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Chapter 11

Acute Toxicity Profiles of Aqueous and Ethanolic Extracts of *Capsicum annum* Seeds from South Western Uganda

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Additional information is available at the end of the chapter

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1. Introduction

Capsicum annum belongs to the Kingdom Plantae plants, subkingdom of Tracheobiota (vascular plants), super-division of Spermatophyta (seed plants), division of Magnoliophyta (flowering plants), class Magnoliopsida (dicotyledons), subclass Asteridae, order Solanels, family Solanaceae (potato family), genus Capsicum L. (pepper), species *Capsicum annum* L. (Cayenne pepper) and variety *Capsicum annum* L. var annum (Cayenne pepper).

Capsicuma annum is a perennial shrub growing up to 2 m (6′) in height, having a woody trunk. It has green fruits that ripen to red. The active ingredient in the plant is *capsaicin* that is used for the management of various medical conditions. The varieties of this "fruit" vary greatly in size, colour and pungency. The plant extract that provides therapeutic action is the seed oil.

Globally, *Capsicum annum* has many uses. It is used as a feed additive and as spices. The corrosive nature of capsaicin, its gross irritating effects and its toxicities are well documented. A qualitative assessment of bioactive chemical compounds in *Capsicum annum* established the presence of reducing compounds, saponins, alkaloid salts, alkaloids, quarternary bases, anthracenosides, flavanosides, flavonds, coumourin derivatives, steroid glycosides and anthocyanosides

It is indeed true that *Capsicum annum* possesses many medicinal plant bioactive substances that are active against certain bacteria, viruses and protozoa [1-3]. In-vitro studies indicated that *Capsicum annum* fruits are efficacious against common bacteria e.g. Staphylococcus, Streptococcus species etc. The widespread use of *Capsicum annum* as an herbal remedy by smallholder poultry farmers is based on a wide range of doses [3]. A wide range of medici-

nal plant users do not believe that plant extracts can be toxic, yet standard laboratory tests [1, 3-5] indicate profound toxicity from some medicinal plants.

The smallholder farmers in the South Western Agro-ecological Zone of Uganda (SWAEZ) use *Capsicum annum* [6] as a medicinal plant for controlling various poultry diseases like Newcastle disease. They would pick 3-4 seeds of Capsicum annum seeds from their gardens or neighbours gardens and crush the harvested seeds in a cup and then mixed it in various proportions of water, from half a litre to up to two litres. The mixed extract is then given to the suspected sick birds infected with Newcastle disease. The birds may be assisted to drink the extract or the birds may drink it freely at will. The shelf life of the prepared extracts is usually one to at least two days then fresh mixtures are constituted depending on the severity and improvements in the health conditions of the bird(s).

Despite the plant and its compounds being used by the farmers, the acute toxicities of *Capsicum annum* extracted under aqueous and ethanolic solvent systems are not yet known. The study hypothesized that medicinal plant with unknown acute toxicity levels exist in the SWAEZ of Uganda. The study assessed the acute toxicity levels of *Capsicum annum* in the SWAEZ under aqueous and ethanolic solvent systems.

2. Materials and methods

2.1. Plant seed collection and preparation

The *Capsicum annum* seeds were collected from Rubindi Sub County of Mbarara district in the South Western Agro-Ecological Zone (SWAEZ). A total of 2 kilograms of the sample was harvested and labelled with the laboratory number (CA 001 Lag), date and kept in black a polythene bag in a cupboard. The seeds were packed in a paper box wrapped in polythene and taken to the Division of Pharmacology and Physiological Sciences, College of Veterinary Medicine, Animal Resources and Bio-security (COVAB), Makerere University (Uganda). The seeds were then dried at room temperature of 25^0C for 7 days. Afterwards, 200 gr were weighed for water extraction and another 200 gr for ethanolic extraction. The seeds were manually ground to fine powder using a mortar and pestle.

Weighing and extraction process: A total of 160 gr of the powdered sample was weighed and soaked in a dark bottle containing 1 litre of 70% ethanol with intermittent shaking for 72 hours. Similarly, 160 gr were weighed for extraction in 1 litre of water. After three days of extraction the extract was filtered using cotton wool fixed within a funnel. The filtrate was collected in a glass conical flask and labelled, and the residue discarded. The filtrate was then subjected to a rotary evaporator at reduced pressure and temperature for concentration to a constant volume. After concentration the extract was put in an oven at 45^0c until a semi-solid form of extract was obtained. This was put in bijous bottle, well labelled, and kept in a refrigerator at 4^0c for future use. The percentage yield (%yield) =10g×100/160=6.25%. The percentage yield (%yield) was 6.25% of the fruit extract.

