

DETERMINATION OF MEDIAN LETHAL DOSE OF ETHANOLIC EXTRACT OF *SESAMUM INDICUM* SEEDS IN ADULT WISTAR RATS

Yusuf M.K, Ibegbu AO, Agbon AN, Evang A

Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria, Nigeria

Corresponding Author: Yusuf M.K

Email: maylkabiru2008@yahoo.com

ABSTRACT

The aim of this research was to determine the median lethal dose of *sesamum indicum* seeds in Wistar rats.

Methods: Twelve Wistar rats within the ages of five to six weeks and of weights, ranging from (98-160) g of both sexes were used for the experiment. They were categorized into two phases of I and II. Phase I consisted three (3) groups of three (3) animals each. While phase II consisted three (3) groups of one (1) animal each. The phase I group was orogastrically intubated with (10, 100 and 1000) mg/kg body weight of ethanolic extract of *sesamum indicum* seeds respectively with the aid of a 2ml syringe with an intubation needle of size 24 mounted on it. The animals in phase II were similarly administered 1600, 2900 and 5000mg/kg body weights of the extract respectively and observed for one hour and ten minutes after every two hours for twenty four hours.

Result: No mortality or any physical signs of toxicity were observed in any of the groups.

Conclusion: The LD50 of ethanolic extract of *sesamum indicum* seeds is above 5000mg/kg body weight in adult Wistar rats.

Key Words: LD50, Sesamum seeds, Wistar rats

INTRODUCTION

Background Information

Over the years, there has been increased scientific research to minimize the health hazards potentiated by toxins that are present in some consumables of man. These were done on phytochemicals extracted from plant species (Bankole et al., 2007). Flavonoids, anthocyanins, vitamins C and E, phenolic compounds, dietary fiber and carotenoids have been reported to be the chief constituents present in fruits and vegetables (Amado, et al., 1990). One of such vegetable plants is *Sesame indicum* whose taxonomic Order is *lamiales*, Family is *pedaliaceae*, Genus is *sesamum* and Species is *indicum*. Sesame seeds exist in brown, black and yellow forms. Sesame seed has been reported to contain high level of unsaturated fatty acids which are Oleic acid-38.84% and linolenic acid-46.26 % (Nzioku et al., 2009). Trace elements such as Calcium, Iron, Magnesium, Zinc, Copper and Phosphorous are

reported to be contained in it (Obiajunwa et al., 2005). Sesame seed is also reported to be rich in phytoestrogenic lignans which is an important phytochemical known to man (Thompson et al., 1991). Sesame seeds oil has been used as healing oil for thousands of years; it naturally possesses antibacterial property for common skin pathogen such as staphylococcus and streptococcus (Amir, 2008). It also possesses anti-fungal, antiviral and anti-inflammatory effects (Amir, 2008). The entire sesame plant is very valuable and serves as staple food in most ethnic groups in north central and south western part of Nigeria and this may be attributed to the high level of fecundity among adult males in these regions (Akpan et al., 2006). Research on the median lethal dose of the ethanolic extract of *sesamum indicum* seed in wistar rats are not readily available as related research were conducted either on mice or the leaf extract of sesame plant. Therefore, the need

to conduct a research on the seeds extract on rat models is required.

METHODS

Animals

A total number of twelve adult Wistar rats within the age of (5-6) weeks and of weight ranging from (98-168) g of both sexes were used for the experiment, they were procured from the Department of Human Anatomy, ABU Zaria and kept under good laboratory conditions and allowed free access to food and water.

Plant

Sesamum indicum seed was obtained from samaru market- Zaria, Nigeria and authenticated in the herbarium of the Department of Biological Sciences, ABU Zaria with voucher number 4. The sesame seed was macerated using 95% ethanol to obtain its extract at the Department of Pharmacology, ABU Zaria and preserved in an air tight container at room temperature.

Ethanollic Extract of *Sesamum Indicum* Seeds

453g of *sesamum indicum* seeds was macerated after determining the weight using a chemical balance. A scale pan of 25×75cm in dimension was initially weighed and the pan was filled with sesame seed and re weighed. The weight of the sesame seed was determined from the formula: Weight of extract+ weight of scale pan- weight of scale pan= (473-20) g = 453g. A total yield of 45.1g was obtained.

