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The Dose and its Acute Toxicology: A Systematic Review Article in the First Phase of Experimental Pharmacology

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Abstract

Background: Hundreds and thousands of acute toxicity studies are conducted in experimental pharmacology with assumption hypothesis every year. It is usually concluded with inadequately validated data during the period of investigation within 24 hours.

Objectives: The purpose of this review is to extract information from published articles, textbooks, workshop reports and conference presentations to find answer for the questions that follow. (1) Does the dose determine the harmful effect of a test substance? (2) What makes the toxicity of a dose?' (3) Is 24 hours investigation time adequate for single dose toxicity trial? (4) Why different substances with the same dose have different length of time at which its undesired effect manifested in treated study animal?

Methods: The techniques used for data collection were: revision of different literatures, data selection, and data processing. Data analysis was done both manually and electronically using a computer package and calculator. Data was thematically compiled for validation of the findings from each of the sources.

Results: The result of different acute toxicity studies showed that the dose had never limited the harmful effect of test substance but the magnitude of adverse effect and length of time at which adverse effect was manifested in treated Balb c mice. The harmful effect of tested chemicals was however determined by the biological response called toxic reaction rate in the biological process of study animal. One day was inadequate to evaluate the toxic nature of most test materials with acute toxicity trial. Adequate investigation time for acute toxicity study was therefore essential to analyse comprehensive pharmacological properties of test substances.

Keywords: Acute Toxicity; Single Dose; Experimental Pharmacology; Toxic Nature; Lethal Effect

Introduction

Value of Single Dose Acute Toxicology

Acute toxicology in Preclinical trial is the basic step in drug discovery and development in which its procedures are still a controversy in terms of ethical and scientific grounds. It has limited clinical value due to the fact that its lethal dose is the endpoint for conclusion. Countless resources are being wasted every year and harmful pharmaceutical products are infiltrating to the public for consumption due to unscientific grounds of single dose acute toxicology by which the lethal dose is the endpoint [1]. A dose which is highly toxic to one species could not have the same pharmacological effect on another species [1]. The dose of a test substance which has undesired effect in animals within 24 hours, the same dose from another test substance could have undesired effect after three days or more. There is no specific minimum lethal dose and maximum non-lethal dose in acute toxicology that could strictly manifest its undesired effect within 24 hours.

The strategy for toxicity investigation has been changed significantly over the last many years in order to ensure that early toxicological data can help to make decisions on the best compounds to progress as valuable human medicines [1,2]. A key step in ensuring safety of test substance is by conducting toxicity trial in which acute toxicology is the primary regulatory procedure in experimental pharmacology [3]. However, the standard regulatory procedures in acute toxicology still needs to be improved to better ensure quality of safety and minimize wastage of time and resources in experimental pharmacology.

Single Dose Toxicology

The dose, in toxicology, is a single amount of test substance administered into laboratory animal using one of the routes of drug administration to be able to investigate its adverse effect in the course of metabolism. Hundreds and thousands of acute toxicological

screening tests are conducted in experimental pharmacology with inadequate duration of investigation and description of safety exclusion every year [4]. It is usually concluded during the period of investigation which is not exceeding 24 hours [4].

Acute toxicity screening test in laboratory animals is a regulatory requirement for any drug intended for human use [5]. It is usually conducted using two different animal species prior initiating studies of a new drug on human subject who includes extensive pre-clinical data collection on organ system toxicity of test compound following acute, sub-acute and chronic exposure [5].

Multiphase trials of test substance on laboratory animals have to be conducted to evaluate the mutagenic and carcinogenic potentials, effects on reproductive health and data on potential effectiveness of new drug if animal model of human disease exists [5]. Two routes of drug administration are normally recommended for acute toxicity evaluation which is intended for intravenous and other routes of human administration [5]. When intravenous administration is the proposed route for human subject, the use of this route in animal toxicity trial is usually sufficient [5]. The acute toxicological evaluation of new substance on laboratory animal however needs to be carried out for adequate duration of time to proceed with sufficiently validated data to the next stage of investigation. It could be wastage of time and resources to proceed and design the next stage of investigation without having complete investigation of the experiment in the first stage. There are a wide range of substances with different nature of toxicity whose acute toxic effect in the organism might not be evaluated within a period of 24 hrs. The period of time to manifest its undesired effect in an organism treated with the same dose also varies because of the different nature of toxic component in different test compounds. A single dose toxicity study conducted by Belay Y, 2011 showed that ethanol and ether test extracts from the dried seeds of *Aristolochia elegans* Mast at different doses didn't manifest any adverse effect on 45 treated Balb c mice for the first 3 days of investigation [6]. However, all treated Balb c mice died as if they were sleeping peacefully without any vigorous movement against the death within 4-9 days after the dose was given orally [6]. Concluding acute toxicity investigation within 24 hours with inadequate information about the toxic nature of test extract might end up proceeding with unsafe test substance to the next phase of trial which leads into wastage of time, resources and infiltration of harmful products for public consumption.

