, а затем проверены с помощью независимого выборочного Т-теста. Существенная разница ($p<0,05$) наблюдалась в продолжительности появления, формирования, сохранения и расслабления ригидности трупа между контрольной и лечебной группами. Между тем, не было значительной разницы ($p>0,05$) в продолжительности стойкой ригидности трупа между контрольной и лечебной группами, хотя была небольшая разница в среднем значении. Между обычными крысами и крысами, умершими в результате индукции фосфорорганическими соединениями, наблюдалась значительная разница в продолжительности появления, совершенного формирования и расслабления ригидности трупа. Однако не было значительной разницы в продолжительности стойкой ригидности.

**Ключевые слова:** Органофосфаты; отравление; ригидность трупа; трупное окоченение



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## INTRODUCTION

Pesticide poisoning is a common global health problem. Of the 141 countries in the world, there are 385 million cases of pesticide poisoning, with the highest number of reports coming from South Asia, East Africa, and Southeast Asia with a mortality rate of >70%.[1]

Indonesia is one of the largest agricultural countries in the world, where most of the population still relies on agriculture as the primary economic sector.[2] Conventional techniques that are still carried out to increase agricultural yields include the use of pesticide agrochemicals as an effort to defend and control pests. That is why pesticide poisoning is still a cause of morbidity and mortality in developing agricultural countries such as Indonesia.[3]

Based on their chemical compounds, pesticides can be classified into organophosphates, organochlorines, chloralose, and carbamates.[4] Organophosphates still dominate in terms of use and are easily accessible to the public. Organophosphate compounds are potent poisons that can cause rapid and severe clinical symptoms even with minimal exposure. This is what causes the many deadly cases that occur in organophosphate poisoning.[5] The mortality rate is estimated to reach 6-30% of the 3 million cases of organophosphate poisoning per year.[6] Regulations and research on efficiency and effectiveness have been carried out.[3] However, the community's lack of understanding and application is still a significant obstacle to using organophosphates, causing poisoning.[6]

Many deaths due to organophosphate poisoning have been reported in emergency conditions[7] due to hypersecretion, respiratory muscle paresis, bronchoconstriction and bronchospasm, depression of the breath regulation center in the brain, and respiratory failure.[8] This is closely related to the effects of organophosphates that can modulate the imbalance of impulses to muscles to the performance of the body's nervous system. In addition, long-term complications can also occur after an acute attack.[9]

A person's death can be judged by the changes in his or her body condition after death. Secondary signs of death are definitive signs to estimate the cause and time of death.[10] Corpse bruising, corpse stiffness, and decomposition are three secondary signs of death often used in forensic analysis.[11] Medico-legally corpse bruising, corpse stiffness, and decomposition can be definitive signs of death, estimate the length of death, and the cause of death.[12]

The body will become stiff 1-2 hours after the cessation of the three central systems. The stiffness of the corpse is called rigor mortis. Corpse stiffness is caused by biochemical processes that occur in the body. The cessation of metabolism means that ATP is no longer produced. As a result, the bond between actin and myosin cannot be released, so the muscles cannot relax and experience stiffness. Corpse stiffness will peak by radiating throughout the body after 12 hours. Then it will gradually disappear in the following 12 hours.[13]

Corpse stiffening plays an essential role in forensic analysis. In medicolegal aspects, corpse stiffness can prove the exact sign of death, determine the duration of death, and estimate the cause of death. The duration of the onset, persistence, and re-relaxation of stiffness are the parameters in determining the stiffness of a corpse.[12] The induction of organophosphates may be a differentiator of parameters.

In this study, cadavers were the ideal sample. However, because it is impossible with real cadavers, without reducing the validity and scientificity, researchers use test animals in the form of Wistar rats. Wistar rats are mammals with a body physiological system similar to humans so that they can be used in toxicology and neurobehavioral research.[14]

The effects of organophosphate toxicity have been widely studied. However, research on the effects of organophosphate toxicity on corpse stiffness has not been studied further, so researchers want to know this using the parameters of the duration of the appearance of corpse stiffness, the duration of the formation of perfect stiffness, the persistence

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of stiffness, and the start of the relaxation of corpse stiffness. Previous studies still have limited samples and have not used stiffness assessment tools. Based on this, this study aims to determine the differences in corpse stiffness found in organophosphate-intoxicated Wistar rats with objective stiffness parameters.

## MATERIAL AND METHODS

This study is a laboratory experimental with postest-only control group design using the stiffness parameter (*rigor mortis*) of the corpse after death by treatment. This research was conducted at the Laboratory of Animal Experiments, Faculty of Medicine, Sebelas Maret University in March-April 2023.

