Toxicological evaluation of a complementary medicine for insomnia in rodents

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ABSTRACT

Background: *Relaxing Tea* is a herbal remedy widely used in Nigeria in the management of insomnia. Given than literature data have highlighted the possibility of adverse effects, sometimes life-threatening, arising from the use of herbal remedies, we conducted an extensive toxicological evaluation of this complementary medicine.

Method: In an acute toxicity test, mice were orally administered *Relaxing Tea* up to 6000 mg/kg. Analyses of rat tissue and serum biochemical, haematological, and semen parameters were carried out following daily administration of *Relaxing Tea* at 100, 200, and 400 mg/kg orally for 90 days. Histology of critical organs was also conducted.

Result: *Relaxing Tea* at 6000 mg/kg was non-lethal in mice. Daily dosing of *Relaxing Tea* for 90 days resulted in increased serum levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), creatinine, urea, and triglycerides, as well as an increase in sperm percentage abnormality. In contrast, there were decreases in kidney catalase, glutathione, and sperm counts compared to the control group (saline 10 ml/kg). However, there were no significant differences in weight, haematological parameters, serum and liver anti-oxidant parameters. Treated rats also showed signs of oedema in the brain, congestion of liver blood vessels, and degeneration of the seminiferous tubules, but no toxic effect on kidneys histologically.

Conclusion: These results suggest that *Relaxing Tea* may not be considered safe following long-term exposure.

Keywords: herbal medicine; insomnia; *Hypericum perforatum; Matricaria chamomilla; toxicity; Relaxing Tea.*

Évaluation toxicologique d'un médicament complémentaire contre l'insomnie chez les rongeurs

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RÉSUMÉ

Contexte: *La tisane relaxante* est un remède à base de plantes largement utilisé au Nigéria pour le traitement de l'insomnie. Compte tenu des données publiées soulignant la possibilité d'effets indésirables, parfois mortels, liés à l'utilisation de remèdes à base de plantes, nous avons mené une évaluation toxicologique approfondie de cette médecine complémentaire.

Méthode: Lors d'un test de toxicité aiguë, des souris ont reçu par voie orale une tisane relaxante jusqu'à une dose de 6 000 mg/kg. Des analyses biochimiques, hématologiques et spermatiques ont été réalisées sur des tissus et du sérum de rats après administration quotidienne de *tisane relaxante* par voie orale à des doses de 100, 200 et 400 mg/kg pendant 90 jours. Une analyse histologique des organes vitaux a également été effectuée.

Résultats: *L'infusion relaxante* à la dose de 6 000 mg/kg n'était pas létale chez la souris. L'administration quotidienne de cette infusion pendant 90 jours a entraîné une augmentation des taux sériques d'aspartate aminotransférase (AST), d'alanine aminotransférase (ALT), de créatinine, d'urée et de triglycérides, ainsi qu'une augmentation du pourcentage d'anomalies spermatiques. En revanche, on a observé une diminution de la catalase rénale, du glutathion et du nombre de spermatozoïdes par rapport au groupe témoin (solution saline à 10 ml/kg). Cependant, aucune différence significative n'a été constatée en ce qui concerne le poids, les paramètres hématologiques, ainsi que les paramètres antioxydants sériques et hépatiques. Les rats traités ont également présenté des signes d'œdème cérébral, de congestion des vaisseaux sanguins hépatiques et de dégénérescence des tubes séminifères, sans effet toxique observable sur les reins à l'examen histologique.

Conclusion: Ces résultats suggèrent que *la tisane relaxante* pourrait ne pas être considérée comme sûre en cas d'exposition prolongée.

Mots-clés: phytothérapie ; insomnie ; *Hypericum perforatum ; Matricaria chamomilla ; toxicité ; tisane relaxante.*

INTRODUCTION

The widespread use of herbal products for promoting health and well-being is well-documented and continues to gain global attention. Across various cultures and medical systems, including traditional Chinese medicine, Ayurveda, and African ethnomedicine, plant-based remedies have played a pivotal role in the prevention and treatment of numerous diseases. Herbal products are often perceived as safer and more holistic alternatives to synthetic drugs, owing to their natural origin and long history of use. In many developing countries, up to 80 % of the population relies on traditional medicine as their primary source of healthcare, primarily due to its accessibility, affordability, and cultural acceptance. Additionally, a growing body of scientific research supports the therapeutic potential of numerous medicinal plants, validating their traditional uses through pharmacological and clinical studies.[1-7] In recent decades, a resurgence of interest in herbal medicine has also occurred in developed countries, driven by a shift toward natural health products, self-care, and preventive medicine. This renewed interest is also fueled by the rising incidence of chronic diseases such as diabetes, cardiovascular disorders, and liver diseases, for which many herbal preparations offer promising bioactive compounds. As a result, herbal supplements, teas, extracts, and essential oils are now commonly marketed and consumed for their anti-anxiety, antioxidant, anti-inflammatory, antidiabetic, and hepatoprotective effects.[5,8] Across the globe, a common theme that encourages their widespread use has been the perception that these products, being derived from nature, are safe and have little to no adverse effects, as evidenced by their testimonial use throughout generations.[9] Despite their popularity, however, the safety, efficacy, and quality control of herbal products remain subjects of ongoing research and regulatory scrutiny. However, studies have demonstrated that the notion that all traditional medicines from nature are safe is not accurate.[10,11] Thus, natural remedies must be screened for efficacy as well as safety, as the population must be protected from risks that may be associated with their use.[12]

Relaxing Tea is a Nigerian herbal blend of two plants: Matricaria chamomilla (leaves and flowers) and Hypericum perforatum (leaves) in a 60/40 proportion. The manufacturer claims that it has a relaxing effect and helps treat insomnia. *Matricaria chamomilla*, commonly known as *German Chamomil*e, is an ancient medicinal herb. The dried flowers of chamomile contain many terpenoids and flavonoids. While numerous preparations of chamomile have been developed, the most popular is in the form of herbal tea, of which more than one million cups are consumed every day.[13] *St. John's* wort (*Hypericum perforatum*) has been valued for its medicinal properties for more than 2,000 years, and like Chamomile, it has traditionally been used to improve or facilitate sleep.[14] The therapeutic popularity of *H. perforatum* is largely attributed to its diverse array of bioactive constituents, including hypericin, pseudohypericin, hyperforin, flavonoids, and various phenolic acids.[15] St John's wort is the most popular and well-studied herbal treatment for psychiatric problems (insomnia inclusive) in the West in recent years, with extensively studied applications in the management of mild to moderate depression, where it has shown comparable efficacy to conventional antidepressants such as selective serotonin reuptake inhibitors (SSRIs), but with fewer side effects. The antidepressant activity is primarily associated with hyperforin and hypericin, which modulate neurotransmitter levels, including serotonin, dopamine, and norepinephrine.[16]

Despite the widespread use of *Relaxing Tea* among the populace, there is a lack of toxicological data available to demonstrate the safety or otherwise of this product. Therefore, this study aimed to conduct an extensive toxicological evaluation of *Relaxing Tea*. In this article, we present our results and observations following an acute and 90-day toxicity evaluation of a sample of Relaxing Tea, including its effects on serum and tissue parameters, as well as the histology of vital organs.

