Toxicology: Open Access

Saganuwan, Toxicol Open Access 2018, 4:1
DOI: 10.4172/2476-2067.1000136

Research Article Open Access

Safety Evaluation of Rayner's Flavor Banana® in Swiss Albino Mice

Saganuwan Alhaji Saganuwan*

Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, P.M.B. 2373, Makurdi, Benue State, Nigeria

***Corresponding author:** Saganuwan Alhaji Saganuwan, Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, P.M.B. 2373, Makurdi, Benue State, Nigeria, Tel: +2348027444269, +2347039309400; E-mail: pharn_saga2006@yahoo.com

Received date: June 27, 2018; **Accepted date:** August 03, 2018; **Published date:** August 06, 2018

Abstract

Safety evaluation of Rayner's Flavor was carried out in Swiss albino mice and 52 mice were used for the evaluation. Forty out of 52 mice were used for determination of median lethal dose (LD_{50}) using modified arithmetical method of Reed and Muench. The LD_{50} was determined at dose rate of 0.35 ml. Another 12 mice were divided into two groups of six per group. The first and second groups were administered Rayner's flavor® (0.05 ml) and water (0.05 ml) respectively. Blood samples were collected for hematology and biochemistry. Haematology revealed significant ($P<0.05$) increased PCV and neutrophils of the group administered Flavor. But biochemistry showed increased levels ($P<0.05$) of SG0T, sodium ion and decreased levels of bicarbonate ion (HCO^-_3), chloride ion (Cl^-) and urea, respectively. More so, Rayner's flavor® caused dullness, dizziness, gasping for air, jerking movement, lying in ventral recumbency, shallow respiration and death. Hence, caution should be exercised when using Rayner's flavor®.

Keywords: Safety; Toxicity; Rayner's flavor®; Albino mice

Introduction

Flavor agents are synthetic chemicals that mimic natural flavors, which are characterized by combination of smell, taste and mouth-feel. Cooking and processing food involves the use of chemical technology to create a harmonious product using color, smell, taste, texture and mouth-feel [1]. However, Rayner's flavor® contains propylene glycol, water, E102 and E110 manufactured in England by Rayner Company Limited. Flavor agents are typically named after initial extract prepared from the source material and have been classified into three classes: those with simple chemical structures, efficient modes of metabolism and low order of toxicity (class 1) and class II agents have structural features that are less innocuous than those in class I and class III agents have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity [2]. Nevertheless, acute toxicity tests are now used to establish an approximate lethal dose of a compound in different species using different routes. The intravenous route should usually be used, since it gives best estimate of intrinsic acute toxicity uncomplicated by such factor like absorption, as the plasma concentration of a compound is a more relevant parameter than the administered dose [3].

Propylene glycol, a component of Rayner's flavor is a clear, viscous liquid, miscible with water used in cosmetics and as occlusive dressing for ichthyosis [4]. The physical properties of propylene glycol are similar to those of ethyl alcohol but it is much less toxic. Hence, it is used as solvent, cosmetic, lotion, ointment, and in food material. Its elimination is slower and its actions prolonged [5]. Percutaneously absorbed propylene glycol is oxidized in liver to lactic acid and pyruvic acid with subsequent utilization in general body metabolism [6].

Since many people are consuming Rayner's flavor, either according to the dosage guides given by Rayner's company or otherwise there is need for determination of safety level of the flavor.

Methodology

Rayner's flavor banana® was purchased from a provision shop in Makurdi, Benue State, Nigeria. The contents of the flavor were propylene glycol, water, E102 (tartrazine) and E110 (synthetic coal tar). Fifty-two Swiss albino mice of either sex weighing 25 ± 2.5 g were used for the study. The mice of about 8-weeks-old were purchased from Veterinary Research Institute (NVRI) Vom, Plateau state, Nigeria. They were acclimatized for a period of 2 weeks and fed Grower's marsh® formulated by Grand Cereals and Oil-mills Limited in Jos, Nigeria. Forty out of 52 mice were used for determination of median lethal dose (LD_{50}) using modified arithmetical method of Reed and Muench [7]. Forty mice were divided into four groups of ten each. The 1st, 2nd, 3rd and 4th group was administered the flavor at 0.00, 0.05, 010 and 0.75 ml/25 g of mice, intravenously. All the animals were observed for a period of 48hrs and thereafter for 12 days for observation of toxicity signs including death. The remaining 12 mice were divided into two groups of six each. First group was administered 0.05 ml of Rayner's flavor® intravenously while the 2nd group was administered distilled water (0.05 ml) intravenously. One day after, blood samples (0.5 ml) were collected into ethylene diammine tetraacetate (EDTA) bottles for hematology and serum biochemistry. All the experimental animals were observed for toxicity signs. Total blood cells count was done using the method of Baker [8]; whereas biochemical parameters were quantitatively determined using hemanalyzer [9].