The study population involved male albino rats (Rattus norvegicus) of the Wistar strain that were 2-3 months old and weighed between 150 and 200 grams. These rats will then go through an acclimatization period for five days. During the acclimatization period, researchers monitored the body weight of each sample daily to ensure that inclusion criteria were met. The characteristics of the subjects were chosen for practical reasons and because they were similar to the human physiological system.[15] If the rats showed signs of illness during this adaptation phase, such as piloerection, hair loss and greasiness, drastic weight loss, and other symptoms, the researcher would remove the animals from the study. Routine check-ups are conducted daily to monitor the condition of the rats periodically.[16]

Samples were taken by purposive sampling with Federer's formula ((n-1) (t-1) ≥ 15). This study will use two groups: the control group (deserebration) and the test group (organophosphate induction). Thus, (t) is the number of two groups. So the final calculation from the formula above is (n) or the number of rats used is 32. These 32 rats will be divided into 2 groups randomly, with each group consisting of 16 rats in the control group (deserebration) and the treatment group (organophosphate induction). Induction in test animals was carried out by gastric sonde method as much as the lethal dose of 1.16 ml in one administration.[17]

Rats declared dead are then placed on the carcass and labeled according to the group. After that, an initial pendulum was given to determine the load needed for the rat's legs to extend fully. The total weight of the pendulum will be a fixed unit in the observation of cadaver stiffness. Observations will be made by placing and removing the pendulum on the rat's leg joints every 15 minutes, starting from the rat's death until the stiffness disappears completely, while still paying attention to the parameters in the dependent variable. The stiffness parameter is assessed when there is no extension of the leg joints when given a pendulum.

In this study, the independent variable is organophosphates, specifically diazinon. Diazinon will be administered to the test animals using a gastric sonde with a volume of 1.16 ml per administration. The dose is based on the dose conversion guide by Nair and Jacob. The maximum dose approach was used to determine the optimal dose for Wistar rats, resulting in an optimal dose of 350 mg/kgBB for Wistar rats. The solution concentration was calculated from the net weight of diazinon (10% multiplied by 600 mg/ml), and after converting the rat weight to 0.2 kg, the induction volume was (ml)= (0.2 kg x 350 mg/kg)/ (60 mg/ml) = 1.16 ml.[17]

The parameters of corpse stiffness (rigor mortis) used include 4 aspects: Corpse Stiffness Appearance, Perfect Formation of Corpse Stiffness, Persistent Corpse Stiffness, and Relaxation of Corpse Stiffness. Observations were made every 15 minutes after death until the corpse stiffness disappeared by applying a 20-gram pendulum weight to the leg of the rat until perfect extension. Then, the elongation measurement that can be achieved by the rat's leg in the extension state is carried out. Determination of the 20-gram load is based on the weight of the load on the pendulum until it can perfectly extend the rat's leg. This experiment found that the rat's leg could be fully extended after being given a load of 20 grams, with an

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extension of 4 cm. The duration of stiffness appearance was calculated since the shortening of the rat's leg during extension when given a load (<4 cm). The duration of perfect stiffness was calculated from the time there was a shortening of the leg when it was extended (<4 cm) until there was no more extension when the leg was given a load (0 cm). The duration of persistence of corpse stiffness began when there was no more elongation of the rat's leg extension when given a 20-gram weight (0 cm). The absence of extension indicated that the leg joints were completely rigid and could no longer be extended. The duration of the stiffening of the corpse is calculated from the appearance of the extension of the rat's leg when given a load (>0 cm) until it extends completely or returns to the initial length shortly after the rat dies. An increase in length when the leg is given a load indicates that the corpse stiffness is slowly disappearing and the leg has begun to extend fully.

Data Analysis. The data obtained from the study were analyzed using an independent sample T-test using the IBM *Statistical Package for the Social Sciences* (SPSS) *Statistics.* This method analyzes the difference in means between 2 unpaired groups (conducted on different subjects). Before that, the distribution was tested with the *Shapiro-Wilk* test, and homogeneity was tested using *Levene's Test* as a parametric requirement.[18] This study has received ethical clearance from the Health Research Ethics Commission of Dr. Moewardi Hospital with number 369/III/HREC/2023.

## RESULT

Before conducting a different test using the Independent Sample T-test, the normality test was carried out using the Shapiro-Wilk test and homogeneity using Levene's Test as a parametric requirement. From the analysis results, it was stated that all data were ordinary distributed and homogeneous.