METHODS

Relaxing Tea (RT), an anti-insomnia brand used in this study, was obtained from the manufacturer in Lagos, Nigeria, in September 2018. The product is widely available in Pharmacies and Supermarkets in Nigeria. The product is a brownish, powdered mixture with a fine texture, a sour taste, and a pungent smell. The label advises preparation by adding the powdered mix to a cup of hot water. It is recommended for daily use by adults.

Extraction and preparation of final formulation

Relaxing Tea (235.96 g) was steeped in 1 L of boiled water in a conical flask for approximately 5 minutes with constant stirring. The mixture was then left to cool and settle and was decanted and filtered. This process was repeated 5 times. The combined filtrate was evaporated to dryness in an oven at 40 ^{o}C. A brownish-black paste solid extract was obtained. The dried extract was

weighed, yielding 21.15 % (w/w), and then reconstituted in distilled water (pH 6.8).

Yield (%) = (Weight of solid extract÷
Weight of dried pulverized plant extract) × 100 ..... (1)

Dosage determination

The 'dose by factor method,' as described by Reigner and Bleisch,[17] was used to convert the human doses prescribed by the manufacturer of RT to equivalent animal doses. A factor of 12.3 and 6.2 was used for the extrapolation of mouse and rat doses, respectively.[18]

Rat dose (mg/kg) =
Human dose (mg/kg) X 6.2 ..... (2)

Mice dose (mg/kg) =
Human dose (mg/kg) X 12.3..... (3)

The manufacturer recommends half a teaspoon of Relaxing Tea in a cup of hot water. Half a teaspoon of Relaxing Tea = 10.099 g; this is equivalent to 2.14 g of the dried extract (using the percentage yield formula). Therefore, the recommended adult human dose is 35.6 mg/kg, and the rat dose is 220 mg/kg (human dose * 6.2).

Experimental animals

Albino mice (15-25 g) and rats (90-150 g) of both sexes used in this study were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos. Nigeria. The animals were maintained under standard environmental conditions (25 ^{o}C, 12 h light/12 h dark cycle) and fed standard rodent pellets (Livestock Feed Plc, Lagos, Nigeria) and tap water ad libitum. However, food was not allowed for 12 hours before each experiment to avoid the potential effect of food on the activities of the orally administered plant extracts. Experiments were conducted in accordance with the ethical standards. They were approved by the Health and Research Committee of the College of Medicine, University of Lagos, Lagos, Nigeria, in compliance with the United States National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals in Biomedical Research.

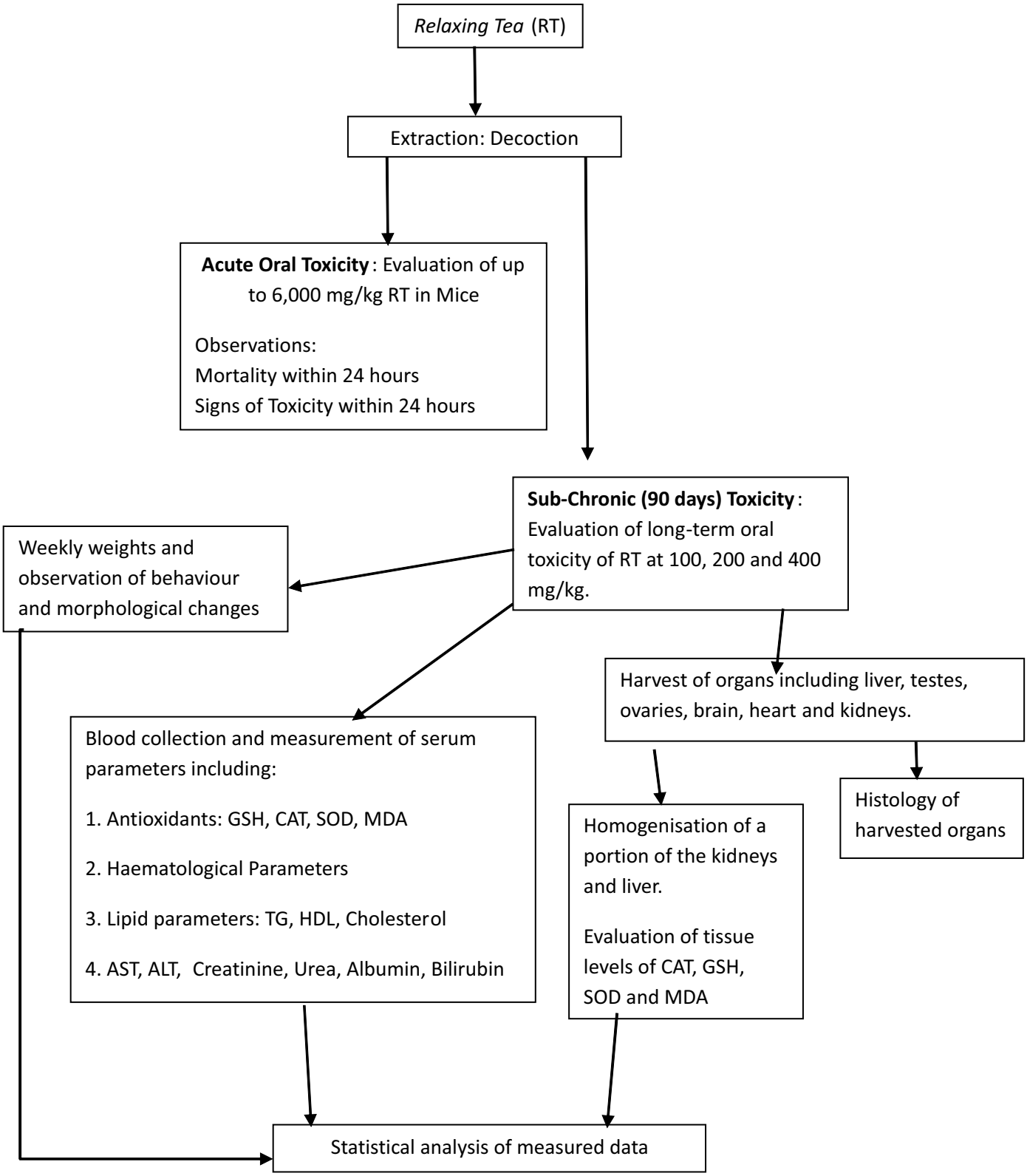


Figure 1: Flowchart depicting the adopted methodology in this study

Acute oral toxicity

Mice were randomly divided into four groups of 5 mice each. They were fasted overnight prior to the experiment. Animals were administered oral doses of RT as follows:

- Group I: RT, 750 mg/kg
- Group II: RT, 1500 mg/kg
- Group III: RT, 3000 mg/kg
- Group IV: RT, 6000 mg/kg

After oral RT administration, each mouse was observed for the first 5 minutes for signs of possible regurgitation. Further observations were conducted every 15 minutes in the first 4 hours after dosing and then at 24 hours after dosing to monitor for signs of toxicity. Additionally, observations were made 14 days after RT dosing to assess for signs of delayed toxicity. Behavioural manifestations of acute oral toxicity, such as restlessness, hyperactivity, stretching, sedation, writhing, rearing, grooming, and dullness, were observed. All observations were systematically recorded, with individual records being maintained for each mouse.

Sub-chronic toxicity test

Sixteen mature male and sixteen mature female rats were randomly divided into four treatment groups: four groups of males (n = 4) and four groups of females (n = 4). Ninety (90) days of oral administration were done as follows:

- Group I: Normal saline, 10 ml/kg
- Group II: RT, 100 mg/kg
- Group III: RT, 200 mg/kg
- Group IV: RT, 400 mg/kg

The rats were weighed weekly throughout the experiment. The animals were closely observed for behavioural changes such as restlessness, hyperactivity, dullness and general morphological changes.

Collection of blood samples and tissues for assays

At the end of the 90-day treatment period, the rats were sacrificed under diethyl ether anaesthesia. By dissection of the muscles and fasciae, the contents of both the thorax and abdomen were exposed. A blood sample was collected directly from the rat's heart via cardiac puncture with a 21G needle mounted on a 5 ml syringe plunger into EDTA sample bottles for haematological analysis and into plain sample bottles for biochemical analysis. Vital organs, including the heart, brain, kidneys, liver, testes, and ovaries, were harvested and weighed. A portion of the organs was preserved in 70 % formal saline for histological assessment, while the other portion was weighed and homogenised for biochemical assays.

The fully automated clinical chemistry analyser (Hitachi 912, Boehringer Mannheim, Germany) was used to determine the levels of serum biochemical parameters, including Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), urea, creatinine, albumin, total bilirubin, Triglycerides, Cholesterol, and High-Density Lipoproteins (HDL). In serum and homogenized liver and kidney tissues, lipid peroxidation activity was determined by measuring the formation of thiobarbituric acid reactive substances according to the method of Varshney and Kale;[19] the method of Beutler *et al*.[20] was used for the determination of activity of reduced glutathione while glutathione S-transferase activity was determined according to the method described by Habig *et al*.[21] The levels of superoxide dismutase and catalase activities were determined by the methods of Misra and Fridovich[22] and Sinha[23], respectively. The fully automated clinical haematological analyzer (Pentra-XL 80, Horiba ABX, USA) was used to determine the levels of white blood cells, red blood cells, haemoglobin, hematocrit (packed cell volume), and platelets. The sperm analysis (motility, count, and abnormal morphology) was performed using the method described by Morakinyo.[24]

Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM). Significant differences among the groups were determined by one-way analysis of variance, and Tukey's multiple comparisons test was performed for intergroup comparison using GraphPad Prism 6. Results were considered statistically significant at $p < 0.05$.

RESULTS

Acute toxicity test

The animals showed no mortality at the given doses, and there were also no toxic changes observed at 750-1500 mg/kg. RT at 3000 mg/kg showed signs of reduced locomotion some minutes after treatment, while a dose of 6000 mg/kg manifested weakness, calmness and sedation in treated mice in the first two hours post-treatment. The results indicate that oral RT is safe on acute exposure as LD50 > 6000 mg/kg.

Table 1: Observations from acute oral toxicity test of *Relaxing Tea* in treated mice.

Group	Dose (mg/kg) (n=5)	2 Hours Post-Administration	24 Hours Post-Administration	Signs of Toxicity
I	750	Survival	Survival	No toxicity observed
II	1500	Survival	Survival	No toxicity observed
III	3000	Survival	Survival	Reduced locomotion observed in the first 2 hours
IV	6000	Survival	Survival	Marked weakness sedation was observed in the first 2 hours

Table describes the results and observations from the acute oral toxicity test of *Relaxing Tea* in mice (n=5). LD_{50} > 6000 mg/kg

Body Weight

Body weight measurements are an important task in toxicity assessments. Significant decreases in body weight at the end of the study period indicate toxicity. As shown in Table 2, no significant difference in the body weight compared to control animals was observed following a 90-day oral administration of *Relaxing Tea.*

Table 2: Effects of 90 days of oral administration of Relaxing Tea on the body weight of rats

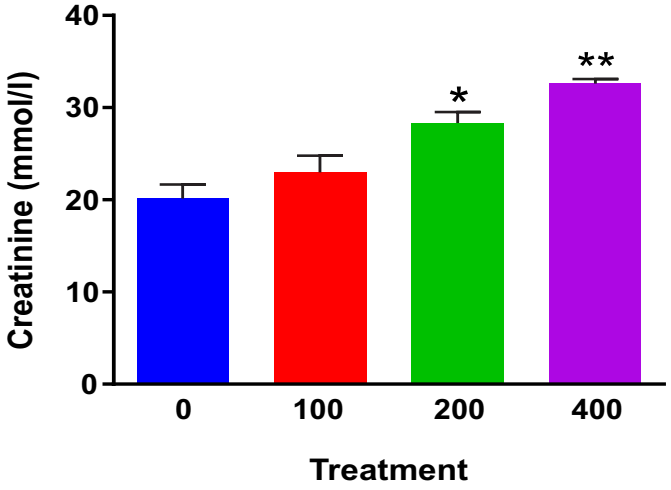
	Normal saline (10 ml/kg) (Control)	RT 100 mg/kg	RT 200 mg/kg	RT 400 mg/kg
Weight day 0 (g)	151.3 ±7.77	155.5 ± 7.63	150.1 ± 7.59	152.3 ±10.60
Weight day 90 (g)	190.3 ± 8.12	184.8 ± 6.51	182.0 ± 3.51	179.3 ± 19.22

Table describes the effect of 90-day oral administration of treatments on rat body weight. Values are expressed as mean ± SEM (n=5). RT is *Relaxing Tea*

Biochemical Assays

Renal function test

Following 90 days of oral administration of *Relaxing Tea*, serum creatinine levels were significantly increased in the 200 and 400 mg/kg groups ($p<0.05$), a dose-dependent effect. (Figure 2). Also, urea levels were raised significantly at the administered dose of 200 mg/kg ($p<0.05$) when compared with the control. This effect was not observed at the 100 and 400 mg/kg group.