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2.2. Acute toxicity study protocol

The ethical committee of Mbarara University of Science and Technology (MUST) approved the use of experimental animals for the experiments.

Swiss albino mice of 16-25 gr bodyweight from both sexes were selected and labelled using tail markers of different colours. The animals (n=30) were divided into five groups of six mice each and kept in different cages for easy observation.

The dose levels were determined after a preliminary acute toxicity trial, which had been carried out earlier. The doses rates were as shown in table 1.

Groups	Extracts Dose levels (mg/kg)		*n*	Nº dead	
	Aqueous (a)	*Ethanolic (b)*		Aqueous (a)	*Ethanolic (b)*
Group 1	10,000	3500	6	0	0
Group 2	12,000	5000	6	2	2
Group 3	14,000	6500	6	4	4
Group 4	16,000	8000	6	6	6
Control	1ml dH_20		6	0	0

Table 1. Preliminary acute toxicity levels of crude extracts of *Capsicum annum*

The volumes of the drug doses administered to the mice were calculated using the following formula [4]: Volume (ml) = body weight (kg) x dose levels (mg/kg)/stock drug concentration (mg/ml). The extracts were administered by gavage orally.

The derived dose levels for ethanolic extracts were 3,500, 5000, 6500 and 8,000 mg/kg and for aqueous extracts were 10,000, 12,000, 14,000 and 16,000 mg/ml.

Postmortem procedure: The numbers of mice which died after being subjected to the medicinal plant crude extracts were noted. Randomly selected animals (n=2) from each group were submitted to necropsy for organ collection. The surviving mice were sacrificed at the end of the experiment after observation for 7 days and subjected to post mortem analysis. Within 24hrs, organs from mice that died or were sacrificed were collected and fixed in jars containing formalin (10% formaldehyde) and subsequently submitted to routine paraffin embedding. The liver, kidney, lungs, intestines and brain were collected for histopathology analysis. Four (4) micrometer sections of the collected organs were routinely stained with haematoxylin and eosin for histological examination, performed by a Pathologist. Putative histopathological changes in the structural organization of the liver, kidney, lungs, intestines and brain were observed and recorded.

Data collection and analysis: Mortality (number of dead mice) were counted in each group and recorded. The median lethal dose that killed 50% of the test animals was determined

using the graphical and Probit analysis. Any signs of toxicity observed in the collected organs were also recorded.

Quality assurance: The calyces were shade dried to prevent loss of essential chemical components and the voucher specimen were taken to the herbarium (Biology Department, Mbarara University of Science and Technology) for identification and also to ensure the quality of the techniques adopted from world Health Organization (WHO) guidelines on herbal quality control.

Distilled water was used for extraction to prevent contamination of the extract. Both the aqueous and ethanolic extracts of *Capsicum annum* seeds were kept in sterilized bottles and placed in a refrigerator at 4°C to prevent mould formation. All the reagents used were analytical grade.

The Swiss albino mice of the same age were used for the study to minimize variation in the test results. The control group given 1 ml of distilled water was used for comparison with the groups given the plant extract.

3. Results

3.1. Aqueous acute toxicity profile of *Capsicum annum* seeds

The aqueous acute toxicity profile of *Capsicum annum* seeds under various dose levels is shown in table 2.

Extracts	Group	Dose (mg/kg)	log dose	dead/total	dead%	Probit
Aqueous	1	10,000	4.00	0/6	0	3.04
	2	12,000	4.08	2/6	33.33	4.53
	3	14,000	4.15	3/6	50.00	4.95
	4	16,000	4.20	4/6	66.66	5.36
	5	18,000	4.26	6/6	100.00	6.75
Ethanolic	1	3500	3.54	0/6	0	3.04
	2	5000	3.70	2/6	33.33	4.53
	3	6500	3.81	4/6	66.67	5.36
	4	8000	3.90	6/6	100	6.75

Table 2. Acute toxicity profile of aqueous and ethanolic *Capsicum annum extracts*

It was observed that it would take 12043 mg/kg to kill 50% of the test animals (Graph 1). This gives a wide safety margin for the lethal dose of *Capsicum annum* fruits extracts in test animals. This is the LD 50 for the aqueous extract; which is the most commonly method used by the local farmers.