Every nation around the world has national drug policy to ensure that safe and efficacious drugs are made available to the entire population to be able to provide satisfactory and safe health care [7]. However, research and drug development industries on the one hand have debatable, multiphase drug screening procedures in which harmful products could still be infiltrated and reached Pharmaceutical market as health service delivery to the public. This happens because the door is open for harmful products to be on market in the way that follows:

A wide range of test substances have delayed manifestation of undesired effect on study subject with the time to undesired effect after acute exposure being weeks and months. Acute toxicology has also limited clinical value due to the fact that its lethal dose is the endpoint for conclusion in which death sometimes occurs after scheduled period of acute toxicology as a result of loss of body mass induced from supressed appetite. The principal use of collected data from acute toxicology is to support regulatory categorization and harmful labelling decisions that the data can also be used to derive safe use threshold levels which may lead to use unsafe material [8,9]. The criterion for classification and labelling also differs among countries, sometimes among authorities within the same country [8]. The principle of toxicology on the other hand is vaguely stated that 'all chemical substances are potential poisons depending on the amount and duration of exposure' in which the nature of any chemical substance could not be changed or eliminated by simply quantification [10]. All xenobiotics are poisons at any amount with different intensity that could be measured using integrated biological parameters. If the higher dose is lethal to the study subject, the lower dose is most likely to have health impact in the long run. There is no scientific ground to categorise the different doses of a test substance as safe dose (ED_{50}) and lethal dose (LD_{50}) to the study subject within the period of the experiment. The toxic effect of a chemical substance might be manifested either at the cellular or organismal level depending on the amount and duration of exposure which might not cause death within scheduled period of drug trial.

The diversity of toxic property of chemical substances and the variety of its adverse effect however make it very difficult to analyse the toxicological nature in a specialized manner [10]. The toxic effect may be considered harmful only when it causes functional or structural damage in which there is no limited time and limited dose to cause this harmful effect. For some doses of chemical compounds, the tolerable exposure may be close to zero whereas other doses of chemical compounds, the tolerable exposure may be days or weeks [11]. The amount which causes recognizable adverse effect within a given time period also varies from one chemical substance to another which makes toxicology having very complex procedure.

Lots of study have already performed on the toxicity and mechanism of toxicity of many chemical substances in the biological process of study animal without having fundamental concept that explain the probable cause of this toxicity in the natural process of an organism. Analysis of toxicity or mechanism of toxicity of a chemical substance without having well defined concept about the probable cause of toxicity makes toxicological study complex for assessment. If we don't know the cause of a disease, it is always not simple to diagnose it because clinical signs and symptoms of a disease are often similar for different diseases. The same applies to the toxicity of a chemical compound because it is chemically induced disease in which clinical signs and symptoms are also often similar for different chemical substances.

This review was therefore conducted to extract information from different literature sources to answer the questions that follow: (1) Does the dose determine the toxicity of a substance? (2) What makes the toxicity of a dose? (3) Why different substances

with the same dose have different length of time at which its adverse effect manifested on exposed animal? These questions were answered using the information extracted from relevant experimental studies which may help to define the fundamental concepts of experimental toxicology. The finding has been presented under different themes in the result and discussion sections of this review.

The Dose and Biological Response Relationship

The magnitude of adverse effect of a certain chemical on a living thing depends on the dose which is one of the basic concepts of toxicology known as dose-response relationship [11]. The biological response is the ultimate effect of an interaction between a dose of a chemical substance and a target molecule in the biology of a living thing [10]. In the toxicity study, the dose-biological response relationship provides the basis of assessment of harmful effect caused by toxic elements which is used for safety evaluation and regulatory requirements [10].