Statistical analysis

Hematological and biochemical parameters were expressed as mean ± S.D. Tests for significance between mean parameters in respect of control and experimental values were performed using student t-test unpaired [10].

Results

The results of median lethal dose (LD_{50}) determination showed 6, 2 and 5 deaths after 48 hours when 0.1, 0.05 and 0.75 ml was administered. LD_{50} of Rayner's flavor was determined at dose rate of 0.35 ml (Table 1). Other toxicity signs observed are weakness, dullness, dizziness, gasping for air, jerking movement, lying ventrally, shallow respiration and death.

Group	Dose (ml/25 g mouse)	No. of Mice	No. of death	No. of survivals
1	0.00	10	0	10
2	0.05	10	2	8
3	0.10	10	6	4
4	0.75	10	5	5

Table 1: Median lethal dose (LD_{50}) determination of Rayner's flavor in Swiss albino mice.

$$Median\ lethal\ dose\ (LD_{50}) = \frac{0.00 + 0.05 + 0.10 + 0.75}{0 + 2 + 6 + 5} \times 5$$
$$= 0.35 \quad ml$$

LD_{50} of the Rayner's flavor was 0.35 ml per 25 g of mouse. This translates to 14 ml per 1 kg signifying high safety profile of the flavor.

Parameters (%)	Control	Experimental
Packed cell volume	32.00 ± 3.46	38.66 ± 5.16[a]
White blood cells	40.50 ± 14.40	44.16 ± 12.10
Neutrophils	43.00 ± 7.48	48.16 ± 12.04[a]
Lymphocytes	54.66 ± 4.54	48.00 ± 12.24
Eosinophils	2.00 ± 0.89	1.83 ± 0.75
Monocytes	2.00 ± 0.89	2.00 ± 0.89
Basophils	0.00 ± 0.00	0.00 ± 0.00
t-test level of significance=5%		

Table 2: Effects of single intravenous bolus of Rayner's flavor on hematological parameters of Swiss albino mice.

Parameters	Control	Experimental
Total protein (g/L)	74.80 ± 8.81	71.50 ± 3.11[b]
Albumin (g/L)	3.34 ± 0.48	3.60 ± 0.42
Alkaline phosphatase (µg/L)	112.2 ± 8.31	104.25 ± 19.82[b]
Total bilirubin (µmol/L)	14.02 ± 1.40	14.12 ± 0.85
Conjugated bilirubin (µmol/L)	2.75 ± 0.49	3.10 ± 0.28
Serum glutamic oxaloacetic transaminase (ug/L)	7.17 ± 1.89	9.27 ± 3.45[a]
Serum glutamic pyruvic transaminase (ug/L)	9.25 ± 1.89	6.05 ± 2.92[b]
Sodium ion (mmol/L)	117.00 ± 7.21	135.20 ± 4.55[a]
Potassium ion (mmol/L)	3.72 ± 0.94	3.94 ± 0.6
Chloride ion (mmol/L)	111.6 ± 12.13	102.20 ± 2.05[b]
Bicarbonate ion (mml/L)	25.6 ± 1.34	21.40 + 2.07[b]
Urea (mmol/L)	99.2 ± 16.39	81.10 + 18.87[b]
t-test level of significance=5%; [a]Significantly higher (P<0.05); [b]Significantly lower (P<0.05)		

Table 3: Effects of Rayner's flavor®on biochemical parameters of Swiss albino mice.

Hematology revealed significantly (P<0.05) increased level of packed cell volume and neutrophils in the group administered Rayner's Flavor. Other hematological parameters were not affected significantly (p>0.005) (Table 2). Serum biochemistry revealed significantly (P<0.05) increased SGOT, sodium ion and decreased alkaline phosphatase, SGPT, chloride ion, bicarbonate ion and urea, respectively (Table 3).

Discussion

The median lethal dose (0.35 ml) of Rayner's flavor® determined in Swiss albino mice agrees with the report of Dreisbach et al. indicating that LD_{50} is the amount of chemical that will kill approximately 50% of a group of test animals. Humburger reported that the ability of a compound to cause death in half of the animals when a certain dose is administered defines the toxicity of the compound. However, the dangerous dose for humans may be 1%-10% more or less of the indicated dose [11]. In the present study the LD_{50} (0.35 ml) translates to 14 ml/kg, suggesting relative safety of the flavor. Despite the fact that the concentration of Rayner's flavor in gram was not known, our finding agrees with the report of Newbold, indicating that chemical does not become a medicine until the potential utility observed in the laboratory has been proven in clinical trials and regulatory agencies have accepted the results of exhaustive tests for efficacy, safety and quality [12]. Our observation of other toxicity signs such as weakness, dullness, dizziness, gasping for air, jerking movement, lying in ventral recumbency, shallow respiration and death disagrees with the report of WHO indicating that class 1 flavor agents have simple chemical structure, efficient modes of metabolism and low order of toxicity. But the long observed central nervous system (CNS) effects in the experimental animals may be due to the presence of propylene glycol. This agrees with the report of Hardman et al. indicating that elimination of propylene glycol is slower and its actions are thus prolonged. This in-turn is confirmed by the report of Dreisbach et al. indicating that propylene glycol has a fatal dose of 100 mg/day with CNS effects including convulsions. Hence, the concentration of propylene glycol in Rayner's Flavor may be relatively high. Moreover, the coloring agents (E102 and E110) may also be relatively toxic. E110 approved by European Union (EU) as synthetic colorant, is a yellow synthetic coal tar, highly soluble in water. Saganuwan reported that coal tar may be converted to central nervous system depressant. E102 (tartrazine), a synthetic azo dye may cause CNS depression and allergic reaction [13]. Therefore, toxicity study of chemicals is a scientific process necessary for identification of a potential toxicant [14,15]. Hence, there is need for Rayner Company Limited to give elaborate explanation of the contents.