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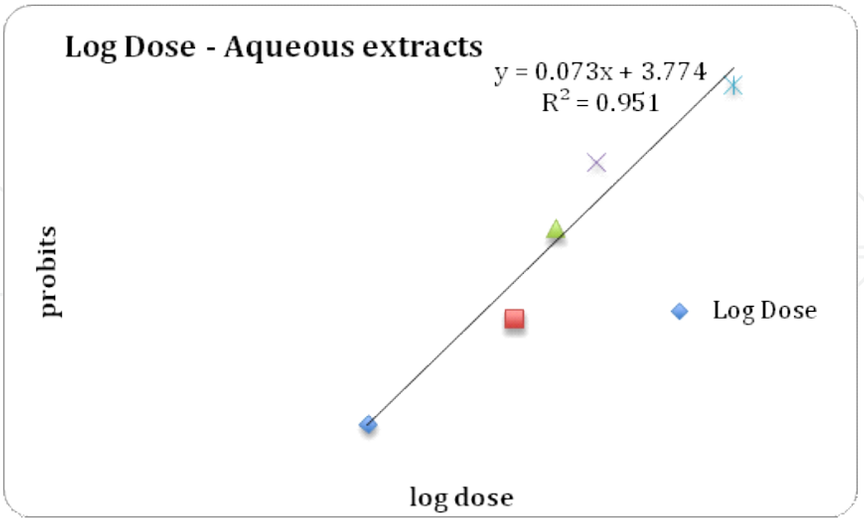


Graph 1. Probit for aqueous *Capsicum annum* extracts. From the equation 1**Y=mx-c**,where: **Y**=0.0737(4.15)+3.7749 = 0.305855+ 3.7749 = 4.080755; **Ld_{50}** = antilog of 4.080755 =12,043.563 mg/kg.

3.2. Toxicity signs observed with dose increase within the groups

There were notable side effects associated to higher doses of *Capsicum* extracts, such as limb movements, gut irritation, loss of balance, GIT muscle twitching. Hyperactivity was noted within 2 minutes after administration. Vocalization, urination, drowsiness, reddening of lips, dyspnoea and circling movement were noted and convulsions occurred before death.

3.3. Acute toxicity of 70% ethanolic extract of *Capsicum annum*

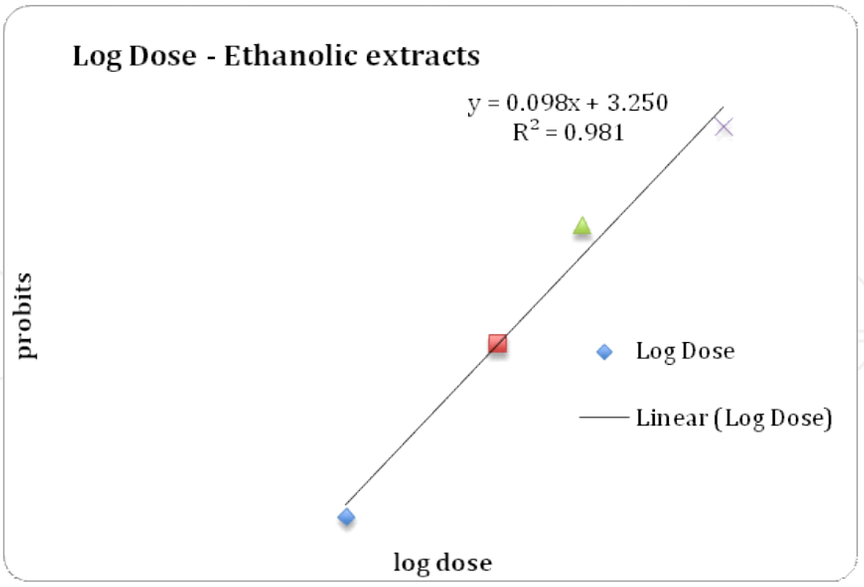
The ethanolic acute toxicity profile of *Capsicum annum* seeds under various dose levels is shown in table 2. The LD50 was calculated according to Fisher's and Yates as in Table 2. The lethal dose was established to be 5,492mg/kg (Graph 2).

3.4. Signs of acute toxicity observed

For the ethanolic extracts at a dosage of 3500mg/kg and 5000mg/kg, the following signs were observed: irritation shown by the use of forelimbs to scratch the areas of the mouth, gasping for air and dyspnoea. For aqueous extracts at a dosage of 6500mg/kg and 8000mg/kg there were observed twitching of GIT muscles, urination, dry mouth, reddening of lips and convulsions.

