RESEARCH ARTICLE

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Corresponding Author
Aurel Constant ALLABI, Campus du champ de Foire-FSS,, 01 BP 188 Cotonou, BENIN
Email:acallabi@hotmail.com

CONFLICT OF INTEREST NONE DECLARED

Acute and Sub-Acute Oral Toxicity Studies of TR-Y017 in Wistar Albino Rats.

Ferdinand Menakpo Adounkpe[1], Aurel Constant Allabi*[1,2], Jacques Vigan[3], Jean Eude Degbelo[6], Anatole Laleye[5].

1- Laboratoire National des Stupéfiants et de Toxicologie (LNST), Centre Béninois de la Recherche Scientifique et de l'Innovation (CBRSI), 01 BP 348 Cotonou.
2- Laboratoire de Pharmacologie-Toxicologie, Faculté des Sciences de la Santé (FSS), Université d'Abomey-Calavi (UAC), 01 BP 918 Cotonou.
3- Unité de Néphrologie du Centre National Hospitalier Universitaire Hubert K. MAGA (CNHU-HKM). Av. Pape Jean-Paul II, 01 BP 386 Cotonou.
4- Laboratoire d'analyses biomédicales, Centre Hospitalier Universitaire de Abomey-Calavi, 04 BP 223 Abomey-Calavi.
5- Laboratoire de Cytogénétique. Faculté des Sciences de la Santé (FSS), Université d'Abomey-Calavi (UAC). ISBA, 01 BP 918 Cotonou.

ABSTRACT

Introduction: *TR-Y017* is a phytomedicine widely used in Benin and which has been claimed effective against anemia, hepatitis, allergies, urinary infections and cancer by the manufacturer. In order to establish the safety use of TR-Y017, this study aims to evaluate the effects of its acute and sub-acute oral administration to Wistar albino rats.

Methods: Acute oral toxicity tests were performed on Wistar rats by oral force-feeding with a single dose of 2000 mg/kg in accordance with the Organization for Economic Co-operation and Development (OECD) Guidelines and animals were observed for 14 days to record various clinical signs or death. In sub-acute toxicity study, female rats were divided into 4 groups of 5 animals each. Three doses 12.5; 25 and 37.5 mg/kg body weight for 28 days were received by groups 2 to 4. Physiological behavior and body weight were evaluated. Biochemical, haematological and histological Analysis were realized at the end of each study.

Results: Out of apathy, reduction in mobility and food rejection during the first hour after TR-Y017 acute administration, no mortality was observed. TR-Y017 was found to be non-toxic at high dose and itUY5s lethal dose was estimated to be superior to 2000 mg/kg body weight under present experimental conditions. On the 28th day of treatment, none of the three doses of TR-Y017 affected general behavior or caused death. However, significant differences in body weight, kidney ($p = 0.0438$) and liver ($p = 0.00929$) weights were observed in treated rats when compared to controls. No significant difference was observed on haematological and biochemical parameters. Between the beginning and the 28th day, there was no significant difference on haematological and biochemical parameters of treated animals comparing to the control group. Histopathological examination revealed no alteration in the morphological architecture of renal and hepatic tissues.

Conclusion: Acute and sub-acute oral administration of TR-Y017 to Wistar rat was not associated with any manifestation of toxicity such as global behavior changes, toxic lesions or death. These results suggest that prolonged administration of TR-Y017 did not cause functional or structural changes in rats and did not indicate any toxicity of this product.

Keywords: Acute toxicity, Sub-acute toxicity, Quality control, Phytomedicine, Anemia.

Introduction

A great part of the global populations still depend on herbal medicine for their primary health care needs believing plant remedies are free from undesirable side effects [(1)]. In recent years, the traditional pharmacopoeia and remedies derived directly from our medicinal plants are experiencing a renewal of promotion which the practitioners of the traditional medicine are often the authors. These remedies are

advertised on our media where sometimes networking is organized. In view of the weaknesses in the pharmaceutical regulations of our countries with limited resources, these traditional herbal medicines are sold and dispensed even in pharmacies, without any scientific indication of their possible toxicity. However, some practitioners of traditional medicine express a desire to have their products evaluated. *TR-Y017* is a phytomedicine prepared by a well-known manufacturer in Benin who has been claiming for years that his product is highly effective against anemia, hepatitis, allergies, urinary tract infections and cancer. It is one of the herbal remedies already dispensed in pharmacies in Benin and widely used for the treatment of anemia. Despite a long period of use, safety profile and toxicity index of the phytomedicine for a specific period of time is lacking to enhance its safety of use. Moreover, in an environment where collaboration between researchers and practitioners of traditional medicine is not always easy, the exact composition of the product is unknown to researchers. Therefore, investigations on herbal remedies to incorporate observations of short and long-term toxicity manifestations among rats are useful. Based on the doses used in humans, adequate doses may be defined for preclinical toxicity. Numerous studies have been devoted for years to the toxic effects of medicinal plants [4-19]. Until now, no toxicological investigation has been carried out to clarify the safety of repeated and prolonged use of this phytomedicine. This study aimed to evaluate the effects of acute and sub-acute oral toxicity of *TR-Y017* on Wistar rats.

