REPRODUCTIVE TOXICITY OF *HIBISCUS SABDARIFFA* IN WISTAR RATS

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Abstract: Natural substances in food are been encouraged as drug substitutes; therefore, given that drugs are potential poisons, this study examined the toxicity of *H. sabdariffa* on the reproductive ability of Wistar rats. Using standard laboratory procedures, *H. sabdariffa* extract was administered on thirty-two matured rats. Male rats were sacrificed (by cervical dislocation) for sperm motility and morphology examination while body weight and pup's morphology were observed for the female. Data were analysed using Descriptive and Chi-square statistics; Analysis of Variance and Ordinary Least Squares regression. Male rats placed on 4 g/kg dose of extract for 14 days had the highest percentage of total sperm deformity (55.3%). Pups from control female rats had the highest average weight [1 day (6.7 g), 3 days (10.1 g), 6 days (14.8 g) and 9 days (16.6 g) after birth] compared to the extract dosed rats. Extract administration had significant effect on rats' spermatozoa mutilation ($p<0.05$). There was insignificant difference in pup weight for the dosed groups while litter size had significant effect on pup weight ($p<0.01$). Day of weighing had significant influence on pups' weight ($p<0.01$). Thus, *H. sabdariffa* extract has a boosting effect on male sex cells with probable increase in deformity. However, while it can be taken in pregnancy without any deleterious effect, it has no effect on infant weight at birth.

Keywords: Ddrug, *Hibiscus sabdariffa* L, pup, rats, spermatozoa, toxicity.

Introduction

Use of herbs is probably a natural progression from dietary to health benefits and through the centuries, herbs are commonly used because of availability and accessibility, formulation simplicity, cheapness and ease of usage. Active advent of synthetic chemistry around the mid-20th century shifted interest from plant-based ingredients to synthetic ingredients (Mathur and Hoskins, 2017; Najmi et al., 2022). The global trend in scientific approaches in research into medicinal plants used in various traditional systems revealed the potentials of medicinal plants in the area of pharmacology (Kayode et al., 2022; Temitope et al., 2017; Imieje et al., 2017a; Oseghale et al., 2017; Mangla and Kohli, 2018). This became pertinent because of attendant challenges confronting orthodox medicine such as drug resistance and adverse toxic effects (Sallah et al., 2002; Suboh, 2004; Tse et al., 2019). Some of the medicinal plants used for ages on which modern medicine focus include *U. lobata* L., *O. gratissimum*, *Z. piperitum* DC, *H. indicus* R. Br, *T. trilobatum*, *Dendrobium spp* and *H. sabdariffa* L (Abat et al., 2017; Abubakar et al., 2019; Verma et al., 2021; Wang et al., 2021).

Hibiscus sabdariffa L. (*roselle*) is an annual herb widely known with different identities and utilization. Its extracts from the calyx, seed and root are used in folk medicine as therapy for high blood pressure, liver diseases and fever (Abubakar et al., 2019; Riaz and Chopra, 2018). This is because the extract is reportedly aphrodisiac, antiseptic, choleretic, aperitive, diuretic, emollient, febrifugal, hypotensive, purgative and sedative (Du and Francis, 1973; Duke, 1983; Owulade, 2004; Tseng, 1996; Thien et al, 2019; Wang et al., 2000). The thick, red and fleshy, cup-shaped calyces of the flower are consumed globally as food in many forms like cold beverages and jelly (Akindahunsi and Olaleye, 2003; Keena, 2003; Majiya and Galstyan, constituents were later extracted from herbs and used for drugs preparation; for example, heroine from morphine. This became the foundation of orthodox medicine in the 19th century (Mathur and Hoskins, 2017; Najmi et al., 2022). However, the 2020; Morton, 1987; Obouayeba et al., 2016). However, increased attention is being given to plant toxicity apart from use for traditional medicine (Keena, 2003; Ojulari et al., 2019; Sireeratawong et al., 2013).

Prescot (1987) and Sjoqvist et al. (1987) stated that toxicity is the ability of a chemical agent to cause injury; i.e. the inherent potential of a substance to cause systemic damage to living organisms (Walter et al., 2002). Toxicity may have short term (acute) effects or long term (chronic) effects (Saganuwan, 2017). Extracts of some plant (including *roselle*) are allegedly characterized by a very low degree of toxicity (Abat et al., 2017; Badreldin et al., 2005; Kolawole and Maduenyi, 2004; Morton, 1987; Dollah et al., 2021; Onyenekwe et al., 1999; Orisakwe et al., 2003; Imieje et al., 2017b; Nishitha et al., 2018; Evbakhavbokun et al., 2020; Bose et al., 2021; Tajbakhsh et al., 2021; Tandon and Yadav, 2019; Mendonça et al., 2020; Lu et al., 2021). However, others extracts may not be characterized by low toxicity (Kolawole et al., 2021; Ogbiko et al., 2017; Yakubu et al., 2020; Mayanti et al., 2020; Zhao et al., 2020). Hence, this study examined the toxicity of *H. sabdariffa* on the reproductive ability of rats.