In order to define this relationship, it is necessary to specify a particular response usually a negative one such as death of exposed organism against increasing intensity of test chemical [11]. At relatively lower doses, an organism exhibits no gross response which perhaps limited at the cellular level and at the higher doses, all exposed organisms exhibit gross response. In between, there may be a range of doses over which some of treated organisms respond in a specific manner, as they differ in their response to the toxic effect of a chemical [10]. It is in general difficult to estimate the exact dose that will cause biological response. Therefore, the average effect of doses tested on a study animal is plotted to get a generalized dose-response curve in which different doses of a test chemical are uniformly administered to study population and its response presented as percentage of death which is generated based on several assumption that leads to assumption safety analysis. This response is a function of the log of a dose at which 50% of the study population dies [11]. This response is known as the median lethal dose (LD_{50}) which is statistically calculated doses of test chemical that has caused death to 50% of study subject [12]. This determination does not provide information regarding mechanism of toxicity and complementary or selective pathways of toxicity [12].

Like the lethal dose 50, the effective dose 50 (ED_{50}) is the dose that causes an effect in 50% of the study subject. Similarly, the toxic dose 50 (TD_{50}) is the dose that causes toxic effect to 50% of the study population [11]. In the case of effective dose 50 and toxic dose 50, the response is the one in which none will die by the exposure of test chemical [11]. Generally, TD_{50} is used to describe responses such as reduced enzyme activity, loss of hearing, nausea and ED_{50} is used to treat a particular disease [11]. There are two types of dose-response relationship depending on number of subjects and doses tested [11].

a. The individual dose-response relationship which is also called graded response which means that the measured effect is continuous over a range of doses [11]. In this relationship, the concentration of test chemical is directly proportional to the biological response that has been manifested on study subject.
b. A quantal dose-response relationship which characterizes the distribution of responses to different doses in a study population of individual organisms [11]. This relationship is generally classified as 'all-or-none effect' where the study subjects are quantified as either responders or non-responders to doses of test chemicals [11].

The dose elicits undesired biological response which can range from slight symptoms like salivation or lacrimation to severe symptoms like coma, convulsion and death. The need to express estimates of risk in an understandable manner is however a challenge in toxicological study. Depending on the specific biochemical mechanism of action, a toxic substance may have very widespread effects throughout the body or may cause very limited change in physiological functioning in a particular organ [13]. Because there are complex interrelationships between the systems within the body, a single change in any one system may result in numerous effects in other systems [13]. It would be an understandable method if we consider an integrated biological approach (physical, histological, biochemical and immunological) techniques upon which modern clinical medicine is based to better analyse and understand the toxic property of a chemical substance.

Materials and Methods

Search Plan for Relevant Studies

A relevant literature search was carried out in PubMed library and google Scholar using the following electronic search terms. These were: (1) acute toxicity studies, (2) acute toxicity procedures and (3) acute toxicity guidelines. Original articles, text books, workshop reports and conference presentations relevant to the title of this review article were selected, downloaded and filed into a computer.

Data Selections

The data in the first original article was collected from four cross-sectional studies which were conducted both in the field and laboratories [6]. The data that had been collected only from acute toxicological investigation of test substances in the laboratory were included in this review. Data collection techniques used in this study was: extraction, measuring and toxicity trial of test substance and histopathology of visceral organs [6]. Other acute toxicity studies (*in vitro* and *in vivo*) which were eligible for the study were also included in this review article

Data Selection Criteria

Checklists were developed to determine selection criteria based on the following questions up on which different studies were included or excluded in this review. These were:

1. Was the acute toxicity trial appropriate in terms of study design and sample size?
2. Did the toxicity trial have undesired effect?
3. Did the undesired effect of toxicity study clearly stated?
4. Did the dose accurately quantified?

A total of twenty-three articles and three text books which were published between 1986 and 2018 and one workshop report and one conference presentation, which gave "yes" answer to the questions mentioned earlier, were included in this review.