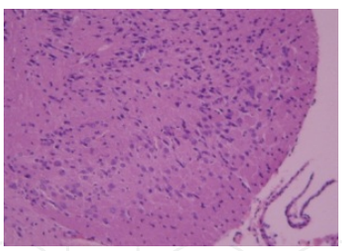
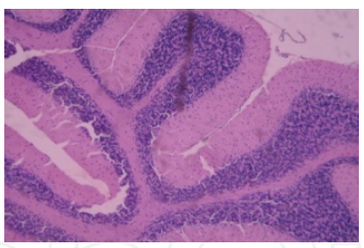
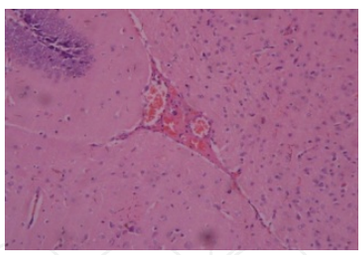
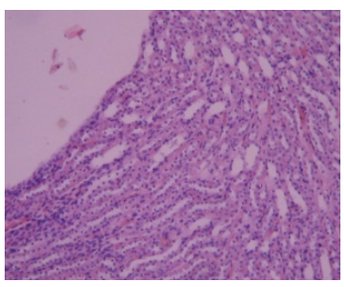
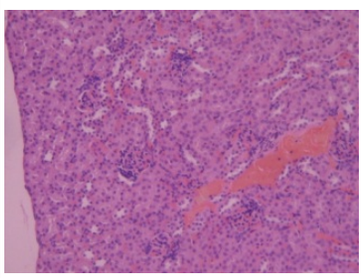
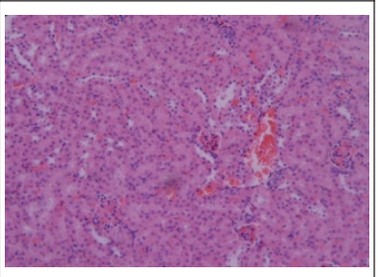
3.5. Comparison of key histo-pathological findings due to aqueous and ethanolic extracts

The key histopathological findings due to the effects of aqueous and 70% ethanolic extracts of *Capsicum annum* are detailed in table 3.



Graph 2. Probit for 70% ethanolic *Capsicum annum* extracts. From the equation 2**y=bx-c**, where **Y**=0.0989 (4.945) + 3.2507 = 0.4890605 + 3.2507 = 3.7397605..**Ld_{50}** = antilog (3.7397605) = 5492.3790

3.6. Histopathology findings

Organs examined	Control group	Toxic effects associated to Capsicum annum aqueous extracts	Toxic effects associated to Capsicum annum 70% alcohol extracts
Brain	 A	 B	 C
	A –Brain (Control). The scale bar is 100µm	B –No significant effects on the brain at 10,000mg/kg. The scale bar is 100µm	C –Slight congestion of the meninges at 3500mg/kg. The scale bar is 100µm
Kidney (Medulla)			 F