Materials and Methods

Dried, powdered, calyx of *H. sabdariffa* (560 g) was extracted with 2 litres of 96% ethanol for 24 hours by conventional Soxhlet extraction (solvent extraction). The extraction was repeated three times and the filtrate was concentrated using rotary evaporator. The (*H. sabdariffa*) extract (5g) was dissolved in (1ml) of water and

administered orally. The calyces of *Hibiscus sabdariffa* L was collected from the university of Ibadan botanical garden in June, 2005, with the identification number UIH – 23181. The calyces were properly dried under regulated room temperature before the extraction. The procedure of Hussaini et al. (2004) was employed in the determination of extract concentration administered to the experimental animals.

[a]Maturity as exclusion criterion, thirty-two (32) matured (male and female) Wistar rats weighing 145 g - 210 g were procured from the animal house of the Faculty of Basic Medical Sciences, University of Ibadan (UI), Ibadan, Nigeria. The animals were acclimated to housing conditions for two (2) weeks before the commencement of the experiment. The animals were maintained, in the animal house of the Department of Clinical Pharmacy, UI, under standard laboratory conditions with free access to feed and water. The animals were kept in the animal house of the Department of Clinical Pharmacy, Faculty of Pharmacy, UI. Feed (standard rat pellets) was obtained from the Department of Biochemistry, UI and drinking water was provided *ad libitum*. Standard and ethical guide for the care and use of laboratory animals established by the UI was strictly followed throughout the experiment in handling the rats. The procedure of Chattopadhyay et al. (2003) was adopted in grouping the experimental animals into weight-matched categories. All these measures (from procurement to weight-matched categories) were steps taken to minimize potential confounders.

The weight-matched animals were divided into three dose-groups[b], which are 2 g/kg (low dose), 4 g/kg (high dose) and 0 g/kg (control). The male extract dose groups were divided further into two period sub-groups (that is, 7 and 14 days of extract administration). There were four (4) rats each in five (5) sub-groups for male rats (4 sub-groups and a control group) and four (4) rats each in three (3) dose groups for female rats. This makes a total of thirty-two (32) rats handled under a completely randomized experimental design.

The physical appearances and activities of the rats were observed daily. All the male animals were sacrificed by cervical dislocation and samples of sperm cells were taken through maceration of the epididymis, which were stained with eosine to study the sperm motility as well as the morphology of the sperm cells under the microscope. The samples were stored in 5ml of 0.9% NaCl under refrigeration. However, for the female animals, the body weight and the (physical) morphology of the pups were determined and observed after delivery. The pups were weighed at three (3) days interval for nine days and the pups' activities such as agility were examined for nine days. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed, and all procedures performed in studies involving animals were in accordance with strict adherence to the University of Ibadan Ethical Policy on the use of laboratory animals. In handling the rats, when this experiment was conducted, the University of Ibadan's Standard Guide and the ethics for the care and use of laboratory animals were strictly followed throughout the experiment.

Descriptive statistics including frequency and percentage were used to summarize the data while Chi-square statistic, analysis of variance (ANOVA) and ordinary least squares (OLS) regression techniques was adopted in evaluating categorical and quantitative variables, with an alpha level (α) of 0.05 ($p \leq 0.05$) statistical significance, and making inferences for the study. Statistical Package for Social Sciences (SPSS) version 16.0 and STATA software were used for the management of sorted and coded data as well as in estimating the inferential analytical tools (i.e. ANOVA and OLS). Each of the analytical tool was computed following Bamgboye (2002).

A. Percentages

This was obtained using the formula below:

$\rho = [n_i(N^{-1})]100$ ---------- (ii)

where:- ρ = percentage;
n_i = i^{th} observation;
N = total observation.

B. Chi-square

$\chi^2 = \sum(O_i - E_i)^2 (E_i)^{-1}$ ---------- (iii)

where:- χ^2 = Chi-square statistic;
$\sum$ = sum of;
O_i = observed frequencies;
E_i = expected frequencies.

C. Analysis of Variance (ANOVA)

The ANOVA model used is based on the following theorem:

First theorem:-

$\sum_{i=j}^{k}\sum_{j=i}^{n}(X_{ij} - x)^2 = \sum_{i=j}^{k}\sum_{j=i}^{n}(X_{ij} - X_j)^2 + n\left[\sum_{j=1}^{k}(X_{ij} - x)^2\right]$ ---------- (iv)

$X_i = \frac{1}{n}\sum_{j=1}^{n} X_{ij}$;
X_{ij} = i^{th} pup weight.

Second theorem:-

If T_l is total of the pup weight for the j^{th} treatment and T is the ground total of all pups weight;

$SST = \sum_{i=1}^{k}\sum_{j=1}^{n} X_{ij}^2 - \frac{1}{kn}(T^2)$ -------- (v)

$SST_r = \frac{1}{n}\sum_{i=1}^{r} T_i^2 - \frac{1}{kn}(T^2)$ ----- (vi)

SSE is then found by the formula:-

$SSE = SST - SST_r$ --------- (vii)

where:- SSE = sum of square error;
SST = sum of square total;
SST_r = sum of square treatment.