Materiel and Methods

Herbal product:

Is sold as a brick red powder in a sachet with a yellowish-colored dilution liquid. The mixture obtained when using the product takes blood color. The manufacturer recommends it to anyone who has lost blood by accident or during childbirth or illness to compensate for blood loss and prevent inflammation.

Animals

Wistar rats used were born and were raised in cages in the animal room of the Human Biology unit under standard conditions (22 to 25 °C, 12 h of obscurity/ light cycle, sanitary environment) with free access to tap water and food.

Acute oral toxicity test

The acute toxicity test of *TR-Y017* was performed in accordance with the OECD guidelines [20-22]. Healthy rats aged 12 to 14 weeks weighting 200 g ± 5g were starved 12 hours, all night before the experimentation. Animals were divided into three groups:

Group I: 5 female rats in this group served as control and received 4 ml of distilled water,

Group II: 5 female rats force-fed with 4 ml of *TR-Y017* at a single dose of 2000 mg/kg,

Group III: 5 male rats force-fed with 4 ml of *TR-Y017* at a single dose of 2000 mg/kg.

After administration, animals were monitored continuously for every two hours for a day and then daily for 14 days to detect acute changes in morphological and behavioral responses, spontaneous activity, irritability, corneal reflex, tremors, convulsion, salivation, diarrhea, lethargy if any, and also monitored for any mortality. Rats were weighed at the beginning of the study and afterwards every seven (07) days and temperatures were taken on the 1st, 4th, 7th, 10th and 14th day. At the end of 14 days, animals were sacrificed and blood sample were collected for biochemical and hematological analyses. Livers and kidneys of both control and treated groups were collected, weighed immediately and transferred to a saline solution. These organs were fixed in 10% buffered formalin for histological examination. The samples were then treated with increasing concentration of ethanol and infiltrated with paraffin and the thin cuts were made and stained with hematoxylin and eosin stains. Then the sections were examined with light microscope and photographed using a microscopic camera.

Sub-acute toxicity study (28 days)

Sub-acute toxicity study was conducted in compliance with OECD guidelines [23; 24]. Twenty healthy female Wistar rats aged 12 to 15 weeks weighting 180 g ± 10 g were randomly distributed into 4 groups of 5 animals each in separate cages under the same conditions as described above. Animals of groups 2 to 4 (test groups) were daily force-fed for 28 days with different doses 12.5; 25 and 37.5 mg/kg body weight of *TR-Y017*, respectively. The control group received 2 ml of distilled water for the same period. During treatment, animals were weekly weighed and daily treated with the corresponding dose of *TR-Y017* (test groups) or distilled water (control) before being allowed to food and water (tap water). Clinical signs were observed at least twice a day during the 28-days treatment period. At the end of the treatment period, blood samples were collected into sterilized dry test tubes or test tubes containing EDTA for biochemical and hematological

analyses. Animals were starved for 12 hours with free access to water cap and were euthanized by lethal inhalation of chloroform vapors. They were dissected then liver, lungs, heart and kidneys of each of them were removed, weighed and prepared for histological examinations.

Complete blood count and Biochemical Analysis

Hematological analyses were performed on blood using an automatic hematological analyzer (System, XP-300). These parameters included red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), blood platelet count (PLT), white blood cell count (WBC), lymphocytes, neutrophils, eosinophils and monocytes counts. Biochemical parameters including Creatinine (CREA), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) were determined using an automatic analyzer (MTN-658F) with specific kits. The parameters values obtained in the experimental groups were compared with those of the control group.

Histopathological study

Livers and kidneys of both experimental groups and control group were immediately stored in 10% paraffin for histological analysis [25; 26]. Paraffin sections were made and stained with hematoxylin and eosin for a thorough histopathological study [27; 28]. Then the sections were examined with light microscope and photographed using a microscopic camera.

Statistical analysis:

The results shown are expressed as means ± standard error of mean (S.E.M.). The data generated by this study were statistically processed using R software R 3.3.2 [29-32]. The analysis of variance (ANOVA) was used to compare means of different groups. Whenever a significant difference ($p<0.05$)was revealed, the ANOVA test is completed by the Tukey post ANOVA test to identify the group with very significant differences compared to the values of the control group.

Results

Acute oral toxicity effects of *TR-Y017* on rats

Clinical signs observed on rats during acute toxicity test

There were no mortality or any serious signs of behavioral changes or toxicity observed after oral administration of *TR-Y017* up to the dose level of 2000mg/kg b.w. in rats. However, apathy, excessive scratching and refusal of food were observed during first one hour after force-feeding (Table 1). While all the rats gained weight during the experimental period, their temperatures dropped after a rise in the first week of the test (figure 1 and figure 2).