Data Processing

The data from different literature sources were extracted and stored in a computer. Then, the data were systematically arranged, processed and analysed using a computer package (adobe reader XI, PDF to word convertor, Microsoft office word 2013, Microsoft visual studio 2013, Word paint and HP photo smart essential along with calculator). The subjects that this study dealt with were identified and organized into meaningful sections and subsections. For completeness, data from different data sources were compiled and used to validate the findings of the study.

Data Presentation

Validated data from different sources were presented in form of tables, pictures and use of descriptive statements under the themes mentioned in the result and discussion sections of this systematic review.

Results

Acute Toxicity Screening Test

A single dose toxicity of ethanol and ether test extracts at different concentration was evaluated on 30 Balb c mice for a maximum period of 10 days for each dose administered orally [6]. There was no signs and symptoms of adverse effect observed in the first batch of laboratory mice which was given a single dose of 1000mg/kg for the first 3- 4 days [6]. The appetite of these mice was, however, gradually depressed and they eventually started dying 7 days after the dose was given in the oral route [6]. All treated mice died without having any vigorous movement against the death [6]. The first sampled and treated laboratory mice died on the 7th, 8th and 9th days after the dose was administered orally (Table 1).

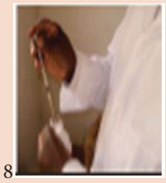
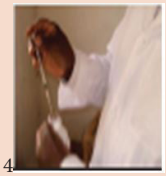
Dose in mg/kg	500&1000	2000&3000	4000&5000	Distilled H20(0.5 ml)	Cooking oil(0.5ml)
Number of treated mice	 8	 8	 8	 4	 2
Adverse effects within 24 hrs.	Nil	Nil	Nil	Nil	Nil
Within 48 hrs	Nil	Nil	Nil	Nil	Nil
Within 72 hrs	Nil	Nil	Depressed Appetite.	Nil	Nil
Within 96 hrs	Nil	Depressed Appetite.	2 mice died	Nil	Nil
Within 120 hrs	Nil	1 mouse died	2 mice died	Nil	Nil
Within 144 hrs	Depressed Appetite	3 mice died	4 mice died	Nil	Nil
Within 168 hrs	2 mice died	4 mice died		Nil	Nil
Within 192 hrs	2 mice died			Nil	Nil
Within 216 hrs	4 mice died			Nil	Nil

Table 1: The length of time at which test extracts caused lethal effect on treated laboratory Balb c mice at different doses (Belay Y 2017)

The second batch of eight Balb c mice which were given a single dose of 2000 and 3000 mg/kg body weight of both test extracts started dying on the 5th day after the dose was administered in the same route [5]. All treated mice died on the 6th and 7th days after treatment (Table 1).

The first two mice from the third batch of eight Balb c mice, which was given a single dose of 4000 and 5000 mg/kg body weight of both test extracts died on the 4th day and the remaining six mice died on the 5th and 6th days after the dose was administered orally (Table 1). But no sampled mice died within 72 hours even with the highest dose administered in this route [6].