D. Ordinary Least Squares Regression Analysis

The model used is as specified hereunder:-

$Y_i = \alpha_0 + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 D_1 + \alpha_4 D_2 + \alpha_5 D_3 + \mu_i$ …..(viii)

where:
Y_i = weight of the i^{th} pup;

[a] Rats become sexually matured at about the sixth week of growth and mating recommended to be from 7 - 10 weeks with body weight range of 122±8g to 321±22g (Masiello et al., 1979 & Finegood et al., 1995 corroborated by Ghasemi et al., 2021; NCKU, 2022; Sengupta, 2013). Sample size was determined following Mead (1988) & Festin & Altman (2002).

[b] This was adopted as a dose expected to produce evident toxicity following OECD (1983, 2001a, 2001b, 2001c, 2008a & 2008b) and Hussaini et al. (2004); corroborated by Aydin et al. (2016).

α_0 = weight of the i^{th} pup in the absence of variables influencing pup weight;
α_1 ---- α_5 = parameter estimates;
X_1 = dose given to sows (g/kg);
X_2 = number of days at which pup weight was obtained;
D_1 = low sized litters (D = 1 if litter size of ≤5, 0 otherwise);
D_2 = medium sized litters (D = 1 if litter size equals 6, D = 0 if otherwise);
D_3 = large sized litters (D = 1 if litter size equals ≥7, 0 otherwise);
μ_i = stochastic random term.

Results and Discussion

Extract may have a boosting effect on sperm count since control rats had the least sperm count while the sperm count for 2 g/kg rats was higher than that of the control and the sperm count for 4 g/kg rats was higher than that of the first two (2) rat categories. Rats that received 4 g/kg of extract for 14 days had the highest percentage of total sperm deformity (55.3%) than the other categories of male rats. Also, male rats that received 4 g/kg of extract for 14 days had the highest percentage of short-headed spermatozoa than other categories of male rats. However, the control rats that received only distilled water had the highest percentage of rudimentary tailed spermatozoa as well as detach tailed spermatozoa. Male rats that received 2 g/kg of extract for 14 days were the only category of males that recorded case of twin headed spermatozoa and curve tailed spermatozoa. Furthermore, rats that received 2 g/kg of extract for 7 days had the highest percentage of bent tailed spermatozoa and loop tailed spermatozoa than other categories of male rats. Chi-square statistic (60.42, $p<0.05$) revealed that extract administration had significant effect on rats' spermatozoa mutilation. Details are presented in Table 1 and Table 2.

In terms of motility of spermatozoa, male rats that received 2 g/kg for 14 days had inactive spermatozoa with slow motility (+50) which is comparable to that of the control animals (+50). The spermatozoa had clustered arrangement while the control is sparsely arranged. Male rats that received 4 g/kg 14 days had spermatozoa with extremely slow motility (+40) which is low when compared with the control animals (+50). The spermatozoa also had clustered arrangement while the control is sparsely arranged. Male rats that received 4 g/kg 7 days had very active spermatozoa with high motility (+70) in comparison to the control animals (+50).

Table 1: Distribution of Deformity in Male Rats by Total Sperm Count

Dose	*Short Head*	*Rudimentary Tail*	*Detached Tail*	*Twin Head*	*Curved Tail*	*Bent Tail*	*Loop Tail*	*Total Deformed Sperm*	*Total Sperm count*
2 g/kg (7days)	26 (18.4)	2 (1.4)	29 (20.6)	0 (0.0)	0 (0.0)	7 (5.0)	3 (2.1)	67 (47.5)	141
4 g/kg (7days)	90 (23.7)	1 (0.3)	40 (10.5)	1 (0.3)	1 (0.3)	8 (2.1)	3 (0.8)	144 (37.9)	380
2 g/kg (14days)	60 (17.1)	0 (0.0)	40 (11.4)	0 (0.0)	0 (0.0)	9 (2.6)	2 (0.6)	111 (31.7)	350
4 g/kg (14days)	170 (36.2)	1 (0.2)	75 (15.9)	0 (0.0)	0 (0.0)	10 (2.1)	4 (0.9)	260 (55.3)	470
0 g/kg (Control)	14 (11.5)	3 (2.5)	30 (24.6)	0 (0.0)	0 (0.0)	4 (3.3)	1 (0.8)	52 (42.6)	122
χ^2	60.42[i]	36.40[ii]	0.05[iii]	-	-	-	-	-	-

NB: values in parenthesis are percentages; i → Chi-square calculated; ii → Chi-square tabulated; iii → Probability level

(Alpha-levels

Table 2: Deformity in Male Rats Sperm Count

Dose	*[y]Short Head*	*[y]Rudimentary Tail*	*[y]Detached Tail*	*[y]Twin Head*	*[x]Curved Tail*	*[y]Bent Tail*	*[y]Loop Tail*	*Total Deformed Sperm*	*[x]Mean Deformity*
2 g/kg (7days)	26 (38.8)	2 (3.0)	29 (43.3)	0 (0.0)	0 (0.0)	7 (10.4)	3 (4.5)	67	10 (12.5)
4 g/kg (7days)	90 (62.5)	1 (0.7)	40 (27.8)	1 (0.7)	1 (0.7)	8 (5.6)	3 (2.1)	144	21 (33.7)
2 g/kg (14days)	60 (54.1)	0 (0.0)	40 (36.0)	0 (0.0)	0 (0.0)	9 (8.1)	2 (1.8)	111	16 (24.2[y])
4 g/kg (14days)	170 (65.4)	1 (0.4)	75 (28.8)	0 (0.0)	0 (0.0)	10 (3.8)	4 (1.5)	260	37.1 (64.5)
0 g/kg (Control)	14 (26.9)	3 (5.8)	30 (57.7)	0 (0.0)	0 (0.0)	4 (7.7)	1 (1.9)	52	7.4 (11.1)