Clinical signs	Response		
	Control (Female) (Distilled water)	**Female** (2000 mg/kg b.w.)	**Male** (2000 mg/kg b.w.)
Apathy	-	+	+
Scratching	-	+	+
Breathing problems	-	-	-
Mouth bleeding	-	-	-
Nasal bleeding	-	-	-
Refusal of food	-	+	+
Contortion	-	-	-
Convulsion	-	-	-
Coma	-	-	-
Mortality	-	-	-

(+): Observed; (-): None Observed

Table 1- Clinical signs observed on rats during acute toxicity test.

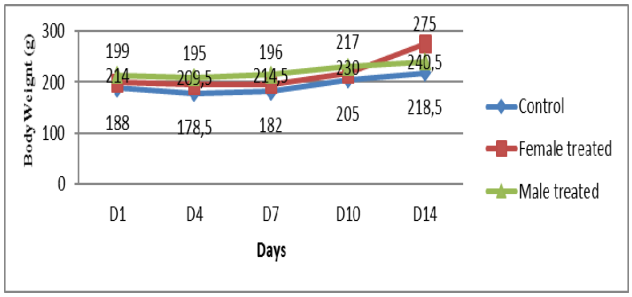


Figure 1-Body weight of Control and treated rats at 2000mg/kg b. w. during acut toxicity test. An increase in body weight of the treated rats compared to the control group was observed with a significant difference ($p<0.001$).

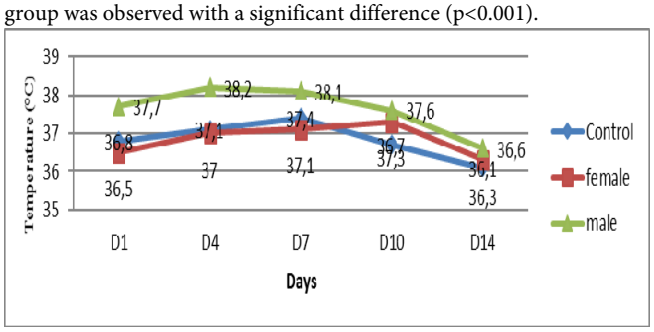


Figure 2-Temperature of Control and treated rats at 2000mg/kg b. w. during acute toxicity test. No significant difference was observed between the body temperature changes of the treated rats compared to the control group ($p> 0.05$)

Biochemical and hematological parameters of rats

The effects of *TR-Y017* administration on biochemical and hematological parameters are presented in Table 2 and Table III, respectively. As shown in Table 2, non-significant difference have been observed in biochemical parameters levels ($p>0.05$) at the 14Th day. No significant difference was observed between control, male and female rats in according to

hematologic parameters like RBC, Haemoglobin, Hematocrit, MCV, MCHC, WBC, Lymphocytes and Platelets. Male rats presented high values of red blood cells, Eosinophils) and Neutrophils with non-significant difference compared to control group between D-0 and D-14.

Biochemical parameters	Days	Control (Female)	Female Treated	Male Treated	Prob (F > F obs)
ASAT(IU/L)	**D-0**	228.7±6.7	254±18.9	233.3±4.5	0.341 ns
	D-14	191.5±1,5	233.5±28.5	221±2.3	0.319 ns
Prob (t > t obs)		*0.032 **	*0.656 ns*	*-0.111 ns*	-
ALAT(IU/L)	**D-0**	154±5.8	150.7±2.1	145.7±12.8	0.777 ns
	D-14	107±5	140±20	115.5±1.5	0.273 ns
Prob (t > t obs)		*0.025 **	*0.689 ns*	*0.144 ns*	-
ALP (IU/L)	**D-0**	151.7±17.5	173±16.3	114.8±10.4	0.085 ns
	D-14	50.2±12.2	69.8 ±9.7	65.2±2.8	0.756 ns
Prob (t > t obs)		*0.041 **	*0.032 **	*0.346 ns*	-
CREA (mg/L)	**D-0**	7.6±0.3	17.3±4.5	14.7±3.2	0.165 ns
	D-14	7±1	12±4	5±1	0.262 ns
Prob (t > t obs)		*0.641 ns*	*0.468 ns*	*0.101 ns*	-

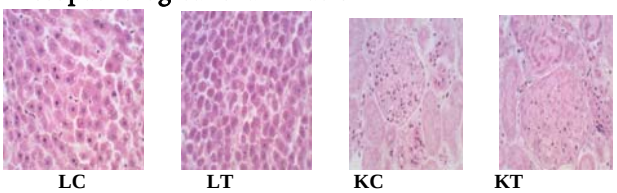
Table 2 -Effect of acute toxicity study of *TR-Y017* on Biochemical parameters in Control and Treated animals. Results were expressed as the mean ± S.E.M of 5 rats in each group.

Biochemical parameters- AST = Aspartate AminoTransferase, ALP = Alkaline Phosphatase, ALT = Alanine AminoTransferase, CREA = Creatinine.

* indicates a significant difference at 5% and ns as non-significant difference at 5%.