Maceration

The sesame seed was poured into a two (2) ml maceration apparatus (pyrex). The macerated seed was later transferred into a beaker.

Addition of Ethanol

3.5l of 95% w/v of ethanol was used to mix the macerated seeds; it was stirred and left for six (6) hours to allow the supernatant to collect above the surface of the ethanol. The supernatant was sieved –off using a metal grid sieve while the filtrate was collected into a separate beaker.

Drying

The supernatant was collected into an evaporating dish and placed on a water bath (Gallen Kamp) and heated to a temperature of 60°C to remove the ethanol. The extract was kept

in an open space and left there for eighteen (18) hours to allow evaporation to take place.

Packaging of Extract/Weight

A spatula was used to collect the extract into a 20ml weighed beaker (pyrex), covered with a foil paper and preserved at normal room temperature. The weight of the extract was determined using a chemical balance.

Determination of the Median Lethal Dose

The median lethal dose of the ethanolic extract of *sesamum indicum* seed was determined using Lorke`s method (1983). The animals were categorized into two phases of I and II. Phase I consisted three (3) groups of three (3) animals each. While phase II consisted three (3) groups of one (1) animal each. The phase I groups were orogastrically intubated with (10, 100 and 1000) mg of ethanolic extract of *sesamum indicum* seed respectively with the aid of a calibrated 2ml syringe with an intubation needle of size 24 mounted on it. The animals in phase II were similarly administered (1600, 2900 and 5000) mg of the extract respectively.

Determination of the Equivalent Dose of *Sesamum Indicum* in Millilitres

The total weight of the extract for the research was obtained by summing up all the administrable dose based on lorke`s method which is calculated as $10 \times 3 + 100 \times 3 + 1,000 \times 3 + 1,600 + 2,900 + 5,000 = 12,830\text{mg}$. 12,830mg was measured using a chemical balance and 1ml of tween -80 (polysorbate) was added to decrease the viscosity or enhance the vehicular movement of the extract considering it`s lipid nature. The concentration obtained was 12,830mg/ml which is equal to 13.297ml when measured using a measuring cylinder. The equivalent dose of 10mg in ml was measured and the value 0.0104ml was obtained. Similar measurement was made for other doses and the values (0.104, 1.04, 1.66, 3.00 and 5.20) ml was obtained respectively. The formula below was also used to confirm the measurement.

$$\text{Volume (ml)} = \frac{\text{Dose} \times \text{weight (kg)}}{\text{Stock concentration}}$$

RESULTS

No mortality was recorded in any of the groups; no physical signs of toxicity were equally recorded. However, some of the general observations made are tabulated below.

Table 1: Observations on intake of ethanolic extract of *sesamum indicum*

No. of Animals	Dose (mg)	General observation	Physical signs of toxicity	Mortality
3	10	Apparently healthy and active	No observable signs of toxicity	Nil
3	100	Apparently healthy and active	No observable sign of toxicity	Nil
3	1000	Apparently healthy and partially inactive	No observable sign of toxicity	Nil
1	1,600	Apparently healthy, inactive and frigid	No observable signs of toxicity	Nil
1	2,900	Apparently healthy and partially in active	No observable sign of toxicity	Nil
1	5000	Apparently healthy and presence of goose hair	No observable signs of toxicity	Nil

DISCUSSION

From the present study, there were no observed physical signs of toxicity at any stage of the experiment; no mortality was equally recorded on administration of all the doses. At a dose of 5000mg/kg body weight, the ethanolic extract of *sesamum indicum* seeds was observed to be safe for consumption in Wistar rats. The United States National Library of Medicine in 2015 reported that Lethality (Death) in living organisms among other factors is a consequence of necrotic processes i.e cell injury. One of the key mechanisms leading to necrotic changes in multicellular organisms is oxidative stress which is caused by either over production of ROS (Reactive Oxygen Species) or under production of antioxidant defense mechanism. Vishwanath et al., (2012), had reported that *sesamum indicum* seeds showed antioxidant activity by inhibiting lipid peroxidation through the release of large scavenging hydroxyl radical and potent scavenger of superoxide anion. The reducing power of the seeds` extract was in substantiation with the anti-oxidant property and the ferrous ion chelating effect by the extract. It can therefore be believed that, sesamin which is an active component of the extract may have released large quantity of superoxide anion and ferrous ion which may have inhibited oxidative stress by its chelating effects, consequently, exhibiting the anti-oxidant effects and thus preventing necrosis which translated into no lethality in the Wistar rats as observed in Table 1. This observation is not in line with the work of Okon et al, 2013 who reported that the LD₅₀ ethanolic extract of