The length of time at which lethal effect was observed on sampled mice was dose dependant [6]. The lethal effect of test extract becomes magnified within a short period of time when large amounts were administered [6]. It also remained after a long period of time when small amounts administered in the same route (Table 1). As a result, it was difficult to determine the lethal dose (LD_{50}) and effective dose (ED_{50}) of the extracts precisely [6]. An experimental study was also conducted on doses of test chemicals prepared from Dichlorvos, Chlorpyrifos and Cypermethrin pesticides at three different level of doses (10, 50 and 90) mg/kg which were administered into Balb c mice and monitored for a maximum period of 5 days [14]. The study revealed that the dose had never limited the toxic property of test chemicals but the magnitude of adverse effect and length of time at which undesired effect was manifested in treated Balb c mice [14]. The length of time at which undesired effect manifested in treated Balb c mice was inversely related to the amount of dose administered in the oral route [14]. The higher the dose of administered test chemical, the shorter the length of time at which the adverse effect manifested in treated Balb c mice (Table 2). This implies that the adverse effect of test chemical is not because of the dose but rather due to its toxic reaction rate which ultimately determined the toxic severity of test chemicals in the biology of treated Balb c mice. The toxic reaction rate (r) and toxic severity (s) of test chemicals were computed using a mathematical formula `$(r=\frac{d}{t}\text{-}\Delta Ig\ plasma\ conc.)mg/sec\ and\ (s=\frac{r}{d}\times 100)\%/sec$` respectively which is explained in detail in the discussion section and recorded in (Table 3 and 4) [15]. The toxic severity and toxic reaction rate of Cypermethrin were more severe than Dichlorvos and chloropyrifos in the three levels of doses prepared from each test chemicals and administered to lab Balb c mice in the oral route (Figure 1 and 2). The toxic severity and toxic reaction rate of the two different level of doses prepared from Dichlorvos were also slightly more severe than Chloropyrifos. However, the toxic severity of each test chemicals was not linearly projected as the dose administered to lab Balb c mice uniformly increased due to differences in the strength of the immune response (Figure 1). The toxic severity of doses (50 and 90) mg/kg prepared from Cypermethrin for instance, had disproportioned difference which was administered to different Balb c mice that had different strength of immune response [14]. The toxic reaction rate of doses (10 and 50) mg/kg prepared from Dichlorvos and Chloropyrifos was slightly different which was not proportional to the dose administered to Balb c mice in the oral route (Table 4). The study revealed that the higher the strength of the immune response, the less toxic severity of test chemicals in the biology of treated Balb c mice (Table 5), (Figure 1). The strength of immune response of Balb c mice treated with 10 mg/kg body weight of Dichlorvos was greater than the Balb c mice treated with 50 mg/kg of the same test chemical which caused much less toxic severity to treated Balb c mice [14]. This means that the level of toxic severity of test chemicals was not only affected by the dose and its toxic reaction rate but also by the strength of immune response of Balb c mice. The toxic severity of test chemicals was more affected than toxic reaction rate by the strength of the immune response of treated Balb c mice (Table 3,4 and 5), (Figure 1 and 2).

Test chemical	Doses tested	№ of Mice	Weight in gm	Time at which test substance administered	Time at which signs of adverse effect clearly manifested	Duration
Dichlorvos	10 mg/kg	1	15.13	10:22	11:22	1 hour
	50 mg/kg	1	17.63	10:23	10:53	30 minutes
	90 mg/kg	1	16.42	10:24	10:39	15 minutes
Chlorpyrifos	10 mg/kg	1	30.41	10:28	13:00	2:30 hours
	50 mg/kg	1	27.12	10:29	12:00	1:30 hours
	90 mg/kg	1	26.84	10:30	11:00	30 minutes
Cypermethrin	10 mg/kg	1	28.42	10:32	10:55	23 minutes
	50 mg/kg	1	30.98	10:33	10:45	12 minutes
	90 mg/kg	1	28.24	10:36	10:45	9 minutes

Table 2: The length of time at which adverse effect significantly manifested on Balb c mice treated with test chemicals orally (Belay Y 2018)

Test chemicals	Doses tested	Toxic severity (s) in %/sec
Dichlorvos	10 mg/kg	-199.0
	50 mg/kg	-19.8
	90 mg/kg	X
Chlorpyrifos	10 mg/kg	-299.0
	50 mg/kg	-39.8
	90 mg/kg	11.1
Cypermethrin	10 mg/kg	-199.0
	50 mg/kg	20.0
	90 mg/kg	33.3

X Represents laboratory Balb c mouse which died earlier than the time for toxic severity evaluation.
Table 3: Toxic severity (s) of test chemicals computed after four hours of dosing (Belay Y 2018)

Test chemicals	Doses tested	Approximate length of time undesired effect significantly manifested	Toxic reaction rate (r) in *mg*/sec
Dichlorvos	10 mg/kg	60 minutes	-19.9
	50 mg/kg	30 minutes	-9.9
	90 mg/kg	15 minutes	X
Chlorpyrifos	10 mg/kg	2:30 hours	-29.9
	50 mg/kg	1:30 hours	-19.9
	90 mg/kg	30 minutes	10.0
Cypermethrin	10 mg/kg	25 minutes	-19.9
	50 mg/kg	12 minutes	10.0
	90 mg/kg	9 minute	30.0

X represents lab Balb c mouse which died earlier than the time for blood specimen collection

Table 4: Toxic reaction rate (r) of test chemicals computed after four hours of dosing (Belay Y 2018)