Means with the same letter are not significant difference at 5%; ** and * indicate respectively a significant difference at 1% and 5% then ns as non-significant difference at 5%. Prob (F > F obs) expressed p-values at D-0 and at D-14; Prob (t > t obs) expresses p-values between D-o and D-14. **(Table 3 - on page 09)**

Histopathological examination



LC **LT** **KC** **KT**

Figure 3: Photomicrographs (haematoxylin-eosin stained, ×400) of liver and kidney tissue. LC=Liver of control rat shows hepatocyts, LT= Liver of treated rats at 2000 mg/kg b.w.; KC= kidney of control rat shows nucleus of cells; KC= kidney of treated rats at 2000 mg/kg

Histopathological examination of the liver and kidneys was performed in both Control and Treated animals. Organ weight revealed that administration of *TR-Y017* at dose of 2000mg/kg body weight did not produce any organ swelling, atrophy and hypertrophy. All tissue sections were within the normal limits and no histopatological sign was observed in the rats treated with TR-Y017 (2000mg/kg b.w.). No significant changes were observed in the histological observation of livers and kidneys of the experimental animals as compared to the Control rats (Figure 3).

Subacute oral toxicity (28 days) effects of *TR-Y017* on female Wistar Rats

Effect of sub-acute oral administration of *TR-Y017* on general behavior

TR-Y017 administered at 12.5; 25 and 37.5 mg/kg b.w. to female Wistar rats did not result in mortality.

During the 28-days treatment, alteration of general behavior, diarrhea, hematuria, autonomic or central systems movements were not observed. However, excessive skin scratching just after force-feeding was observed with treated rats.

Effect of sub-acute administration of TR-Y017 on Control and Treated rats weights

Changes in the body weight evolution of the rats were observed throughout the 28 days of sub-acute toxicity test. As shown in Figure 4, all animals treated with the three different doses 12.5; 25 and 37.5 mg/kg b. w. showed a clear increase in body weights compared to Control animals.

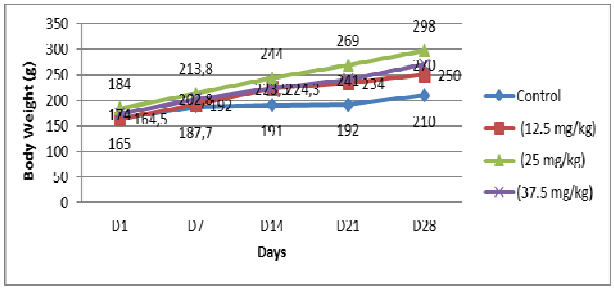
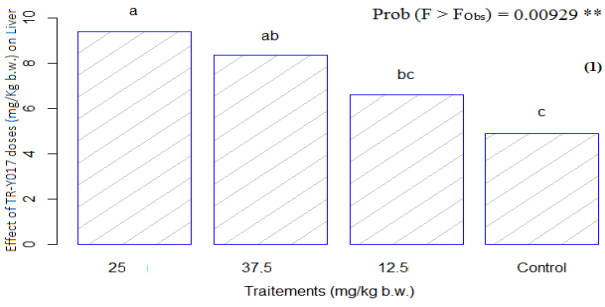


Figure 4: Body weight evolution of Control and treated female rats at 12.5; 25 and 37.5mg/kg b. w. during Sub-acute toxicity test. An increase in body weight of the treated rats compared to the control group was observed with a significant difference ($p<0.001$).

Effects of sub-acute administration of TR-Y017 on organs weights of Control group and Treated rats

A significant difference was noticed in livers ($p<0.01$) and kidneys ($p<0.05$) weights changes of treated rats compared to those of control group (Figure 5).



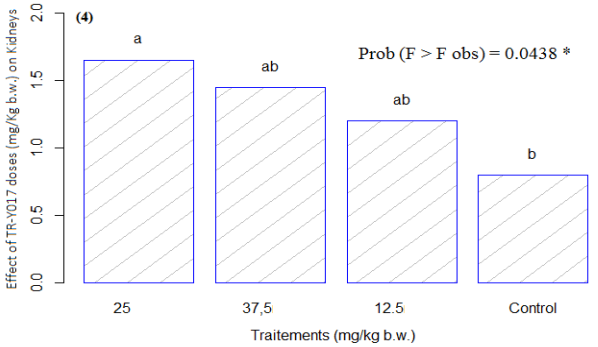


Figure 5: Effect of sub-acute administration of TR-Y017 on organ as Liver (**1**) and Kidneys (**4**) of treated rats at 12.5 mg/kg, 25 mg/kg and 37.5 mg/kg. Results of ANOVA and SNK test represented to barplot (n of treated animals = 5)

Means with the same letter are not significant difference at 5%; ** and * indicate respectively a significant difference at 1% and 5% then ns as non-significant difference at 5%.