Sesamum indicum seeds in Albino rats is 1732.1mg/kg. The possible reason could be as a result of differences in the specie of rats used in the experiment. However, this work is in line with the work reported in USA national technical information service that the LD₅₀ of ethanolic extract of *Sesamum indicum* seeds in mouse via intraperitoneal administration is greater than 5000mg/kg body weight. This research is also similar with the work reported by the United States National Library of Medicine in the journal of American college of toxicological that the LD₅₀ of ethanolic extract of *Sesamum indicum* seeds in rabbit intradermally is above 2000mg/kg bwt.

Conclusion

The LD₅₀ of ethanolic extract of *sesamum indicum* seeds in Wistar rats is greater than 5000mg/kg body weight. It can also be concluded that at a dose of 5000mg/kg body weight, the ethanolic extract of *sesamum indicum* seeds is safe for consumption in Wistar rats and an arbitrarily value may be chosen as a high or low dose for a research on the extract.

REFERENCES

- Akpan IG, Idowu AA, Misani SM (2006). Collection and evaluation of *Sesamum Spp.* germplasm in Nigeria. IGPR/FAO.142: 59-62
- Amado JA, Pesquera C, Gonzalez EM, Otero M, Freijanes J, Alvarez A, (1990). Postgraduate Medical Journal 66 (773): 221-3

- Amir A. (2008). Ketoconazole induced testicular Damage in rats reduced by Gentiana extract. *Experimental Toxicological Pathology* 59(6):377-84
- Bankole MA, Shittu LA, Ahmed TA, Bankole MN, Shittu RK, Ashiru OA (2007). Synergistic antimicrobial activities of phytoestrogen in crude extract of two sesame species against some common pathogenic micro-organisms. *African journal of traditional complimentary Alternative Medicine*.4 (4): 427-433
- Lorke`s D (1983). A new approach to tropical acute toxicity testing. *Archive of toxicology*. 53:275-287
- Nzioku JM, (2009). Chemical composition on the seed and oil of sesame (*Sesame indicum* L.) grown in Congo-Brazzaville. *Advanced Journal of Food and science Technology* 1 (1):6-11
- Okon JE, Umoh NS, (2013). Influence of *Sesamum indicum* L. ethanolic leaf extract on haematological parameters on albino rats in akwa ibom state, Nigeria. <http://www.cibtech.org/jls.htm>. Vol. 3 (1): 106-110
- Okon and Umoh Short Communication 106 *Journal of Fundamental and Applied Life Sciences* ISSN: 2231-6345
- Obiajunwa EI, Adebisi FM, Omode PE (2005). Determination of essential minerals and trace elements in Nigerian sesame seeds using TXRF Technique. *Pakistan Journal of Nutrition* 4 (6):393-395.
- Thompson LU, Robb P, Serraino M, Cheng F (1991). Mamalian lignin production from various foods. *Nutrition Cancer* 16 (1):43-52.
- United States National Library of Medicine (1993). LD₅₀ of ethanolic extract of *Sesamum indicum* seeds in rabbit intradermally. *Journal of American college of toxicological*. Vol. 45, pg.261
- United State National Library of Medicine (2015 Necrotic processes)
- USA National technical information service (1993). LD₅₀ of ethanolic extract of *Sesamum indicum* seeds in mouse via intraperitoneal administration Volume AD: 691-490
- Vishwanath HS, Anilakumar KR, Harsha SN, Khanum F, Bawa AS (2012). Invitro antioxidant activity of *Sesamum indicum* seeds. *Asian Journal of Pharmaceutical and Clinical Research*. ISSN 0974-2441.