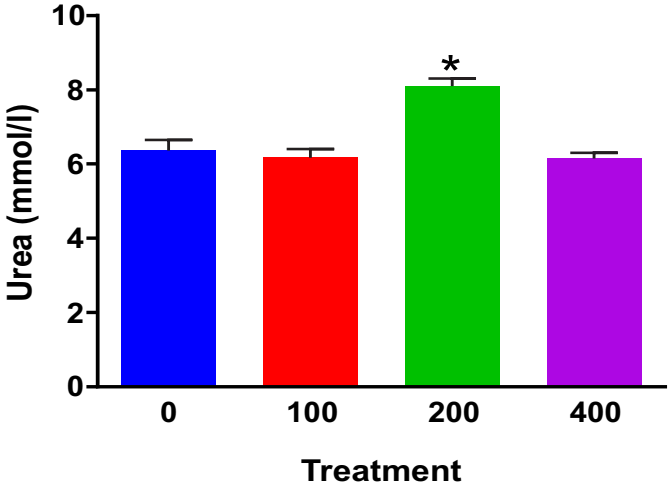
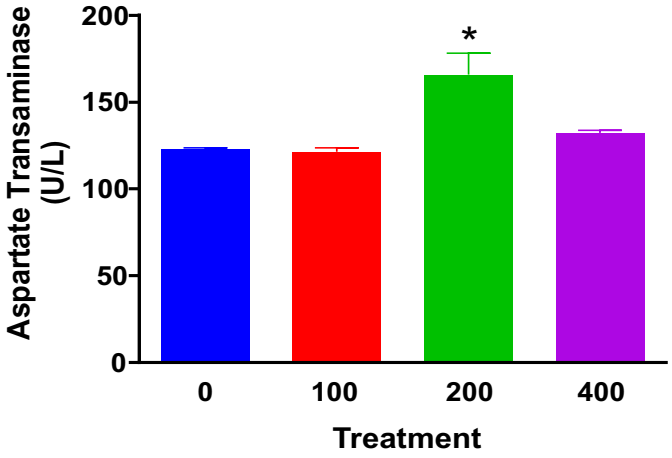
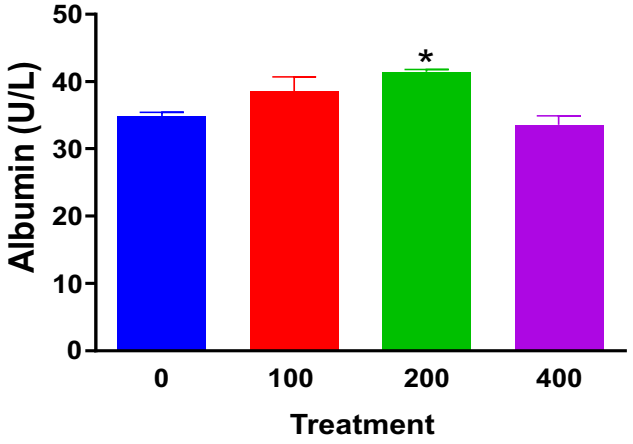


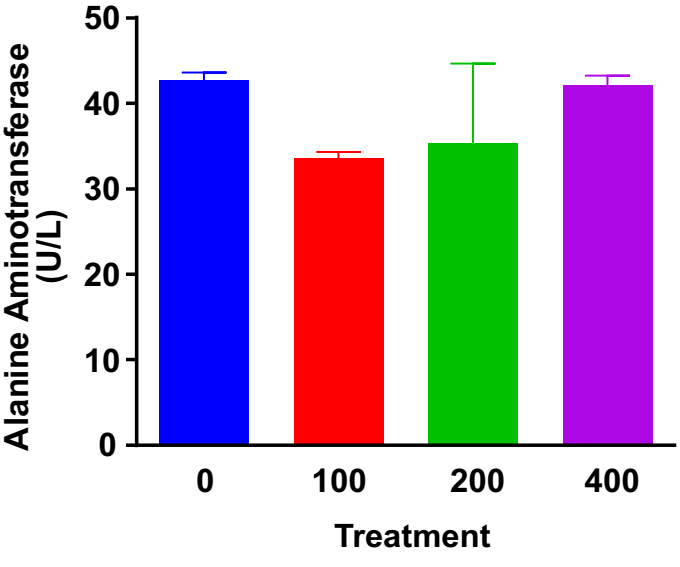
Figure 2: Effect of 90 days of oral administration of *Relaxing Tea* extract on creatinine and urea in rats. Each bar represents Mean ± SEM (n=5). *p<0.05, **p<0.01 vs control using one-way ANOVA followed by Turkey post hoc multiple comparison test.

Liver function test

Long-term (90 days) oral administration of *Relaxing Tea* at 200 mg/kg resulted in a significant increase (p<0.05) in serum Aspartate Transaminase (AST) and Albumin levels when compared to the control group. However, there were no significant differences in the levels of Bilirubin and Alanine Aminotransferase (ALT) when *Relaxing Tea* treated animals were compared to the control.







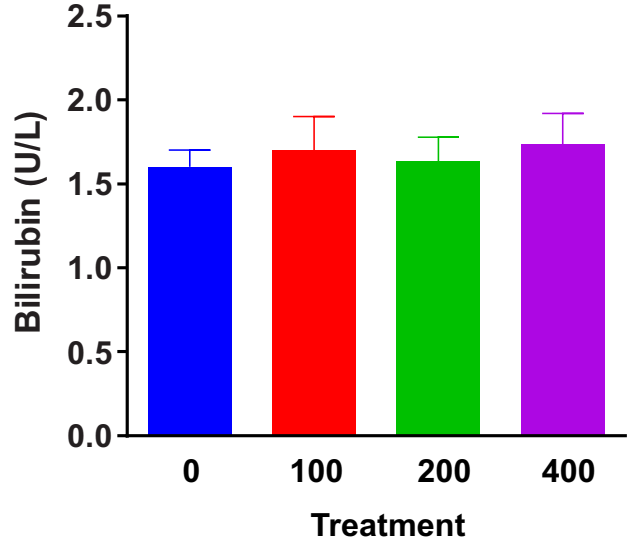


Figure 3: Effects of 90 days of oral administration of *Relaxing Tea* extract on Aspartate Transaminase, Albumin, Bilirubin, and Alanine Aminotransferase (ALT) in rats. Each bar represents Mean ± SEM (n=5). *p<0.05 vs control using one-way ANOVA followed by Turkey post hoc multiple comparison test.