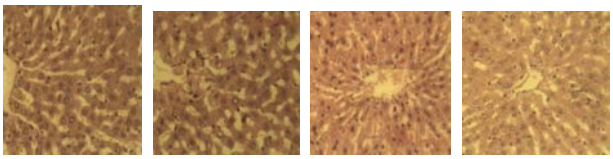
Effect of Sub-acute oral administration of *TR-Y017* on Hematological Parameters

No significant difference (p> 0.05) was observed on hematological parameters. Between the beginning and the 28th day, a significant increase in hemoglobin (p<0.01) and a significant decrease in hematocrit (p<0.001) have been observed with both Treated and Control animals that could not be attributed to the product effect **(Table 5 on page-10**)

Effect of sub-acute oral administration of TR-Y017 on Serum Biochemical Parameters:

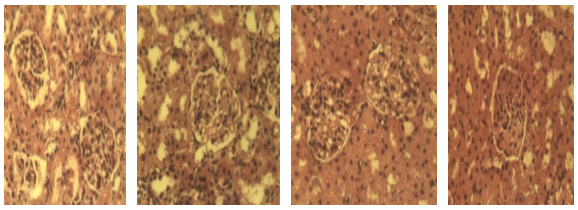
No significant difference (p> 0.05) was observed on biochemical parameters except increasing of Alanine aminotransferase (p = 0.041031) noticed at 37.5 mg/kg in treated animals which is not different from those of Control group at date D-28. Between the beginning and the 28th day, a significant decrease in aspartate amino transferase (ASAT) (p<0.001) and a significant increase in Alanine aminotransferase (p<0.001) have been observed with both treated rats and those of control group which could not also be attributed to the product effect. (**Table 6-on page no.11)**

Histopathological examination of the livers (Figure 5) and kidneys (Figure 6) was performed in both Control and Treated animals.



LC L2 L3 L4

Figure 6: Photomicrographs (HE X400) Liver of control rat (**LC**), with a hepatic lobule showing hepatocyts disposed radially arounda centrolobular vein; Liver of treated rats at 12.5 mg/kg (**L2**); at 25 mg/kg (**L3**) and at 37.5 mg/kg b.w. (**L4**).



KC **K2** **K3** **K4**

Figure 7: Photomicrographs (HE X400) kidney of control rat (**KC**) shows nucleus of cells; treated rats at 12.5 mg/kg (**K2**); at 25 mg/kg (**K3**) and at 37.5 mg/kg b.w. (**K4**).

Discussion

TR-Y017 is a phytomedicine reported to be highly effective against anemia and without side effects according to manufacturer. In the absence of toxicity information, our study is the first to investigate its acute and sub-acute toxicity by oral force-feeding in Wistar rat. This should serve to prevent exposing human subjects to potential toxicity-related health risks while using *TR-Y017*. Toxicity studies in appropriate animal models are commonly used to assess potential health risks in humans. Such toxicity studies assess the hazard and determine the risk level by addressing the probability of exposure to that particular hazard at certain doses or concentrations [33]. In our acute toxicity study, out of apathy, excessive scratching and refusal of food observed during the first one hour after administration, no behavior changes was induced by a single dose of 2000mg/kg b.w. of *TR-Y017*. Excessive scratching and refusal of food observed just after force-feeding could be explained by a normal physiological reaction of rats. In addition, no clinical or histopathological sign of toxicity was induced by a single dose of 2000 mg/kg of TR-Y017 in our experimental conditions, suggesting that its LD_{50} is higher than 2000 mg/kg. It is known that there is no real correlation between acute dose of LD_{50} administration and prediction of side effects of a repetitive doses administration. Many years ago, it is reported that the LD_{50} in animals does not predict the lethal dose of a drug in humans or the symptomatology of acute poisoning after overdose [34]. Nevertheless, knowledge or estimation of the LD_{50} of phytomedicines provides valuable information on the repetitive doses to be advised to consumers. Acute toxicity test is carried out in each animal species as the same route as intended for use in the treatment and provides both dose guidelines on the dose to be use in more prolonged studies and also the basis for which other testing program can be design. An increase in body