NB: x → values in parenthesis are percentages; y → values in parenthesis are standard deviations

Male rats that received 2 g/kg 7 days had active spermatozoa with motility (of +60) which was higher than that of the control animals (+50) but lower than that of 4 g/kg 7 days. The spermatozoa of the extract-dose rats is arranged in partial cluster while that of the control rats is sparsely arranged.

Male rats treated with 4 g/kg of extract for 14 days may be presented with short-headed spermatozoa. However, the extract may not have any significant effect on the male rats' spermatozoa having rudimentary or detached tail since the control had the highest percentage of spermatozoa having these defects. Secondly, the extract may not cause twin headed or curve tailed spermatozoa since only 0.26% had these defects and, more so, only the group treated for 7 days with 4 g/kg of extract had the defects. Furthermore, the extract at 2 g/kg for 7days had the highest proportion of loop and bent tailed spermatozoa. However, Chi-square statistic affirmed that extract administration had significant effect on rats' spermatozoa mutilation.

Generally, it is obvious that male rats that received 4 g/kg of the extract for 14 days had the highest sperm count than other categories of males. However, the longer the duration of dose administration, the more the deviations of the deformed spermatozoa from the mean value. The same pattern was also observed with increase in dose level. Male rats that received 4 g/kg extract for 14 days had the highest percentage of sperm deformity as well as the highest sperm count and extract was found to be a significant factor in observable mutilated spermatozoa. This means that extract can lead to high sperm mutilation. The significant effect of extract administration on rats' spermatozoa mutilation implies that extract is inimical to the health of spermatozoa. However, this can be attributed to the fact that extract boost sperm count, hence the more the sperm count, the more the number of (observable) mutilated spermatozoa. The effect of the (*H. sabdariffa*) extract on sexual features of male rat is similar to the findings of Akomolafe (2022), de Arruda et al. (2016), Mahmoud (2012), Ojekale et al. (2021), Orisakwe (2004), Ramalan et al. (2021) and Soliman et al. (2021).

Pups from the control female rats had the highest average weight (1 day, 3 days and 6 days after delivery) compared to those that received 2 g/kg or 4 g/kg of the extract. However, pups of animals that received 2 g/kg of the extract had the highest weight 9 days after delivery. Except for 1day weight after birth, pups of animals that received 2 g/kg had higher average weight than those of animals that received 4 g/kg. The control animals had the lowest number of pups of five (5) when compared with the litter size of female rats that received 2 g/kg and 4 g/kg of the extract which were 6 and 8 pups respectively. Details are presented in Table 3.

From the physical examination of the body of the pups as well as the activity of the pups (of each treatment group), no physical deformity was observed in the appearance of pups from the treated sows when compared with pups from the control sows. The pups from the treated sows were very active even immediately after delivery. This agility was equally observed in the treated sows (themselves) during the course of treatment as compared with the control group.

ANOVA analysis indicated an insignificant difference in pup weight for the dose groups while litter size had significant effect on pup weight ($p<0.01$). However, there was significant difference in pups' weight three days ($p<0.05$), six days and nine days after birth ($p<0.01$) while there was no significant difference in pups' weight a day after birth. Pups from sows in the control group had the highest average weight than pups of sows from other rats' category except nine (9)-day-after-birth pups.

Table 3: Average Weight of Pups from Sowed Rats (g)

Period	*Low dose*	*High dose*	*Control*
24 hours	5.85 (0.50)	6.08 (0.31)	6.65 (0.58)
3 days	8.93 (0.67)	8.44 (0.33)	10.13 (0.70)
6 days	13.30 (1.26)	11.28 (0.37)	14.83 (0.80)
9 days	17.71 (1.73)	12.32 (0.76)	16.62 (1.40)

NB: Values in parenthesis are standard deviations

Also, pups of sows from the 4g/kg group had higher average weight than those from 2 g/kg group a day after birth. For the two-way analysis, extract was an insignificant factor of average pup weight ($p<0.05$); but litter size and dose period was a significant factor ($p<0.05$). However, in the one-way analysis, extract was a significant factor of average pup weight ($p<0.05$) and an insignificant factor of average pup weight a day after birth ($p>0.05$). Details are presented in Table 4.

Table 4: Relationship between *H. sabdariffa* Extract and Pup Weights

Period	*Source of Variation*	*F*	*F-crit.*	*p-Value*
Pooled data (One-way analysis)	Treatment	0.38	4.26	0.70
Pooled data (Two-way analysis)	Treatment	0.96	4.46	0.42
	Litter Size	17.70***	3.84	0.00
Disaggregated data (One-way analysis)				
1-day	Dose Group	3.49	3.63	0.06
3-days	Dose Group	4.05**	3.63	0. 04
6-days	Dose Group	60.61***	3.63	0.00
9-days	Dose Group	46.06***	3.63	0.00

***Sig. at 1% and **Sig. at 5%

Furthermore, ordinary least square shows that the day of weight determination had a significant direct relationship ($\beta = 1.05$, $p<0.01$) with pups' weight while extract administration had insignificant relationship with pups' weight. Details are presented in Table 5.