Haematological assessment

As shown in Table 3, various doses of RT produced no significant difference in the haematology parameters when compared with the control. However, there was a significant, fifty-per cent (p<0.05) reduction in platelet level in rats administered 400 mg/kg RT for 90 days.

Table 3: Effects of 90 days of oral administration of *Relaxing Tea* on hematological parameters in rats

Parameters	Normal Saline 10 ml/kg (Control)	Treatment		
		RT 100 mg/kg	RT 200 mg/kg	RT 400 mg/kg
WBC (10^9/L)	15.40 ± 5.19	9.80 ± 1.66	7.10 ± 0.79	9.00 ±1.60
HGB (g/L)	14.43 ± 0.72	14.5 ± 0.32	13.93 ± 2.14	14.00 ± 1.25
RBC (10^{12}/L)	10.01 ± 2.56	9.53 ± 2.23	6.90 ± 1.01	7.18 ± 0.59
HTC (%)	45.17 ± 2.24	43.00 ± 0.70	41.07 ± 6.65	43.37 ± 4.12
PLT (10^9/L)	1061.00 ± 218.00	761.70 ± 48.31	575.30 ± 15.98	543.30 ± 124.40*

Table summarizes the effect of 90 days of oral administration of *Relaxing Tea* (RT) on haematological parameters in rats. Values are expressed as Mean ± SEM (n=5). *represents p<0.05 vs control using one-way ANOVA followed by Turkey post hoc multiple comparison test. WBC is White blood cells; HGB is Hemoglobin; RBC is Red blood cells; HTC is Hematocrit; PLT is Platelets.

Serum antioxidants

As shown in Table 4, various doses of *Relaxing Tea* produced no significant differences in serum antioxidant parameters when compared with the control.

Table 4: Effects of 90 days administration of *Relaxing Tea* on serum antioxidant parameters in rats

Treatment	Serum Antioxidant Parameters			
	GSH (µ/mg)	SOD (µ/mg)	CAT (µ/mg)	MDA (µ/mg)
Normal Saline 10 ml/kg	0.17 ± 0.01	1.40 ± 0.16	11.33 ± 1.56	0.01 ± 0.01
RT 100 mg/kg	0.15 ± 0.03	1.23 ± 0.08	8.38 ± 0.38	0.02 ± 0.00
RT 200 mg/kg	0.09 ± 0.03	1.93 ± 0.29	15.24 ± 4.25	0.01 ± 0.01
RT 400 mg/kg	0.13 ± 0.03	1.90 ± 0.01	14.46 ± 0.46	0.02 ± 0.01

Table 4 summarises the effect of 90 days of oral administration of *Relaxing Tea* (RT) on serum antioxidant parameters in rats. Values are expressed as Mean ± SEM (n=5). GSH is Glutathione; SOD is Superoxide dismutase; CAT is Catalase; MDA is Malondialdehyde.

Liver antioxidant parameters

Oral administration of *Relaxing Tea* for 90 days resulted in no significant differences in liver antioxidant parameters compared to the control.

Table 5: Effects of 90 days administration of *Relaxing Tea* on liver antioxidant parameters in rats

Treatment	Liver Antioxidant Parameters			
	GSH (µ/mg)	SOD (µ/mg)	CAT (µ/mg)	MDA (µ/mg)
Normal Saline 10 ml/kg	0.90 ± 0.17	6.64 ± 0.97	33.85 ± 7.19	0.12 ± 0.02
RT 100 mg/kg	0.60 ± 0.07	4.16 ± 0.07	22.52 ± 0.54	0.07 ± 0.03
RT 200 mg/kg	0.49 ± 0.04	5.54 ±1.06	32.70 ± 0.49	0.12 ± 0.03
RT 400 mg/kg	0.80 ± 0.01	5.53 ± 0.16	31.99 ± 6.28	0.13 ± 0.01

Table 5 summarizes the effect of 90 days of oral administration of *Relaxing Tea* (RT) on serum antioxidant parameters in rats. Values are expressed as Mean ± SEM (n=5). GSH is Glutathione; SOD is Superoxide dismutase; CAT is Catalase; MDA is Malondialdehyde.

Kidney antioxidant parameters

Oral administration of RT for 90 days resulted in significant ($p < 0.05$) decreases in the kidney levels of catalase (CAT) and glutathione compared to the control group. However, no significant differences were observed in the levels of kidney superoxide dismutase (SOD) and malondialdehyde (MDA) following oral administration of RT compared with the control.

Table 6: Effects of 90 days administration of *Relaxing Tea* on kidney antioxidant parameters in rats

Treatment	Kidney Antioxidant Parameters			
	GSH (µ/mg)	SOD (µ/mg)	CAT (µ/mg)	MDA (µ/mg)
Normal Saline 10 ml/kg	0.96 ± 0.03	9.21 ± 1.33	62.96 ± 6.44	0.06 ± 0.04
RT 100 mg/kg	0.19 ± 0.05*	4.41 ± 0.10	31.01 ± 2.39*	0.18 ± 0.08
RT 200 mg/kg	0.55 ± 0.23	4.54 ± 0.85	16.82 ± 3.69**	0.16 ± 0.01
RT 400 mg/kg	0.72 ± 0.07	5.08 ± 1.63	32.24 ± 3.10*	0.09 ± 0.01

Table 6 summarizes the effect of 90 days of oral administration of *Relaxing Tea* (RT) on serum antioxidant parameters in rats. Values are expressed as Mean ± SEM (n=5). *p<0.05, **p<0.01 vs control using one-way ANOVA followed by Turkey post hoc multiple comparison test. GSH is Glutathione; SOD is Superoxide dismutase; CAT is Catalase; MDA is Malondialdehyde.

Lipid profile

Oral administration of RT at 200 mg/kg for 90 days resulted in a significant ($p < 0.05$) increase in triglyceride levels compared to the control group. This effect was absent in the groups administered 100 and 400 mg/kg. No significant differences in the levels of other lipid profile parameters, including High-Density Lipoprotein and cholesterol, were observed following long-term oral administration of RT in rats.