weight of the treated rats at 2000 mg/kg b.w. compared to the control group was observed with a significant difference ($p<0.001$). This suggests that TR-Y017 increased rats' weights but the mechanisms of weight increase observed in rats treated were not due to a possible anti-anemic effect as there was no increase in hemoglobin or red blood cell count in these rats. This body weight increase could also be indicative of their overall good health over the entire treatment period. A non-significant difference observed in biochemical and hematological parameters on the 14th day already in agreement with the fact that no histopathological lesions were observed in the liver and kidneys of rats treated at 2000mg/kg b.w. suggests that this product may be administered orally without causing harm to consumers. During the sub-acute toxicity study, rats were submitted on three doses of 12.5 mg/kg, 25 mg/kg and 37.5 mg/kg of *TR-Y017*. 28 consecutive days administration did not produce any death, abnormality or clinical signs of toxicity, any alteration of general behavior. However, excessive skin scratching just after force-feeding was observed with treated rats that could be explain as normal physiological reactions. Changes on body weight evolution and significant differences in kidney ($p = 0.0438$) and liver ($p = 0.00929$) weights were observed in treated rats compared to those of Control group. This suggests that *TR-Y017* does not affect the general aspect of the animals but seems to have favored their weight gain throughout the treatment period. This increase in the weight of the rats treated at the three different doses could probably be explained by another mechanism which would not necessarily be that of the anti-anemic power claimed by the manufacturer of the product. Indeed, this increase in weight was not followed by an increase in the hemoglobin level or the number of red blood cells in the treated animals. No significant difference was also observed on hematological and biochemical parameters except increasing of alkaline phosphatase ($p = 0.041031$) specially noticed at 37.5 mg/kg in treated animals which is not different from those of Control group at date D-28. This alkaline phosphatase increasing was not associated with any other manifestation of toxicity such as increased serum transaminases or toxic lesions, and then it could not logically be attributed to the product effect. As it is known that in pregnant animals, biological changes are remarkable and alkaline phosphatase is made in placenta, especially in late pregnancy [35-41], alkaline phosphatase increase observed in this study could be explain by the fact that rats used were probably pregnant. Between the beginning and the 28th day, a significant decrease in aspartate amino transferase (ASAT) ($p<0.001$) and a significant increase in alkaline phosphatase ($p<0.001$) have been observed with both treated rats and those of Control group which could not also be attributed to the product effect. All these changes could be simply related to the physiological state of the rats and not to renal or hepatic dysfunction. This suggestion seems to be justified since the histopathological examination revealed no alteration in the morphological architecture of renal and hepatic tissues.

Conclusion

This study shows that acute administration of a single dose of 2000 mg/kg of *TR-Y017* caused no evidence of toxicity and no death in Wistar rat. Similarly, sub-acute toxicity study carried out with three increasing doses did not cause any death but significant differences in some biological parameters were observed in both treated rats and control group without kidneys and livers structure disturbance. Based on these results, *TR-Y017* is nontoxic when administered at doses lesser than or equal to 2000 mg/kg. This research provided firsthand information on the acute and sub-acute toxicity of TR-Y017 until further studies were conducted, for example, to elucidate its mechanism for correcting anemia claimed by the manufacturer. Therefore, it is desirable to remain cautious in its prolonged use at high doses.

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Hematologic parameters	Days	Control (Female)	Female Treated	Male Treated	Prob (F > F obs)
RBC ($x10^{12}$/L)	D-0	6.6±0.1	6.5±0.4	6.4±0.1	0.7986652 ns
	D-14	7.6±0.3	7.5±0.3	7.9±0.1	0.8542033 ns
Prob (t > t obs)		*0.0296884514 **	*0.14430348 ns*	*0.0018850221 ***	-
Haemoglobin (g/dL)	D-0	13.6±0.4	13.1±1.1	11.4±1.5	0.3692670 ns
	D-14	14.5±0.8	14.2±0.3	13.8±0.5	0.7140839 ns
Prob (t > t obs)		*0.3274278725 ns*	*0.48892607 ns*	*0.3008657598 ns*	-
Hematocrit (%)	D-0	41.2±0.9	41.6±2.5	39.7±0.5	0.6722863 ns
	D-14	44.5±2.1	43.5±0.4	43.1±0.8	0.7681676 ns
Prob (t > t obs)		*0.1899928583 ns*	*0.58904447 ns*	*0.0290394784 **	-
MCV (fL)	D-0	62.4±0.9	57.9±5.7	62.0±0.7	0.5983522 ns
	D-14	58.4±0.2	57.9±1.5	58.0±0.4	0.9256691 ns
Prob (t > t obs)		*0.0405854233 **	*0.99178585 ns*	*0.0290291583 **	-
MCH (pg)	D-0	20.6± 0.5	20.1±0.7	17.7±2.3	0.3705317 ns
	D-14	19.1±0.3	18.9±0.3	18.6±0.4	0.6090160 ns
Prob (t > t obs)		*0.0933496993 ns*	*0.33573048 ns*	*0.7877406130 ns*	-
MCHC (g/dL)	D-0	33.3±0.5	31.5±1.4	28.6±3.5	0.3962165 ns
	D-14	32.6±0.3	32.7±0.4	32.2±0.5	0.6267302 ns
Prob (t > t obs)		*0.5581532421 ns*	*0.55118241 ns*	*0.4850108418 ns*	*0.0474474501*
WBC ($x10^3$/mm^3)	D-0	8.5±1.4	8.1±1.1	5.9±1.5	0.4138109 ns
	D-14	9.2±1.6	9.6±2.1	10.8±2.3	0.8468380 ns
Prob (t > t obs)		*0.7917253080 ns*	*0.52543673 ns*	*0.1473911863 ns*	-
Lymphocytes ($x10^9$/L)	D-0	5.9±0.9	6.1±0.9	4.6±1.1	0.5231668 ns
	D-14	6.7±0.8	6.2±0.7	6.6±0.9	0.9009679 ns
Prob (t > t obs)		*0.6182950926 ns*	*0.92743363 ns*	*0.2776020865 ns*	-
Platelets ($x10^9$/L)	D-0	831.7±92.6	694.7± 86.0	737.0±81.9	0.5548793 ns
	D-14	794.0±92.0	753.5±55.5	799.5±34.5	0.8678325 ns
Prob (t > t obs)		*0.8024068341 ns*	*0.65363137 ns*	*0.6058453380 ns*	-
Neutrophils ($x10^8$/L)	D-0	1.0±0.3	1.0±0.1	0.6±0.1	0.9400640 ns
	D-14	13.7±4.5	23.8±10.1	27.1±10.9	0.6036191 ns
Prob (t > t obs)		*0.0337727729 **	*0.05448920 ns*	*0.0474474501 **	-
Eosinophils ($x10^8$/L)	D-0	0.4±0.1	4.9±0.1	4.2±0.1	0.9399052 ns
	D-14	3.1±0.1	3.5±0.2	5.6±0.1	0.1696754 ns
Prob (t > t obs)		*0.0007253174 ****	*0.05045042 ns*	*0.0003589718 ****	-
Monocytes ($x10^9$/L)	D-0	5.9±0.1	5.7±0.2	5.8±0.2	0.9555026 ns
	D-14	8.1±0.3	7.4±0.2	9.5±0.1	0.8645517 ns
		0.4602400616 ns	*0.31445383 ns*	*0.3774102532 ns*	