Table 5: Factors Affecting Pup Weights from Experimental Female Rats

Model	*Variable Status*	*Estimate*	*t-value*	*Probability*
Constant	-	24.48**	1.98	0.05
Extract dose	-	4.78	1.12	0.14
Litter size	-	-3.09	-1.49	0.27
Days of weighing	-	1.05***	21.44	0.00
R^2	-	0.74	-	-
Adjusted R^2	-	0.73	-	-
D-W Statistics	-	1.66	-	-
F	-	50.62***	-	0.00

***Sig. at 1% and **Sig. at 5%

There is an indication that other factors apart from extract administration may be responsible for the differences in the weight of the female rats' pups since pups from the control female rats had the highest average weight than those from other categories on the first, third and sixth day of birth. Also, pups of animals that received 2 g/kg of extract had the highest average weight on the ninth day of birth implying that other factors may be responsible for this rather than the extract treatment. This is in consonance with the findings of Husori et al. (2021). However, *H. sabdariffa* may have boosting effect on the implantation sites of treated female rats (both 2 g/kg and 4 g/kg groups) when compared with the control rats; since the average litter size of 4 g/kg, 2 g/kg and control rats were eight (8), six (6) and five (5) pups respectively.

Results from Table 4 bring to limelight that the more the number of days after birth, the higher the weight of the pups. However, the results in Table 5 confirmed the postulations that factors other than extract treatment may be responsible for pups' weight variations.

Conclusion

The results indicate that *H. sabdariffa* has a boosting effect on male animal sex gametes, i.e. spermatozoa, but it can lead to increases in deformed spermatozoa at relatively toxic level and long time. Also, *H. sabdariffa* can be taken in pregnancy without any deleterious effect on the foetus. This is in line with the suggestion that doses (of *H. sabdarifa*) for relatively long periods may have a deleterious effect (Saganuwan, 2017) but contradicted the suggestion of no probable deleterious effect (Badreldin et al., 2012).

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Conflict of Interest

There is no conflict of interest whatsoever at every stage of this study.

References

Abat JK, Kumar S & Mohanty A 2017. Ethnomedicinal, phytochemical and ethnopharmacological aspects of four medicinal plants of malvaceae used in Indian traditional medicines: A review. *Medicines*, 4(4): 75. doi:10.3390/medicines4040075.

Abubakar SM, Ukeyima MT, Spencer JPE & Lovegrove JA 2019. Acute effects of *Hibiscus sabdariffa* calyces on postprandial blood pressure, vascular function, blood lipids, biomarkers of insulin resistance and inflammation in humans. *Nutrients*, 11(2): 341. doi: 10.3390/nu11020341.

Akindahunsi AA & Olaleye MT 2003. Toxicological investigation of the aqueous-methanolic extract of the calyces of *Hibiscus sabdariffa*. *J Ethnopharmacol*., 89(1): 161-164.

Akomolafe SF 2022. *In vitro* erectogenic property of aqueous extract from roasted and raw pepitas in isolated *Corpora cavernosa* of wistar rats. *Trop J Nat Prod Res*., 6(5): 789-794.

Aydın A, Aktay G & Yesilada EA 2016. Guidance manual for the toxicity assessment of traditional herbal medicines. *Nat Prod Commun*., 11(11): 1763-1773.

Badreldin HA, Al-Lawati I, Beegam S, Ziada A, Al Salam S, Nemmar A & Blunden G 2012. Effect of *Hibiscus sabdariffa* and its anthocyanins on some reproductive aspects in rats. *Nat Prod Commun*., 7(1): 41-44.

Badreldin HA, Naser AW & Blunden G 2005. Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: A review. *Phytother Res*., 19(5): 369-375.

Bamgboye EA 2002. A Companion of Medical Statistics. Folbam Publishers, Ibadan Nigeria, pp 235-245.

Bose S, Datta R & Kirlin WG 2021. Toxicity studies related to medicinal plants. In: Mandal SC, Chakraborty R, Sen S. (Ed.). Evidence Based Validation of Traditional Medicines: A Comprehensive Approach. Springer, Singapore, p. 621-647.

Chattopadhyay S, Pal S, Gosh D & Debnath J 2003. Effect of dietary co-administration of sodium selenite on sodium arsenite-induced ovarian disorders in mature albino rats. *Tox Sci*., 75(2): 412-422.

de Arruda A, Cardoso CA, Vieira MC & Arena AC 2016. Safety assessment of *Hibiscus sabdariffa* after maternal exposure on male reproductive parameters in rats. *Drug Chem. Toxicol*., 39(1): 22-27.

Dollah MA, Rozak NI, Nazari P & Parhizkar S 2021. Toxicity Effect of *Phaleria macrocarpa* (Mahkota Dewa) on Liver Function in Sprague Dawley Rats. *Trop J Nat Prod Res*., 5(2): 304-308.