Table 7: Effects of 90 days administration of *Relaxing Tea* on lipid parameters in rats

Treatment	Lipid Parameters		
	Triglyceride (mmol/L)	Cholesterol (mmol/L)	HDL (mmol/L)
Normal Saline 10 ml/kg	0.67 ± 0.04	1.83 ± 0.20	1.47 ± 0.09
RT 100 mg/kg	0.85 ± 0.10$^{\alpha}$	1.66 ± 0.07	1.53 ± 0.05
RT 200 mg/kg	1.48 ± 0.31*	1.79 ± 0.11	1.39 ± 0.13
RT 400 mg/kg	0.67 ± 0.07$^{\alpha}$	1.83 ± 0.19	1.27 ± 0.22

Table 7 summarises the effect of 90 days of oral administration of *Relaxing Tea* (RT) on lipid parameters in rats. Values are expressed as Mean ± SEM (n=5). *p<0.05 vs control, ?p<0.05 vs RT 200 mg/kg using one-way ANOVA followed by Turkey post hoc multiple comparison test. HDL is a High-Density Lipoprotein.

Semen analysis

Oral administration of RT at 100 and 200 mg/kg resulted in significant increases in percentage abnormality (two- and four-fold increases, respectively) compared to the control group. However, this effect was absent at a dose of 400 mg/kg. Additionally, RT at 200 mg/kg resulted in a significant decrease in sperm count compared to the control. However, there was no significant difference in percentage motility at any doses of RT tested when compared with the control.

Table 8: Effects of 90 days administration of *Relaxing Tea* on semen parameters in rats

Treatments	Semen Parameters		
	Motility (%)	Abnormality (%)	Sperm count (10^6sp/ml)
Normal Saline 10 ml/kg	95.00 ± 3.00	11.00 ± 2.00	76.50 ± 0.50
RT 100 mg/kg	96.00 ± 1.00	22.00 ± 2.00*	73.00 ± 1.00
RT 200 mg/kg	86.00 ± 1.00	44.00 ± 1.00***	60.50 ± 1.50**
RT 400 mg/kg	97.50 ± 2.50	7.50 ± 1.50	79.50 ± 0.50

Table 8 summarizes the effect of 90 days of oral administration of *Relaxing Tea* (RT) on lipid parameters in rats. Values are expressed as Mean ± SEM (n=5). *p<0.05, **p<0.01 ***p<0.001 vs control using one-way ANOVA followed by Turkey post hoc multiple comparison test.

Histology

As shown in Plate 1, the meninges of the control (A) are intact with no inflammation. However, oedema/inflammation and gliosis were seen in the treated rats [100mg/kg (B), 200mg/kg (C), and 400mg/kg (D)], ranging from mild to moderate oedema. On examination, the glomeruli and tubules of the kidneys of all the groups were shown to be normal, with no oedema, congestion or haemorrhage (Plate 2).

Plate 3 shows sections of the liver from control (A) and RT-treated rats [100mg/kg (B), 200mg/kg (C), and 400mg/kg

(D)]. Congestion of the parenchyma is observed in treated animals, ranging from mild to severe, with an increasing dose of the treatment. As shown in Plate 4, the seminiferous tubules in the control group are regular and uniformly spaced. In contrast, those in the treated rats are regular but widely spaced (due to oedema), a phenomenon that increases with increasing doses.

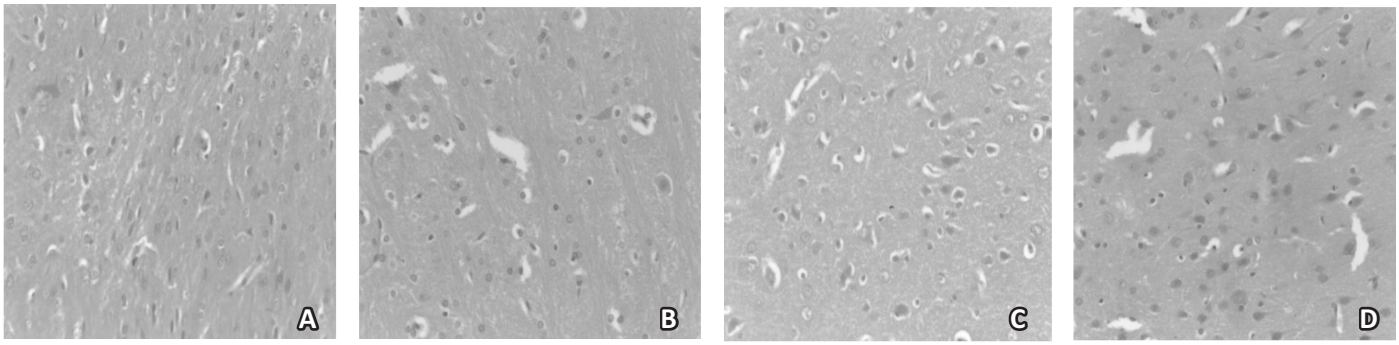


Plate 1: Micrographs of the brain sections obtained from rats untreated and rats treated with various doses of RT. Control (A); RT treated rats [100mg/kg (B), 200mg/kg (C), and 400mg/kg (D)] Magnification 400×.

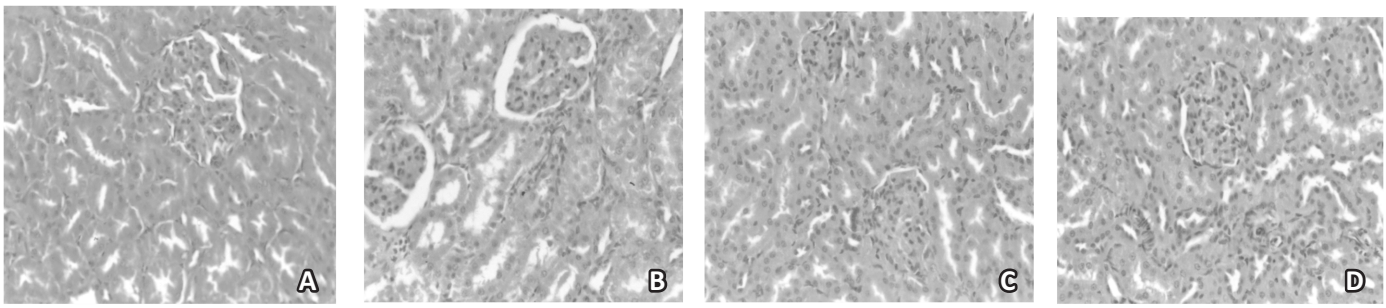


Plate 2: Micrographs of the kidney sections obtained from rats untreated and rats treated with various doses of RT. Control (A); RT treated rats [100mg/kg (B), 200mg/kg (C), and 400mg/kg (D)]-magnification 400×.