Table 3 -Effect of acute toxicity study of *TR-Y017* on Hematologic parameters in Control and Treated animals. Results were expressed as the mean ± S.E.M of 5 rats in each group.

Hematological parameters: RBC: red blood cells, WBC: white blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin levels; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width.

Means with the same letter are not significant difference at 5%; ** and * indicate respectively a significance at 1% and 5% then ns as non-significance at 5%. Prob (F > F obs) expressed p-values at D-0 and at D-14; Prob (t > t obs) expresses p-values between D-o and D-14

Hematologic parameters	**Days**	**Control Group**	***TR-Y017* doses (mg/kg b.w.)**			**Prob (F > F obs)**
RBC ($x10^{12}$/L)	D-0	6.0±0.1	5.7±0.1	5.8±0.2	6,1±0.2	0.945376976 ns
	D-28	6.3±0.2	6.5±0.3	6.4±0.1	6.2±0.1	0.7517396 ns
Prob (t > t obs)		*2.237832e-01 **	*1.372529e-01 ns*	*3.475295e-01 ns*	*5.913796e-01 ns*	-
Haemoglobin (g/dL)	D-0	12.3±0.2	12.4±0.3	11.9±0.7	12.6±0.4	0.790904708 ns
	D-28	14.4±0.3	14.6±0.5	14.9±0.5	13.9±0.4	0.3594851 ns
Prob (t > t obs)		*5.650525e-04 ****	*6.330216e-03 ***	*1.377269e-02 **	*3.464113e-02 **	-
Hematocrit (%)	D-0	32.3±0.6	32.5±0.6	32.5±2.6	33.3±0.6	0.967558163 ns
	D-28	5.1±0.2	5.4±0.5	4.7±0.8	4.8±0.1	0.7801255 ns
Prob (t > t obs)		*1.313519e-08 ****	*3.969029e-08 ****	*5.746089e-05 ****	*9.266896e-09 ****	-
MCV (fL)	D-0	54.3±0.2	58.8±2.4	56.0±0.8	55.5±0.6	0.714243345 ns
	D-28	55.8±0,25	57.8±1,18	56.3±0.8	56.5±0.9	0.4548558 ns
Prob (t > t obs)		*6.037057e-03 ***	*2.333388e-01 ns*	*8.394208e-01 ns*	*4.197531e-01 ns*	-
MCH (pg)	D-0	20.0±0.4	21.0±0.4	20.0±0.4	20.3±0.3	0.242760713 ns
	D-28	25.5±1.6	22.5±0.6	23.7±0.6	22.3±0.8	0.1586694 ns
Prob (t > t obs)		1.703075e-02 *	9.716018e-02 ns	2.452342e-03 **	6.563885e-02 ns	-
MCHC (g/dL)	D-0	37.4±0.5	37.2±0.6	36.0±0.7	37.0±0.4	0.355555840 ns
	D-28	40.5±0.4	39.3±1.1	41.5±0.6	39.3±0.9	0.1834668 ns
Prob (t > t obs)		*1.755278e-03 ***	*1.677142e-01 ns*	*1.210357e-03 ***	*5.497646e-02 ns*	-
WBC ($x10^3$/mm^3)	D-0	6.1±0.0	7.9±1.5	8.1±1.8	7.2±0.8	0.660438537 ns
	D-28	7.3±1.1	9.9±2.0	8.7±0.8	7.4±0.7	0.4522335 ns
Prob (t > t obs)		*2.967244e-01 ns*	*4.459064e-01 ns*	*7.697127e-01 ns*	*8.290795e-01 ns*	-
Lymphocytes ($x10^9$/L)	D-0	4.8±0.1	5.7±1.0	6.2±1.4	5.7±0.5	0.747218995 ns
	D-28	5.6±0.9	6.9±1.1	6.7±1.5	5.8±0.4	0.6963707 ns
Prob (t > t obs)		*3.040782e-01 ns*	*4.557975e-01 ns*	*7.793327e-01 ns*	*9.950061e-01 ns*	-
Platelets ($x10^9$/L)	D-0	624.3±40.4	669.8±40.6	615.5±98.6	462.0±145.0	0.441002764 ns
	D-28	652.0±5.7	687.0±24.5	679.8±37.3	698.3±33.2	0.6908467 ns
Prob (t > t obs)		*5.213032e-01 ns*	*7.284504e-01 ns*	*5.646094e-01 ns*	*1.630226e-01 ns*	-
Neutrophils ($x10^9$/L)	D-0	0.5±0.0	0.7±0.2	0.6±0.1	0.5±0.1	0.428665622 ns
	D-28	0.4±0.1	1.3±0.5	0.8±0.3	0.6±0.2	0.1733055 ns
Prob (t > t obs)		*4.770521e-01 ns*	*2.862938e-01 ns*	*5.132593e-01 ns*	*6.090640e-01 ns*	-
Eosinophils ($x10^9$/L)	D-0	0.2±0.0 ab	0.1±0.0 bc	0.3±0.0 a	0.1±0.0 bc	0.003366999 **
	D-28	0.2±0.0	0.1±0.0	0.1±0.0	0,1275±0,0214	0.5722226 ns
Prob (t > t obs)		*4.471932e-01 ns*	*5.385175e-01 ns*	*1.711702e-01 ns*	*8.978851e-02 ns*	-
Monocytes ($x10^9$/L)	D-0	0.6±0.1	4.0±2.5	1.1±0.1	1.2±0.1	0.261084893 ns
	D-28	1.0±0.0	2.1±0.2	1.1±0.1	0.9±0.1	0.2223005 ns
Prob (t > t obs)		*2.778924e-02 **	*4.016307e-01 ns*	*8.891501e-01 ns*	*5.469250e-01 ns*	