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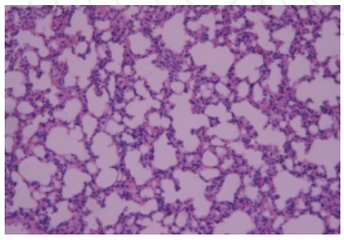
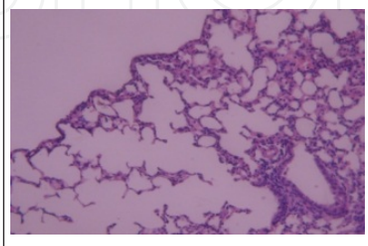
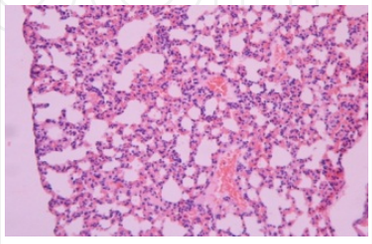
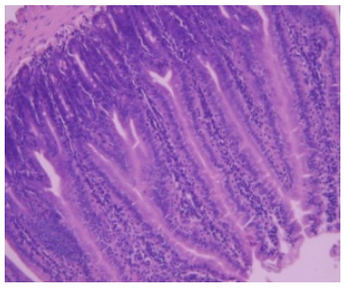
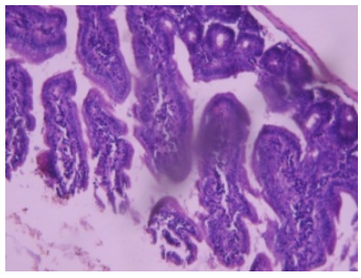
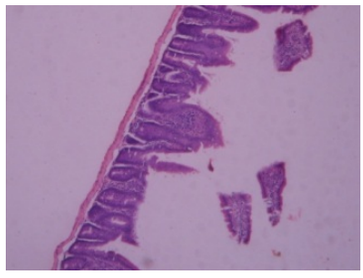
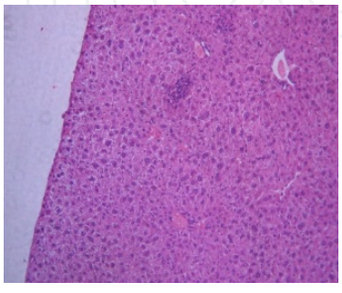
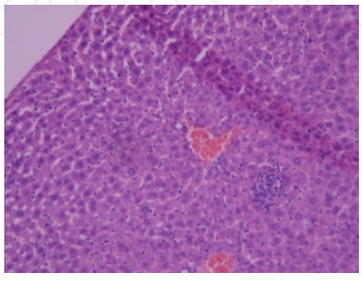
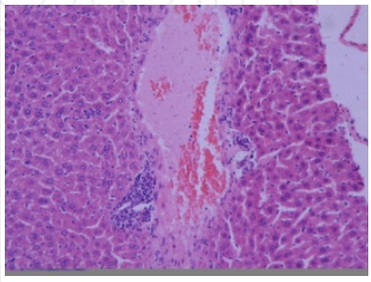
Organs examined	Control group	Toxic effects associated to Capsicum annum aqueous extracts	Toxic effects associated to Capsicum annum 70% alcohol extracts
	D	E	
	D –Kidney (Control) Medulla. The scale bar is 100µm	E –Kidney renal haemorrhages, congestion and tissue degeneration at 12,000mg/kg. The scale bar is 100µm	F –Haemorrhages and congestion at 5,00mg/kg. The scale bar is 100µm
Lungs	 G	 H	 I
	G –Lungs (Control). The scale bar is 100µm	H –Mild Lung emphysema noted at 10,000mg/kg. The scale bar is 100µm	I –Lungs haemorrhages and congestion at 5,000mg/kg. The scale bar is 100µm
Small intestine	 J	 K	 L
	J –Small intestine (Control). The scale bar is 100µm	K –Erosions of the small intestine at 14,000mg/kg. The scale bar is 100µm	L –Intestines villus sloughing off and mucosal erosion at 6,500mg/kg. The scale bar is 100µm
Liver	 M	 N	 O

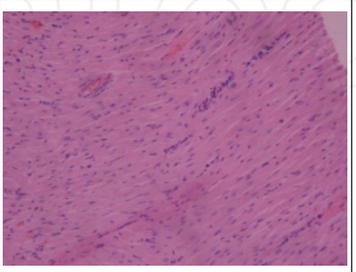
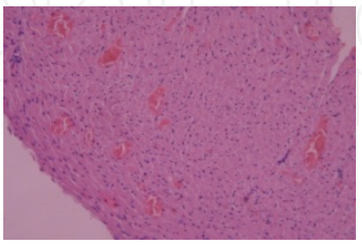
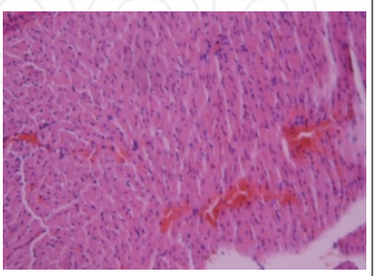
Organs examined	Control group	Toxic effects associated to Capsicum annum aqueous extracts	Toxic effects associated to Capsicum annum 70% alcohol extracts
	M –Liver (Control). The scale bar is 100µm	N –Hepatic degeneration and congestion of the liver at 14,000mg/kg. The scale bar is 100µm	O –Liver wide necrosis and tissue degeneration at 6,500mg/kg. The scale bar is 100µm
Heart	 P	 Q	 R
	P –Heart (Control). The scale bar is 100µm	Q –Myocardial haemorrhages at 12,000mg/kg. The scale bar is 100µm	R –Wide spread haemorrhages on the myocardium at 5,000mg/kg. The scale bar is 100µm

Table 3. Comparison of key histopathological findings of aqueous and 70% ethanolic extracts for the collected organs

4. Discussion

4.1. Lethal dose (LD50) levels

The lethal dose (LD50) of aqueous extracts of *Capsicum annum* was found to be12043 mg/kg. The 70% ethanolic extracts of *Capsicum annum* had a lethal dose (LD50) of 5492 mg/kg. Consequently the *Capsicum annum* is classified as practically non-toxic because the LD50 results fall in the dose range of 5-15g/kg of body weight [4].