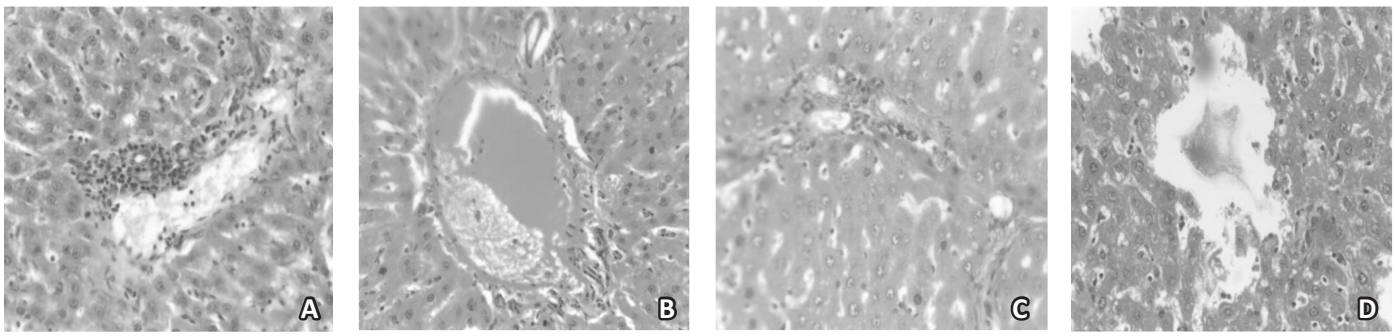


Plate 3: Micrographs of the liver sections obtained from rats untreated and rats treated with various doses of RT. Control (A); RT treated rats [100mg/kg (B), 200mg/kg (C), and 400mg/kg (D)]-magnification 400×.

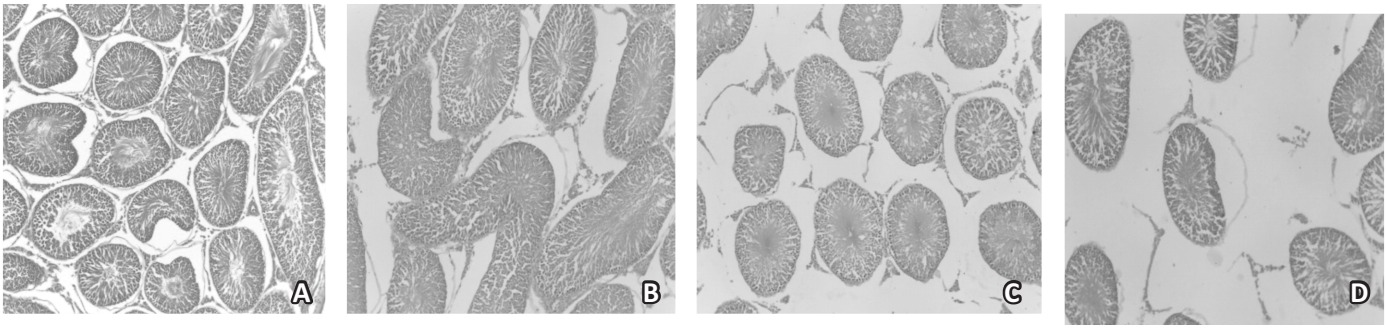


Plate 4: Micrographs of the testis sections obtained from rats untreated and rats treated with various doses of RT. Control (A); RT treated rats [100mg/kg (B), 200mg/kg (C), and 400mg/kg (D)]. Magnification 100×

DISCUSSION

Although data on insomnia prevalence in sub-Saharan Africa is limited, emerging evidence indicates that sleep disorders may be frequently overlooked and underestimated in this area.[25] Insomnia, characterised by challenges with falling or staying asleep or experiencing non-restorative sleep, can severely affect physical health, cognitive abilities, and overall well-being. In sub-Saharan Africa, contributing factors may include socioeconomic pressures, urbanization, mental health issues, substance use, and restricted access to healthcare services. Additionally, cultural attitudes towards sleep and mental health, along with the scarcity of specialized diagnostic tools and trained professionals, further exacerbate the underreporting of insomnia.[26] In Nigeria, data reveals a notably high incidence of insomnia, with prevalence rates reaching up to 31 % among the population. This indicates that sleep disorders are a significant yet frequently neglected public health issue in the country,[27] alongside a high chronicity of insomnia in Nigeria's elderly demographic.[28] Furthermore, multiple studies reveal that insomnia is linked to various clinical morbidities, highlighting its importance as a public health concern. Chronic insomnia has been associated with an elevated risk of cardiovascular diseases, hypertension, type 2 diabetes, obesity, and metabolic syndrome. It also plays a significant role in the development and worsening of mental health disorders, especially depression and anxiety, establishing a bidirectional relationship that complicates treatment outcomes. Additionally, ongoing sleep disturbances can hinder cognitive functioning, weaken immune response, and worsen chronic pain conditions. Over the long term, untreated insomnia is linked with diminished quality of life, increased healthcare use, and higher risks of occupational accidents and motor vehicle incidents accidents,[29,30] and to improve sleep quality and quantity, insomniacs tend to embark on self-management including the use of traditional herbal remedies.[30]