Table 5- Effect of sub-acute toxicity study (28 days) of *TR-Y017* on Hematological parameters in Control and Treated rats. Results were expressed as the mean ± S.E.M. of 5 rats in each group.

Hematological parameters: RBC: red blood cells, WBC: white blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin levels; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width.

Means with the same letter are not significant difference at 5%; ***; ** and * indicate respectively a significance at 0.1%, 1% and 5% then ns as non-significance at 5%. Prob (F > F obs) expresses p-values at D-0 and at D-28; Prob (t > t obs) expresses p-values between D-o and D-28

Hematologic parameters	Days	Control Group	*TR-Y017* doses (mg/kg b.w.)			Prob (F > F obs)
ASAT (IU/L)	D-0	213.9±18.7	245.3±42.1	175.0±3.4	214.3±18.6	0.3073656351 ns
	D-28	123.2±7.6	140.1±8.2	126.2±5.1	120.0±12.3	0.410850739 ns
Prob (t > t obs)		*0.0040907261* **	*0.0493594627* *	*2.057461e-04* ***	*0.0054580155* **	
ALAT (IU/L)	D-0	762.6±44.4	63.05±80.5	833.7±35.3	1173.7±82.9	0.1606346236 ns
	D-28	139.64±8.44	160.5±25.1	140.1±14.1	156.7±17.6	0.757790049 ns
Prob (t > t obs)		*0.4198430968 ns*	*0.2522968060 ns*	*3.291868e-01 ns*	*0.6438624903 ns*	-
ALP (IU/L)	D-0	150.4±9.2 b	120.6±19.1 b	101.5±33.5 b	166.6±10.1 a	0.0004792472 ***
	D-28	275.9±33.1 b	328.5±31.3 ab	340.9±15.4 ab	417.0±36.3 a	0.041031019 *
Prob (t > t obs)		*0.0001202418* ***	*0.0120847033* *	*1.535025e-05* ***	*0.0001593489* ***	-
CREA (mg/L)	D-0	4.3±0.4	6.5±0.4	6.4±0.4	9.5±2.7	0.1296583864 ns
	D-28	4.3±0.4	4.1±0.5	3.9±0.5	4.0±0.8	0.928886502 ns
Prob (t > t obs)		*0.9967564259 ns*	*0.0061602162* **	*5.019761e-03* **	*0.1011117250 ns*	-

Table 6 - Effect of Sub-acute toxicity study of *TR-Y017* on Biochemical parameters in Control and Treated Rats. Results were expressed as the mean ± S.E.M. of 5 rats in each group

Biochemical parameters: CREA= Creatinine; ASAT= Aspartate transaminase; ALAT= Alanine aminotransferase;

Means with the same letter are not significant difference at 5%; *** and ** indicate respectively a significance at 0.1% and 1% then ns as non-significance at 5%. Prob (F > F obs) expresses p-values at D-0 and at D-28; *Prob (t > t obs)* expresses p-values between D-o and D-28