Difference of Corpse Stiffness Appearance and Perfect Formations

In this study, it was found that there was a significant difference in the duration of appearance and perfect formations of stiffness ($p<0.05$) between ordinary and organophosphate-induced dead rats. On average, the stiffness duration in organophosphate-induced dead rats was shorter (19 minutes and 53 minutes) than that of normal dead rats (30 minutes and 121 minutes). This data indicates organophosphate-induced dead rats develop stiffness faster than ordinary dead rats (Table 1 Table 2).

**Table 1**. Duration Difference of Corpse Stiffness Appearance

| | **Corpse Stiffness Appearance (minutes)** | |
|---|---|---|
| | **Decerebration** | **Organophosphate** |
| Mean ± SD | 30 ± 9.4 | 18.75 ± 6.7 |
| p$^{\delta}$ | 0.001 | |

SD: standard deviation, $^{\delta}$ $p < 0.05$ from independent t-test

**Table 2.** Duration Difference of Corpse Stiffness Perfect Formation

| | **Perfect Formation of Corpse Stiffness (minutes)** | |
|---|---|---|
| | **Decerebration** | **Organophosphate** |
| Mean ± SD | 120.8 ± 11.5 | 53.4 ± 7.6 |
| p$^{\delta}$ | 0.000 | |

SD: standard deviation, $^{\delta}$ $p < 0.05$ from independent t-test

**Duration Difference of Persistent Corpse Stiffness**

In the variable analysis test of persistent corpse stiffness, there was no significant difference ($p>0.05$) between the two groups. However, in terms of the mean, there was a slight difference between the duration of rigor mortis of ordinary dead rats (390 minutes) and organophosphate-induced dead rats (382 minutes) (Table 3).

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**Table 3.** Duration Difference of Persistent Corpse Stiffness

| | Corpse Stiffness Appearance (minutes) | |
|---|---|---|
| | **Decerebration** | **Organophosphate** |
| Mean ± SD | 390 ± 14.4 | 382.5 ± 7.7 |
| p$^{\delta}$ | 0.078 | |

SD: standard deviation, $^{\delta}$ p < 0.05 from independent t-test

Duration Difference in Relaxation of Corpse Stiffness

This study found a significant difference (p>0.05) in the duration of relaxation between ordinary dead rats and organophosphate-induced dead rats. On average, the onset of stiff relaxation in organophosphate-induced dead rats (45 minutes) was shorter than that of ordinary dead rats (65 minutes). Based on this data, organophosphate-induced dead rats are faster to return to relaxation than ordinary dead rats (**Table 4**).

**Table 4**. Duration Difference in Relaxation of Corpse Stiffness

| | Corpse Stiffness Appearance (minutes) | |
|---|---|---|
| | **Decerebration** | **Organophosphate** |
| Mean ± SD | 64.6 ± 10.5 | 45 ± 9.4 |
| p$^{\delta}$ | 0.000 | |

SD: standard deviation, $^{\delta}$ p < 0.05 from independent t-test

## DISCUSSION

Death, according to forensic medicine, includes two phases, namely individual death *(somatic death)* and intracellular death *(cellular death)*. Somatic death will occur first compared to cell death. The absence of a pulse characterizes the clinical examination to determine somatic death and the absence of heartbeat and breathing sounds, respiratory movements, and body reflexes. These signs indicate that in somatic death, the three main life support systems, which include the central nervous system, cardiovascular system, and respiratory system, have irreversibly stopped. However, unlike somatic death, the smallest unit of body cells still carries out metabolic processes using the remaining oxygen.[12] The resistance of each cell in responding to limited oxygen conditions also varies. This causes the cellular death of each organ not to occur simultaneously.[19]

After death *(post-mortem)*, the body undergoes several complex changes. These changes are called secondary signs of death. Secondary signs of death arise from physiochemical processes in the body and environmental conditions. In other words, there are internal and external factors. Internal factors include body fat content, sepsis, injury, and toxic reactions that cause death. Meanwhile, external factors include temperature, season, and geographical location where the body experiences death.[13]

Death at the molecular level does not occur simultaneously with somatic death. Every cell and tissue of the body can still survive with limited and no oxygen supply. This causes changes in the form that are evident in the body after death *(post-mortem)*. These changes include Algor Mortis, Rigor Mortis, Livor Mortis, and Decomposition.[12]

Organophosphates, OPE *(Organo Phosphate Esterase),* belong to the organophosphorus compound group with the general structure O = P $(OR)_3$. This compound is also a critical biomolecular constituent for DNA, RNA, and ATP, as well as many herbicides and insecticides.[20] Organophosphates work by inactivating the acetylcholinesterase enzyme responsible for acetylcholine (ACh) metabolism. As a result, Acetylcholine will accumulate at the synapse gap and cause overstimulation of postsynaptic receptors. If this excess of free Acetylcholine in the synapse gap occurs continuously, it will cause clinical symptoms related to the central, autonomic, and neuromuscular nervous systems.[9]