Relaxing Tea, a blend of *Matricaria chamomilla* (German Chamomile) leaves and flowers and Hypericum perforatum (St. John's wort) leaves, is widely used in Nigeria to manage insomnia. Chamomile is listed on the FDA's GRAS (Generally Recognised as Safe) list,[13] with the primary concern regarding its use being a relatively low percentage of people who are sensitive to chamomile and develop allergic reactions.[31] In terms of safety, *St. John's Wort* (*Hypericum perforatum*) is generally well-tolerated when used at recommended doses and for short to moderate durations. Most individuals experience minimal side effects, which are typically mild and may include gastrointestinal discomfort, dizziness, dry mouth, or photosensitivity reactions. However, a primary concern with the use of H. perforatum lies in its potential for clinically significant herb-drug interactions.[32,33] However, other studies have raised concerns about the potential toxicological effects of *St. John's Wort* (*Hypericum perforatum*), particularly in animal models. Preclinical studies indicate that using H. perforatum might cause liver and kidney damage, particularly with long-term use or high doses. These adverse effects have been noted in both adult and nursing animals, representing a potential risk for breastfeeding young due to the transfer of its bioactive components. Moreover, *H. perforatum* has been linked to a decline in the antioxidant defence system within liver and kidney tissues, which may heighten the risk of oxidative stress and organ damage. In addition to liver and kidney toxicity, some studies also show signs of reproductive toxicity, such as changes in sperm quality, lower fertility rates, and hormonal disruptions in animal models.[34,35] Moreover, there are well-established drug interactions associated with *St. John's Wort*, primarily resulting from its effect on the cytochrome P450 enzyme system, specifically CYP3A4, CYP2C9, and CYP2C19, as well as drug transporters such as *P-glycoprotein.* Such interactions can profoundly affect the absorption, distribution, metabolism, and excretion of concurrently administered medications. By stimulating these enzymes and transporters, *H. perforatum* can decrease plasma levels of numerous drugs, including immunosuppressants, antiretrovirals, anticoagulants, anticonvulsants, and oral contraceptives, which may lead to their ineffectiveness. Conversely, it can also hinder drug clearance, raising the potential for toxicity. Therefore, the simultaneous use of *St. John's Wort* with standard medications can either heighten or diminish the risk of adverse reactions or therapeutic failure, contingent on the particular medication involved. These interactions emphasise the essential requirement for healthcare professionals to evaluate the use of herbal supplements in clinical settings, especially for patients undergoing chronic or intricate medication therapies.[36,37] These reports particularly necessitate the toxicity evaluation of *Relaxing Tea*, which is undertaken in this study.

At present, the recommended classification of acute systemic toxicity based on oral LD_{50} values by the Organisation for Economic Co-operation and Development (OECD) puts values ≥ 2000 mg/kg as safe.[38] Our findings suggest that *Relaxing Tea* is safe for acute

oral use, as we observed no signs of toxicity, mortality, or significant physiological changes in the test subjects throughout the study.

Body weight is a fundamental parameter routinely monitored in long-term toxicity studies and serves as a sensitive indicator of systemic toxicity. Changes in body weight can reflect alterations in metabolic function, appetite, organ function, and overall health status, making it a valuable non-invasive endpoint in toxicological evaluations. Consistent weight loss or failure to gain weight during a study period may suggest adverse physiological effects or organ dysfunction induced by the test substance.[39,40] A reduction in weight or suppression of weight gain is an indicator of toxicity.[41] Our findings reveal no notable differences in body weight between the control and treated groups during the study period, suggesting that administering the test substance did not negatively impact the overall growth or metabolic status of the rats. Prolonged oral treatment, however, resulted in significantly elevated serum levels of creatinine and urea compared to the control group. These biochemical alterations suggest a potential nephrotoxic effect associated with chronic exposure to *Relaxing Tea*, consistent with findings reported in previous studies.[34,42] The increase in these renal function markers may indicate impaired glomerular filtration and reduced kidney clearance capacity; therefore, caution should be exercised when using this medication long-term in patients with renal conditions. A notable decrease in renal glutathione and catalase levels was also noted, aligning with findings from Gregoretti.[34] This decline in essential antioxidant enzymes suggests that chronic use of *Relaxing Tea* may lead to increased free radicals and oxidative stress in the kidneys. Such an oxidative imbalance could result in cellular damage, offering a likely explanation for the significant rises in serum urea and creatinine observed in the treated animals. These findings emphasise the potential nephrotoxic effects of extended *Relaxing Tea* exposure and highlight the need for further mechanistic studies to clarify the oxidative pathways involved.

The liver plays a crucial role in metabolising and detoxifying xenobiotics, which include most pharmaceutical and herbal substances. Consequently, changes in hepatic enzyme levels are viewed as reliable signs of drug-induced toxicity and general liver health. Hepatotoxicity, or liver damage, is often characterised by elevated serum levels of liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). These enzymes leak into the bloodstream due to hepatocellular injury or disturbances in membrane permeability. Additionally, liver dysfunction may manifest as lowered serum albumin levels, indicating compromised hepatic synthetic capability.[43] The finding that *Relaxing Tea* significantly altered hepatic enzyme markers, specifically increasing AST and serum albumin levels, suggests a possible hepatotoxic effect associated with long-term exposure. These alterations may indicate hepatocellular damage and reduced synthetic function, underscoring the need for caution when using *Relaxing Tea* for extended periods.

Congestive hepatopathy, marked by vascular congestion in the liver and potentially leading to fibrosis or cirrhosis,44 was seen in the liver histological sections of rats administered *Relaxing Tea*. This pathological observation indicates that prolonged exposure to the formulation could hinder hepatic blood flow, resulting in structural liver damage and supporting biochemical evidence of hepatotoxicity.

This study found that prolonged exposure to *Relaxing Tea* resulted in a significant increase in the percentage of abnormal sperm morphology and a notable decrease in sperm count. These changes in sperm quality indicate impaired spermatogenesis. Additionally, histological analysis of the testicular tissues from the treated rats revealed extensively dilated seminiferous tubules, indicating degenerative changes and structural disruption in the spermatogenic epithelium. Such morphological abnormalities strongly indicate reproductive toxicity, which could negatively impact male fertility. These results align with earlier reports 45 and underscore the need for caution when using *Relaxing Tea*, particularly in individuals of reproductive age. Although it remains unclear whether the male reproductive toxicity observed in this study is solely attributable to the effects of *St. John's Wort*, as previously documented,[35] or possibly due to a synergistic interaction with Chamomile, the findings highlight a potential reproductive risk associated with the prolonged use of *Relaxing Tea*. These results warrant caution in the long-term use of this polyherbal formulation, particularly among men of reproductive age, and emphasise the need for further mechanistic and toxicological studies to clarify the roles of individual components and their combined effects.

CONCLUSION

Based on our observations in animal models, *Relaxing Tea*, a herbal mixture of Matricaria chamomilla (leaves and flowers) and Hypericum perforatum (leaves), may not be considered safe following long-term exposure and should be avoided in patients with renal insufficiency or other kidney diseases. Also, long-term exposure in men should be avoided as there were observed structural and possibly functional toxicity risks to the testes. As the components of this mixture are commonly employed in traditional remedies and commercial natural products, further studies on the underlying mechanisms of these toxicities are encouraged.

ETHICAL APPROVAL

The researchers acquired ethical clearance from the College of Medicine of the University of Lagos, Health Research Ethics Committee (CMUL/HREC), to use animals for this investigation. The project was approved, and authorization was granted .

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