Test chemical	Tested doses	Quantitative immunoassay before treatment as reference test		Quantitative immunoassay four hours after treatment for comparison		Δ Ig serum conc.
		IgG	IgM	IgG	IgM	Δ Ig
Dichlorvos	10 mg/kg	<1100 mg/L	70 mg/L	<1100 mg/L	90 mg/L	+20 mg/L
	50 mg/kg	<1100 mg/L	70 mg/L	<1100 mg/L	80 mg/L	+10 mg/L
	90mg/kg	X	X	X	X	X
Chlorpyrifos	10 mg/kg	<1100 mg/L	90 mg/L	<1100 mg/L	120 mg/L	+30 mg/L
	50 mg/kg	<1100 mg/L	50 mg/L	<1100 mg/L	70 mg/L	+20 mg/L
	90mg/kg	<1100 mg/L	90 mg/L	<1100 mg/L	80 mg/L	-10 mg/L
Cypermethrin	10mg/kg	<1100 mg/L	70 mg/L	<1100 mg/L	90 mg/L	+20 mg/L
	50 mg/kg	<1100 mg/L	80 mg/L	<1100 mg/L	70 mg/L	-10 mg/L
	90 mg/kg	<1100 mg/L	80 mg/L	<1100 mg/L	50 mg/L	-30 mg/L

X Treated mouse died much earlier than the time for blood specimen collection.

Table 5: Plasma immunoglobulins change (Δ Ig) after treatment of Balb-c mice with different doses of test chemicals (Belay Y 2018)

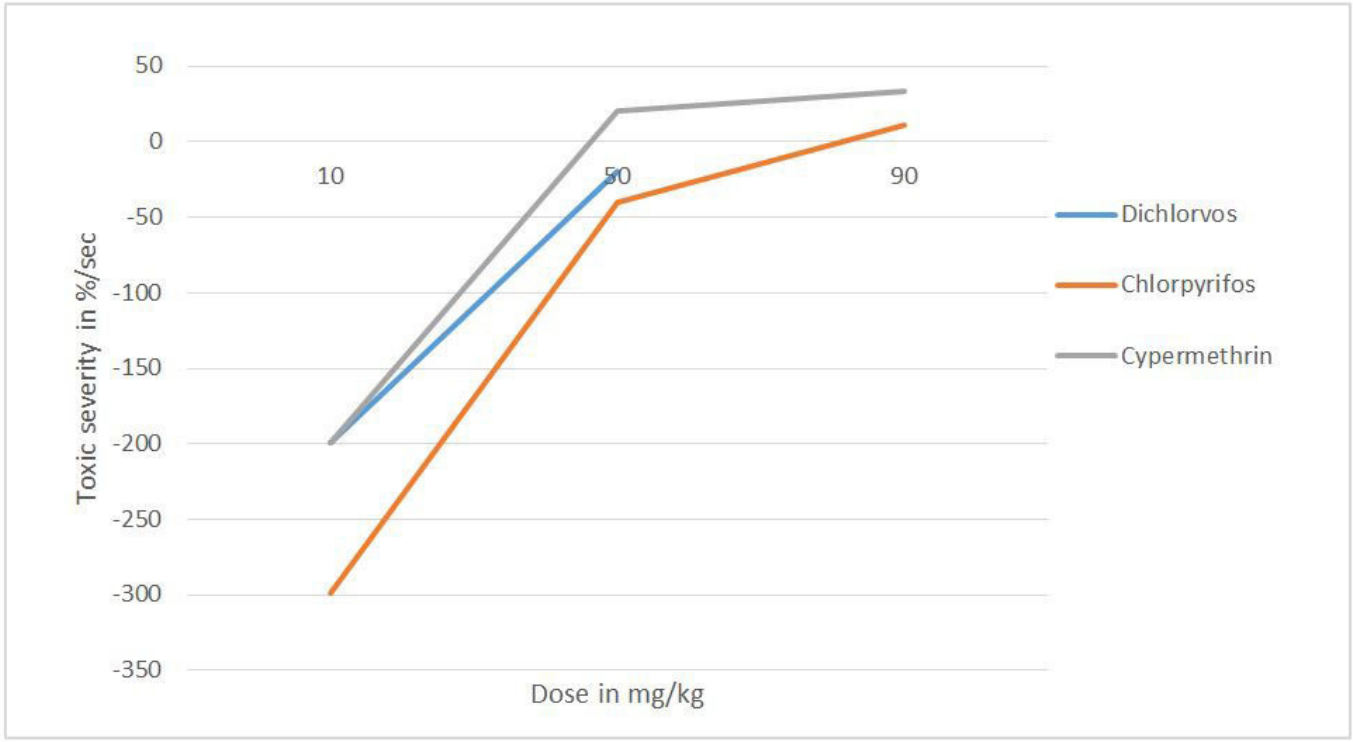


Figure 1: Toxic severity of tested doses prepared from Dichlorvos, Chlorpyrifos and Cypermethrin (Belay Y 2018)