The increased packed cell volume observed in the group administered flavor (38.66% ± 5.16%) as compared to the group

administered water (32.00% ± 3.46%) is suggestive of hemopoietic effect of the flavor. But the increased neutrophils observed may be suggestive of immune-stimulatory activity of the flavor. The significant increased level of SGOT and sodium ion in the flavor group as compared to the water group, may suggest that the flavor have effect on liver and heart. But the significant decreased level of bicarbonate ion may suggest the ability of Rayner's flavor® to cause metabolic acidosis. But the decreased urea, chloride ion and bicarbonate ion may suggest safety of the kidney. Hence, the use of Rayner's flavor® by public should be considered as an issue that is worthy of caution.

Conclusion

The median lethal dose of Rayner's flavor was estimated to be 0.35 ml in Swiss albino mice. Other toxicity signs include weakness, dullness, dizziness, gasping for air, jerking movement, lying in ventral recumbency, shallow respiration and death. The Flavor caused increase packed cell volume, sodium ion (Na^+) and decreased bicarbonate ion (HCO_3^-), chloride (Cl^-) ion and urea. Hence the use of Rayner's flavor® by public should be reviewed. I recommend that the Rayner Company Limited should conduct a study on safety evaluation and also give explanation in relation to the contents of Rayners' flavor®.

References

1. Foskett D, Ceserani V, Kinton R (2003) The Theory of Catering. (10th edn), Hodder & Stoughton, London, UK.
2. WHO (2005) Evaluation of certain food additives. 63rd Report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva.
3. Brimblecombe RW, Dayan AD (1993) Preclinical toxicity testing In: Burley DM, Clarke JM, Lusanga L. Pharmaceutical Medicine. Great Britain.
4. Tripathi KD (2003) Drugs acting on skin and mucous membrane.In: Essentials of Medical Pharmacology. (5th edn), JP Medical Ltd, London, UK.
5. Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG (1996) Non-metallic environmental toxicants. In: Goodman & Gilman's The Pharmacological Basis of Therapeutics. (9th edn), McGraw-Hill, London, UK.
6. Katzung BG (2004) Dermatologic pharmacology. In: Basic and Clinical Pharmacology. (9th edn.) McGraw-Hill, London, UK.
7. Saganuwan SA (2011) A modified arithmetical method of Reed and Muench for determination of a relatively ideal median lethal dose (LD50). Afr J Pharm Pharmacol 5: 1543-1546.
8. Baker FJ (1985) The Full blood counts. In: Baker FJ, Silverton RE (eds.) Introduction to Medical Laboratory Technology. (6th edn). Butterworth & Co. Ltd, London, UK.
9. Willard MD, Tvedten H, Turnwald GH (1989) Small Animal Clinical Diagnosis by Laboratory Methods. (5th edn), WB Saunders Company, Philadelphia.
10. Petrie A, Watson P (2002) Hypothesis test 1 – The test comparing one or two means. Statistics for Veterinary Science. (3rd edn), Blackwell science Ltd., London, UK.
11. Dreisbach RH, Robertson WO (1987) Alcohols and glycols. Handbook of Poisoning: Prevention, Diagnosis & Treatment. (13th edn), Appleton & large, London, UK.
12. Newbold BB (1993) How are medicines discovered. In: Burley DM, Clarke JM, Lusanga L (eds.) Pharmaceutical Medicine, Great Britain.
13. Saganuwan SA (2017) Functional chemical groups that may likely become source for synthesis of novel central nervous system (CNS) acting drugs. Cent Nerv Syst Agents Med Chem 17: 1-9.
14. Saganuwan SA (2017) Therapeutic causes of Stevens Johnson syndrome: A mini review. Open Acc J of Toxicol 1: 1-4.
15. Saganuwan SA (2017) Toxicity studies of drugs and chemicals in animals: An overview. Bulg J Vet Med 18: 1-18.