Not all organophosphates will react directly to provide an inhibitory effect. Based on the need for activation, organophosphates can be divided into direct inhibitors (containing oxygen groups) and indirect (containing sulfur groups). Direct inhibitors can provide direct

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inhibitory effects, while indirect inhibitors require bioactivation first before they can provide inhibitory effects. Organophosphate indirect inhibitors have more harmful effects on health. Some examples are diazinon, malathion, chlorpyrifos, and parathion.[21]

Organophosphates are more toxic and cause toxic effects on humans, among other types of pesticides. Humans can be exposed through direct skin contact, entry into the digestive system, such as lambing, and inhalation.[22] Organophosphate direct inhibitors can cause toxic effects when in direct contact with skin or sweat, stimulate bronchospasm when inhalation contact, and miosis or *pinpoint* pupil when eye contact. It was also reported that organophosphates in specific amounts can cause death.[23]

Organophosphate intoxication can occur acutely. It begins when acetylcholinesterase is successfully inhibited, resulting in clinical manifestations related to the nervous system, including the sympathetic nervous system, with gastrointestinal symptoms (nausea, vomiting, and abdominal cramps), urinary incontinence, excessive sweating, hypersalivation, hyper lacrimation, blurred vision, miosis (*pinpoint* pupils), and bradycardia. In the sympathetic and motor nervous system, hypertension, tachycardia, muscle fasciculations and cramps, paralysis, and motor weakness. In the central nervous system, confusion, anxiety, tremors, dizziness, headache, insomnia, ataxia, and coma.[24]

Rigor mortis is the stiffness experienced by the dead body due to a biochemical process. Rigor mortis begins about 1-2 hours after death and spreads throughout the body. The peak is around 12 hours after death, and the entire body of the corpse experiences rigor mortis. After that, the stiffness of the corpse will gradually disappear in the next 12 hours, or 24-36 hours after death.[12]

The process of corpse stiffness is divided into three stages: the first is the period of primary relaxation (primary flaccidity), characterized by muscle irritability that still exists but muscle tone has disappeared. All body muscles are relaxed and can be moved in all directions. This phase occurs after death and lasts 2-3 hours. The second is the rigor mortis core, where cell-level death occurs in this phase. Electrical activity and muscle impulses no longer exist. As a result, muscle stiffness occurs. This phase will occur 2-3 hours after the primary relaxation phase.[11] Then, the third Secondary relaxation period. In this phase, the protein has been completely broken down, and no more physical and chemical reactions occur so that the muscles return to being limp and easy to move. In some cases, cadaver stiffness is so rapid that it is difficult to distinguish between primary and secondary relaxation.[15]

The condition of the corpse, body temperature, environment, and the last physical activity performed will affect the process of corpse stiffening. A thin body, high body temperature, and increased activity before death will accelerate the onset of corpse stiffness. In addition, conditions related to specific causes of death, such as electrocution, striking poisoning, and malnutrition, will also accelerate the onset of corpse stiffness. Corpse stiffness occurs gradually from one part to the whole body. Corpse stiffness will start from the muscles around the eyes, the back of the neck, the face, and downwards.[26]

Human exposure to organophosphates can occur through skin contact, inhalation, and the digestive system. Organophosphates that enter the body inhibit the activation of the enzyme acetylcholinesterase (AChE), which should work to hydrolyze acetylcholine to acetate and choline at postsynaptic receptors. If acetylcholinesterase does not work, acetylcholine will continue to bind to muscarinic and nicotinic receptors and cause overstimulation of the peripheral and central nervous system.[21] Uncontrolled continuous stimulation will lead to various clinical manifestations and even degeneration and apoptosis of nerve cells, especially in the brain, due to oxidative stress, causing death.[27]

Excessive signal stimulation in the nervous system will also affect muscle impulses. The inhibited acetylcholinesterase enzyme will also cause ACh to continue to adhere and not

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terminate the end plate potential (EPP), preventing the muscle cell membrane from returning to its resting potential. As a result, the muscle will continue to contract, causing ATP depletion. Eventually, with limited ATP conditions, actin-myosin filaments cannot bind to discrete sites for relaxation. The muscles will clump together and become stiff.[28]

Organophosphates cause toxicity that affects multiple systems in the body. The number of effects caused can affect post-mortem conditions in the event of death. One of the secondary signs of death is a corpse stiffness.[7]