Du CT & Francis FJ 1973. Anthocyanins of roselle (*Hibiscus sabdariffa* L). *J Food Sci*., 38: 310-312.

Duke JA 1983. *Hibiscus sabdariffa* (Malvaceae, Roselle). In: Hand book of energy crops.

hort.purdue.edu/newcrop/duke_energy/Hibiscus_sabdariffa.html. Accessed 17 Nov 2005.

Evbakhavbokun O, Ekrakene T, Daniel EO & Olisaka FN 2020. Phytochemical screening liver toxicity and antibacterial properties of aqueous and ethanol extracts of a local herbal mixture "aju Mbaise". *Trop J Nat Prod Res*., 4(6): 237-242.

Festing MF & Altman DG 2002. Guidelines for the design and statistical analysis of experiments using laboratory animals. *ILAR J*.; 43(4): 244-258.

Finegood DT, Scaglia L & Bonner-Weir S 1995. Dynamics of beta-cell mass in the growing rat pancreas: Estimation with a simple mathematical model. *Diabetes*, 44(3): 249-256

Ghasemi A, Jeddi S & Kashfi K 2021. The laboratory rat: Age and body weight matter. *EXCLI J*., 20: 1431-1445.

Husori DI, Patilaya MP & Febrika AN 2021. Acute toxicity studies of a combination of ethanol extracts of *Andrographis paniculata*, *Centella asiatica*, and *Curcuma heyneana* in rats. *Trop J Nat Prod Res*., 5(12): 2074-2078.

Hussaini DC, Orisakwe OE, Akunyili DN, Njan AA, Akumka DD & Udemezue OO 2004. Subchronic administration of Nigerian specie of aqueous extract of *Hibiscus sabdariffa* in rats did not produce cardiotoxicity. *Eur Bull Drug Res*., 12(1): 1-4.

Imieje OV, Zaki AA, Oseghale IO, Iheanacho CM, Fasinu PS, Khan IA, Langer P & Falodun A 2017a. *Pyrenacantha staudtii* Hutch and Dalz (*Icacinaceae*): A review of phytochemistry, pharmacology and future perspectives. *Trop J Nat Prod Res*.; 1(2): 49-57.

Imieje V, Zaki AA, Fasinu PS, Ali Z, Khan IA, Tekwani B, Khan SI, Nosa EO & Falodun A 2017b. Antiprotozoal and cytotoxicity studies of fractions and compounds from *Enantia chlorantha*. *Trop J Nat Prod Res*., 1(2): 89-94.

Kayode OT, Ohanaka NJ, Femi-Olabisi FJ, Agboola AO & Oyebanji EO 2022. *Parquetina nigrescens* Species: A concise review of phytochemistry and pharmacology. *Trop J Nat Prod Res*., 6(4): 454-461.

Keena C. Hibiscus: To eat or not to eat? 2003. http://www.hibiscus.org/privacy.php. Accessed 17 Nov 2005.

Kolawole JA & Maduenyi A 2004. Effect of zobo drink (*Hibiscus sabdariffa* water extract) on the pharmacokinetics of acetaminophen in human volunteers. *Eur J Drug Metab Pharmacokinet.*, 29(1): 25-29.

Kolawole OT, Adeyeba OA, Awodele O, Ayankunle AA, Wakeel OK, Oluogun WA & Olaniyi OS 2021. Evaluation of Chronic Toxicity of *Newbouldia laevis* Leaf Extract in Rats. *Trop J Nat Prod Res*., 5(11): 1889-1895.

Lu QY, Zhang JH, Li YY, Pu XX, Zhang CS, Liu S, Wan JJ, Di YT & Hao XJ 2021. New Secodaphnane-type alkaloids with cytotoxic activities from *Daphniphyllum angustifolium* Hutch. *Nat Prod Bioprospect*., 11(4): 453-457.

Mahmoud YI 2012. Effect of extract of hibiscus on the ultrastructure of the testis in adult mice. *Acta Histochem*., 114(4): 342-348.

Majiya H & Galstyan A 2020. Dye extract of calyces of *Hibsiscus sabdariffa* has photodynamic antibacterial activity: A prospect for sunlight-driven fresh produce sanitation. *Food Sci Nutr*., 8(7): 3200-3211.

Mangla B & Kohli K 2018. Pharmaceutical and therapeutic potential of miraculin and miracle berry. *Trop J Nat Prod Res*., 2(1): 12-17.

Masiello P, De Paoli AA & Bergamini E 1979. Influence of age on the sensivity of the rat to streptozotocin. *Hormone Res*., 11(5): 262-274.

Mathur S & Hoskins C 2017. Drug development: Lessons from nature (review). *Biomed*. *Rep*., 6(6): 612-614.

Mayanti T, Darwati D, Al Anshori J, Supratman U, Madihah M, Lesmana R, Dinata DI & Gaffar S 2020. Toxicity evaluation of ethanol extract of *Lansium domesticum* cv kokossan seeds in female wistar rats. *Trop J Nat Prod Res*., 4(8): 348-354.