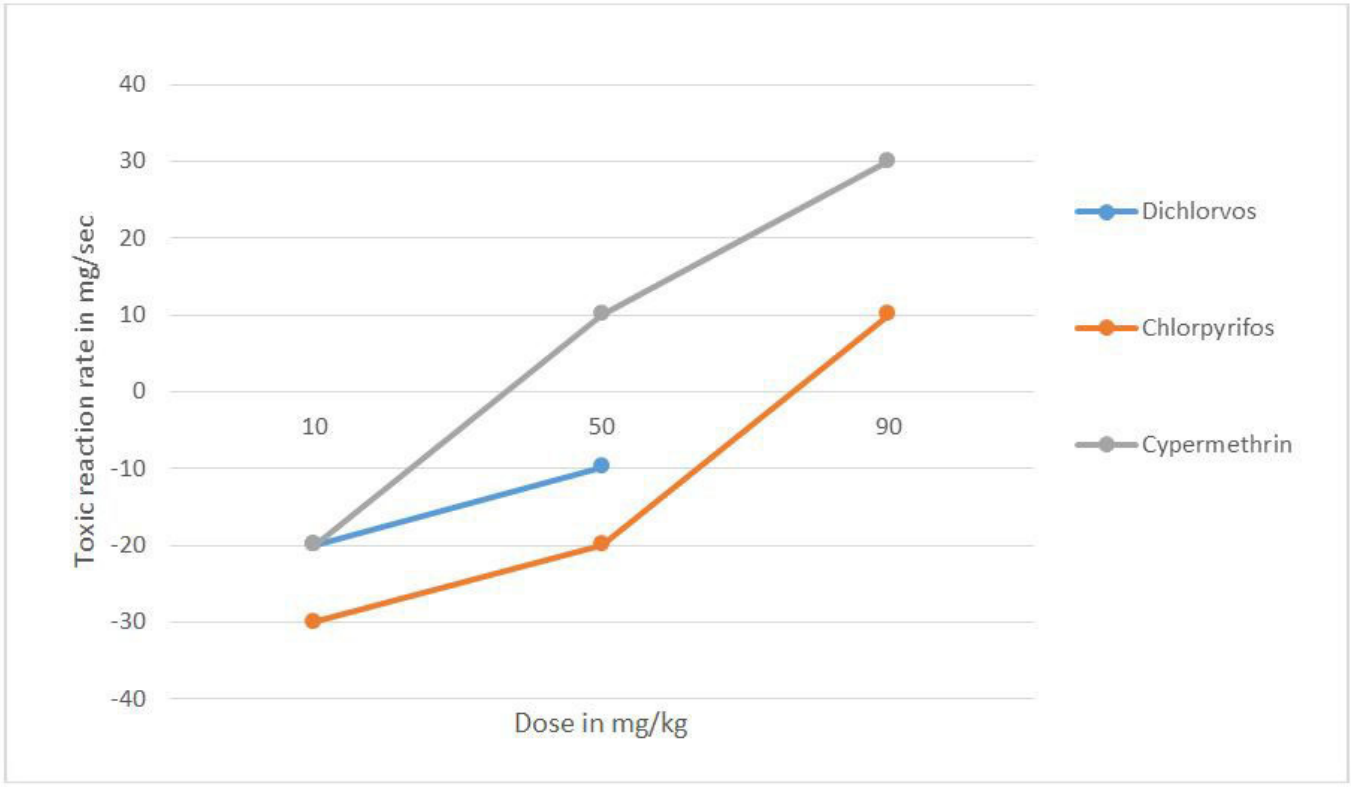


Figure 2: Toxic reaction rate (r) of tesed doses prepared from Dichlorvos, Chlorpyrifos and Cypermethrin (Belay Y 2018)