In humans, corpse stiffness occurs 1-2 hours after death due to muscle contraction of the corpse. However, in rats, it can be faster because the cross-sectional area of the body is smaller than in humans. Physiologically, the process of muscle contraction is strongly influenced by the release of ACh at the synapse gap, which will signal the release of calcium ions to the active side of actin (troponin). After actin is active, the myosin head can bind and with energy from ATP, it can cause muscle contraction.[11] In this study, it was found that there was a significant difference in the duration of appearance and perfect formations stiffness ($p<0.05$) between ordinary and organophosphate-induced dead rats. In cases of organophosphate poisoning or induction, the AChE enzyme fails to regulate the release of ACh signals at the synapse gap. That failure is because organophosphates have a mechanism that can bind to the AChE enzyme. As a result, the clearance of ACh at the motor and plate fails so that calcium ions will flood the muscle fiber and link with the active side of actin. That is what makes contractions occur quickly and can cause dyspnea, bronchospasm, and rapid vasoconstriction that will cause death. The condition of unstoppable ACh will also cause stiff corpses to appear and form completely faster.[9 24]

Corpse stiffness (Rigor Mortis) of humans will remain for 12-24 hours. However, it can be faster in rats because of the smaller cross-sectional area of the body.[13] Physiologically, relaxation occurs when ATP pumps out calcium ions so that they release their bond with the active side of actin. After death, ATP reserves will continue to deplete and eventually run out. As a result, calcium ions fail to be pumped back to the sarcoplasmic reticulum because there is no encouragement from ATP. That is what causes muscle relaxation to fail to occur.[11] In this phase, there is continuous contraction until it causes persistent stiffness in the corpse. This study's slight difference in duration may be due to the ATP reserves of organophosphate-induced dead rats being depleted faster because they were triggered earlier during the stiffness phase.[24]

In humans, generally, after 24 hours, the protein has been completely broken down and no more physical and chemical reactions occur, so the muscles become limp and easy to move. It may be faster in rats due to the smaller cross-sectional area of the body and energy reserves.[25] The proteins involved in muscle contraction will break down and cause the stiffness of the corpse to disappear slowly. This study found a significant difference ($p>0.05$) in the duration of relaxation between ordinary dead rats and organophosphate-induced dead rats. In organophosphate intoxication, the chemical and ionic balance of the body will be disturbed, allowing the denaturation process and protein breakdown in muscles to occur faster. This is consistent with the fact that corpse stiffness in rats that die from organophosphate induction disappears faster than ordinary death.[22]

This study has been carried out optimally, but some limitations remain. First, the dose variation in this study only used one administration, so it could not determine the optimal lethal dose in the death of test animals. Second, this study's rigor mortis observation time interval was only 15 minutes, so a more effective rigor mortis method and measuring instrument can be formulated for duration accuracy. Third, in this study, rigor was only measured in 1 part of the rat's body, so it is hoped that further research can be done on rigor mortis distribution in organophosphate intoxication cases. A confounding factor in this

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study was the use of ketamine anesthesia at a dose of 0.3 per oral in the sample as an effort to meet animal welfare principles. Although the use of this substance did not affect the results, further research is needed in this regard.

## CONCLUSION

Based on the research and the results of the analytical tests carried out, it can be concluded that there are significant differences in the duration of the appearance of stiffness, the duration of perfect formation of stiffness, and the duration of relaxation of stiffening between ordinary dead (decerebrated) rats and dead rats due to organophosphate-induced. There is no significant difference for the duration of persistent stiffness, although there is a slight difference on average.

Improvements are still needed in this corpse stiffness research. Among them are the observation of corpse stiffness with a shorter time interval to be more accurate, the discovery of more effective methods and tools for measuring corpse stiffness, and further research on the distribution of corpse stiffness.

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## DECLARATIONS

The concepts presented in this paper were developed jointly by NNA and ZAF. NNA was vital in formulating the theoretical framework and performing the necessary calculations. Meanwhile, ZAF, WH, and AWD contributed by detailing and rigorously validating the methods and analyses used in this study. In addition, NNA actively encouraged ZAF to explore further research avenues and supervised the entire research process. All authors engaged in in-depth discussions of the findings and their implications, ultimately shaping the final manuscript. Their collective expertise and contributions enriched the scientific discourse and strengthened the overall quality of the research. The presented concept was formulated by NNA and ZAF. NNA formulated the theory and conducted the calculations. ZAF, WH, and AWD validated the analytical methods. NNA motivated SMSWAW to explore and oversaw the discovery process. All authors participated in result discussions and made contributions to the final manuscript.

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