Mead R 1988. The design of experiments. Cambridge University Press, Cambridge, UK.

Mendonça LABM, Matias R, Zanella DFP, Porto KRA, Guilhermino JF, Moreira DL, Roel A, Pott RA & Carvalho CME 2020. Toxicity and phytochemistry of eight species used in the traditional medicine of sul-mato-grossense, Brazil. *Braz J Biol*., 80(3): 574-581.

Morton J 1987. Roselle (*Hibiscus sabdariffa* L.). In: Morton JF, Miami, FL (Eds.). Fruits of Warm Climates. http://www.hort.purdue.edu/newcrop/morton/ roselle.html. Accessed 17 Nov 2005.

Najmi A, Javed SA, Al-Bratty M & Alhazmi HA 2022. Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. *Molecules*, 27(2): 349. https://doi.org/10.3390/molecules27020349.

NCKU 2015. Weight to age-in-week table for rat. https://animal.ncku.edu.tw/ p/412-1130-16363.php?Lang=en. Accessed 10 Jan 2022.

Nishitha G, Latha GA, Tejaswini L, Mounica A, Rekha K, Nikitha KR, Jyothirmayi B, Kesana NS & Unissa R 2018. *In vitro* cytotoxic activity of ethyl acetate fraction of *Hibiscus vitifolius* flowers against HeLa cell line. *Trop J Nat Prod Res*., 2(3): 122-125.

Obouayeba AP, Soumahin EF, Diarrassouba M, Koffi KE, Kouakou TH, Djaman AJ & N'guessan JD 2016. *Hibiscus sabdariffa*: Food and medicinal plant of the Ivorian pharmacopeia - an important source of bioactive substances. *J Pharm Chem Biol Sci*., 4(3): 321-335.

OECD 1983. The OECD guidelines for the testing of chemicals: 415. One-generation reproduction toxicity study. Organization for Economic Cooperation and Development, Paris, France.

OECD 2001a. The OECD guidelines for the testing of chemicals: 414. Prenatal developmental toxicity study - proposal for updating guideline 414. Organization for Economic Cooperation and Development, Paris, France.

OECD 2001b. The OECD guidelines for the testing of chemicals: 423. Acute oral toxicity – Acute toxic class method. Organization for Economic Cooperation and Development, Paris, France.

OECD 2001c. The OECD guidelines for the testing of chemicals: 420. Acute oral toxicity – Fixed dose procedure. Organization for Economic Cooperation and Development, Paris, France.

OECD 2008a. The OECD guidelines for the testing of chemicals: 425. Acute oral toxicity – Up-and-down-procedure (UDP). Organization for Economic Cooperation and Development, Paris, France.

OECD 2008b. The OECD guidelines for the testing of chemicals: 407. Repeated dose 28-day oral toxicity study in rodents. Organization for Economic Cooperation and Development, Paris, France.

Ogbiko C, Eboka JC, Igbe I & Usman DM 2017. Anti-ulcer activity of methanol extract of *Plantago rugelii* Decne (*Plantaginaceae*). *Trop J Nat Prod Res*., 1(2): 84-88.

Ojekale A, Jewo P, Kanmodi R, Lasisi M, Oguntola, Emuejevoke T, Adele A, Acheampong A, Osundare B, Alawiye O & Victor A 2021. Ethanol leaf extract of *Cyathula prostrata* enhances spermatogenesis in wistar rats via expression of AKAP4, DAZL and PRM2. *Trop J Nat Prod Res*., 5(7): 1309-1314.

Ojulari OV, Lee SG & Nam J 2019. Beneficial effects of natural bioactive compounds from *Hibiscus sabdariffa* L. on

obesity. *Molecules*, 24(1): 210. doi.org/10.3390/molecules2 4010210.

Onyenekwe PC, Ajani EO, Ameh DA & Gamaliel KS 1999. Anti-hypertensive effect of roselle (*H. sabdariffa*) calyx infusion in spontaneously hypertensive rats and a comparison of its toxicity with that in wistar rats. *Cell Biochem Funct*., 17(3): 199-206.

Orisakwe OE, Hussaini DC & Afonne OJ 2004. Testicular effects of sub-chronic administration of *Hibiscus sabdariffa* calyx aqueous extract in rats. *Reprod Toxicol*., 18(2): 295-298.

Orisakwe OE, Hussaini DC, Orish UN, Obi E & Udemezue OO 2003. Nephrotoxic effects of *H. sabdariffa* calyx in rats. *Eur Bull Drug Res*., 11(4): 99-103.

Oseghale IO, Imieje VO, Erharuyi O, Iheanacho C & Falodun A 2017. A review of the phytochemistry and pharmacology of *Eleusine coracana* Linn (*Poaceae*): A popular Nigerian edible grain. *Trop J Nat Prod Res*., 1(6): 227-235.

Owulade MO 2004. Effects of aqueous extracts of *Hibiscus sabdariffa* calyces and *Ocimum gratissimum* leaves on intestinal transit in rats. *Afr J Biochem Res*., 7(1): 31-33.

Prescott LF 1987. Drug overdose and poisoning. In: Speight TM (Ed); Avery's drug treatment. ADIS, UK, pp. 283-302.