Local smallholder farmers are using aqueous extracts of *Capsicum annum* seeds to manage various disorders. This study has confirmed that the use of the aqueous extract of *Capsicum annum* by the local poultry farmers is safe. Save to note here that the smallholder farmer's dose levels greatly vary from one farmer to another and in varying levels of concoctions [6]. The estimate dose for local farmers range between 4014-6022mg/kg for aqueous extracts. The farmers' dose levels are two-three times below the determined LD50 of 12043mg/Kg body weight for aqueous extracts.

The Organization for Economic Cooperation Development (OECD) Guidelines for the Testing of Chemicals [7] recommended that the maximum dose levels for any chemical compounds should not exceed 5000 mg/kg of the animal body weight. The ethanolic extraction of Capsicum annum has advantages compared to the aqueous because ethanol is better solvent for extraction of bioactive compounds.

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The extracts derived from ethanolic solvents have lower LD50 of 5492mg/kg because ethanol is less polar. The ethanolic extracts have yet another advantage: the longer shelf life for use because of the preservative effects of alcohol on the extracts when compared to that of the water in aqueous extracts [5]. It can be noted here that extracts, which seem to show high toxicity in many circumstances, have no traditional uses, which is in agreement with previous findings [1,8].

After administration of the higher extract dosages there were often observed notable clinical signs in test animals, due to the side effects of the extracts. Key clinical signs noted were irritation, evidenced by the use of fore limbs to scratch the areas of the mouth, gasping for air, dyspnoea, twitching of GIT muscle, urination, dry mouth, reddening of lips and convulsions. The effects of bioavailability of the drugs on major organs like the intestines, liver, lungs, kidneys and brain showed clear manifestations of the presence of the active substances. Bioavailability is the rate and extent to which the active drug ingredient is absorbed from a drug product and becomes available at the site of drug action. The effects of the active substances in the body of the mice could affect by the rate of disintegration of the drug product, dissolution of the drug in the fluids at the absorption site or the transfer of drug molecule across the membrane lining the gastrointestinal tract into the systemic circulation.

The key physiologic factors that could affect the availability of the active substances in the body include: variations in absorption power along GI tract, variations in pH of GI fluids, gastric emptying rate, intestinal motility, perfusion of GI tract, presystemic and first-pass metabolism, age, sex, weight and disease states. The interactions with other substances like food, fluid volume and other drugs or chemicals could also play an important role.

There exist some reports of seeds side effects by *Capsicum annum* on chickens reared under the traditional use system. This finding concurs with the experimental trials. Aware of this fact, is to note that mice, chickens compared to other mammals hence exhibit a large surface area compared to other mammals.

The histopathological findings showed significant effects on brain, kidney, lungs, small intestines, liver and heart. Studies in India indicated that necropsy examination is paramount in linking the general and target organ specific toxic effects of phytomedicine [9]. Many others are in agreement OECD [10-12]. Absence of any significant gross pathological lesions in treated rats and mice at the terminal sacrifice indicates the justifiable harmless nature of the phytomedicine [9].

5. Conclusion

Results of study indicated that the lethal dose (LD50) of aqueous extracts of *Capsicum annum* was12043 mg/kg compared with 5,492mg/kg for 70% ethanolic extracts. The clinical signs noted depended on the level of concentration of the plant extracts.The study concludes that 70% ethanol extracts of *Capsicum annum and aqueous extracts* of capsicum annum are safe to use and are classified as practically non-toxic (5-15g/kg body weight). Both extracts are suit-

able for traditional poultry disease management by farmers. Further toxicity studies using chicken as animal species are necessary. Sub-acute and chronic toxicity tests are recommended in order to determine the long-term effects of the extract.

Conflict of interest

The authors declare here that there is no conflict of interest.

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Appendix

%	0	1	2	3	4	5	6	7	8	9
0		2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.49	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

The percentage dead for 0 and 100 will be corrected before determination of LD50 using the following formula [4]: *For 0% dead: 100(0.25/n); for 100% dead: 100(n-0.25/n).*

Table 4. Probit conversion tables. Source: [4]

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