Ramalan MA, Shuaibu AB, Abdussalam US & Yaro AH 2021. Aphrodisiac effect of methanol hypocotyls extracts of *Borassus aethiopum* Mart. in male wistar rats. *Trop J Nat Prod Res*., 5(10): 1852-1858.

Riaz G & Chopra R 2018. A review on phytochemistry and therapeutic uses of *Hibiscus sabdariffa* L. *Biomed Pharmacother*., 102: 575-586.

Saganuwan SA 2017. Toxicity studies of drugs and chemicals in animals: An overview. *Bulg J Vet Med*., 20(4): 291-318.

Sallah AM, Gathumbi J & Vierling W 2002. Inhibition of intestinal motility by methanolic extract of *H. sabdariffa L Malvaceae* in rats. *Phytother Res*., 16(3): 283-285.

Sengupta P 2013. The laboratory rat: Relating its age with human's. *Int J Prev. Med*., 4(6): 624-630.

Sireeratawong S, Itharat A, Khonsung P, Lertprasertsuke N & Jaijoy K 2013. Toxicity studies of the water extract from the calyces of *Hibiscus Sabdariffa* L. in rats. *Afr J Tradit Complement Altern Med*., 10(4): 122-127.

Sjoqvist F, Borgu O & Orme MLE 1987. Fundamentals of clinical pharmacology. In: Speight TM (Ed), Avery's drug treatment. ADIS, UK. p. 1-64.

Soliman NA, EL-Gaafary MN, Shalaby AA, Hussein OM & Taha NM 2021. Physiological and histopathological effects of two seed extracts (*Phoenix dactylifera* and *Raphanus sativus*) and L-Carnitine drug on semen quality and testicular sexual hormonal changes in adult male rabbits. *Trop J Nat Prod Res*., 5(10): 1709-1715.

Suboh SM 2004. Protective effects of selected medicinal plants against protein degradation, lipid peroxidation and deformability loss of oxidately stressed human erythrocytes. *Phytother Res*., 18(4): 280-284.

Tajbakhsh E, Kwenti TE, Kheyri P, Nezaratizade S, Lindsay DS & Khamesipour, F 2021. Antiplasmodial, antimalarial activities and toxicity of African medicinal plants: A systematic review of literature. *Malar J*.; 20(1): 349. https://doi.org/10.1186/s12936-021-03866-0.

Tandon N & Yadav SS 2019. Safety and toxicity of medicinal plants: Concerns and overview. In: Ghosh D & Mukherjee PK (Eds), Natural medicines: Clinical efficacy, safety and quality. Taylor & Francis Group, London, UK. p. 158-176.

Temitope AO, Gowon JA & Oluwatayo AZ 2017. Protective effect of ethanol extract of *Senna occidentalis* leaf against cadmium induced Hepat. *Trop J Nat Prod Res*., 1(1): 17-21.

Thien VNT, Do LTM., Dang PH, Huynhs NV, Dang HP, Nguyen TT, Tran KT, Huu DMN & That QT 2019. A new Lignan from the flowers of *Hibiscus sabdariffa* L. (Malvaceae). *Nat Prod Res*., 35(13): 2218-2223.

Tse EG, Korsik M & Todd MH 2019. The past, present and future of anti-malarial medicines. *Malar J*., 18(1): 93. https://doi.org/10.1186/s12936-019-2724-z.

Tseng TH, Wang CJ, Kao ES & Chu HY 1996. Hibiscus protocatechuic acid protects against oxidative damage induced by *tert*-butyl hydroperoxide in rat primary hepatocytes. *Chem.-Biol Interact*.; 101: 137-148.

Verma T, Sinha M, Bansal N, Yadav SR, Shah K & Chauhan NS 2021. Plants Used as Antihypertensive. *Nat Prod Bioprospect*., 11(2): 155-184.

Walter H, Consolaro F, Gramatica P, Scholze M & Altenburger R 2002. Mixture toxicity of priority pollutants at no observed effect concentrations (NOECs). *Ecotoxicology*, 11(5): 299-310.

Wang CJ, Wang JM, Lin WL, Chu CY & Tseng TH 2000. Protective effect of hibiscus anthocyanins against *tert*-butyl hydroperoxide-induced hepatic toxicity in rats. *Food Chem Toxicol*., 38(5): 411-416.

Wang YH 2021. Traditional Uses and Pharmacologically Active Constituents of *Dendrobium* Plants for Dermatological Disorders: A Review. *Nat Prod Bioprospect*., 11(5): 465-487.

Yakubu J, Abdulrahman FI & Sodipo OA 2020. Evaluation of the toxicity profile and antidiabetic potentials of the methanol extracts of *Boswellia dalzielii* (Frankincense tree) in alloxan-induced diabetic rats. *Trop J Nat Prod Res*., 4(5): 190-194.

Zhao YL, Su M, Shang JH, Wang X, Njetang GSS, Bao GL, Ma J, Sun QD, Yuan F, Wang JK & Luo XD 2020. Acute and sub-chronic toxicity of indole alkaloids extract from leaves of *Alstonia scholaris* (L.) R. Br. in beagle dogs. *Nat Prod Bioprospect.*, 10(4): 209-220.