Adverse Effects of Tasted Doses

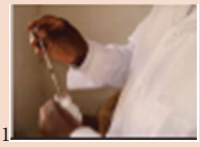
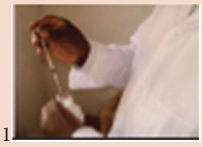
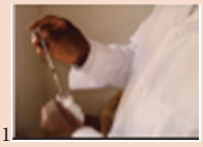
Dose in mg/kg	10	50	90
Number of mice treated	 1	 1	 1
Adverse effects within 24 hrs	Hypo-activity Weak Slow respiration	Hypo-activity weak Slow respiration	Hypo-activity Weak Slow respiration Died after 1:36 hours
Adverse effects within 48 hrs	Increased activity	Slightly increased activity	
Adverse effects within 72 hrs	Recovered	Slightly recovered	
Adverse effects within 96 hrs	Completely recovered	Recovered	
Adverse effects within 120 hrs	Weight loss	Weight loss	

Table 6: The effect of different doses of Dichlorvos on Balb c mice which were administered orally using interagasteral tube (Belay Y 2018)

Test chemicals	Doses tested	Weight before dosing	Weight on 5th day after dosing
Dichlorvos	10 mg/kg	15.13 g	13.37 g
	50 mg/kg	17.63 g	14.72 g
	90 mg/kg	16.42 g	X
Chlorpyrifos	10 mg/kg	30.41g	26.58 g
	50 mg/kg	27.12 g	23.37 g
	90 mg/kg	26.84 g	X
Cypermethrin	10 mg/kg	28.42 g	23.58 g
	50 mg/kg	30.98 g	X
	90 mg/kg	28.24 g	X

X Represents laboratory Balb c mice which were died earlier than two days after dosing

Table 7: Body weight of Balb c mice which was weighed before and five days after dosing (Belay Y 2018)

Dichlorvos Pesticide: The three Balb c mice treated with different level of doses of Dichlorvos (10, 50 and 90) mg/kg body weight were developed slow respiration and hypo-activity immediately after oral administration (Table 6). Lethal effect was observed at 1:36 hours after oral treatment of laboratory Bulb c mice with 90 mg/kg body weight of test chemical [14]. The signs and symptoms of adverse effect were manifested almost at the same time but with different magnitude for the three different levels of doses [14]. Slow respiration and hypo-activity was significantly manifested within 15 to 60 minutes after treatment depending on the amount of test chemicals administered in the oral route [14]. A sign of recovery such as increased activity in the survived two Balb c mice was observed about seven hours after treatment with 10 and 50 mg/kg body weight of Dichlorvos. Even though the two Balb c mice look completely recovered in the third day after treatment, the body weight which was weighed on the 5th day after dosing remained significantly below the body weight they had before dosing (Table 7).

Chlorpyrifos pesticide: The three Balb c mice which were treated with three different level of doses (10, 50 and 90) mg/kg body weight of Chlorpyrifos pesticide were also evaluated with acute toxicity trial for five days (Table 8). The main toxicity signs and symptoms significantly manifested after treatment were salivation, lacrimation, miosis (pinpoint eyes), trembling, breathing difficulty and general weakness which were clearly manifested within about 30 minutes to 3 hours after treatment depending on the amount of dose administered orally [14]. Salivation, breathing difficulty and trembling were the worst in the Balb c mice treated with the highest two doses (50 and 90 mg/kg body weight) especially about three hours after oral administration of test chemical. Despite the fact that the magnitude of adverse effect and length of time at which the undesired effect manifested in treated Balb c mice, the dose had never limited the toxic property of test chemicals [14]. The Balb c mouse which was treated with the highest dose (90 mg/kg body weight) died at 12:36 hours after treatment and the other two Balb c mice treated with the lower two doses (10 and 50 mg/kg body weight) developed a sign of recovery such as increased activity and reduced trembling and salivation in the second and third day after treatment respectively (Table 8). Even though treated Balb c mice look completely recovered in the fifth day after dosing orally, significant weight loss observed to each treated Balb c mice (Table 7).

Dose in mg/kg	10	50	90
№ of mice treated	 1	 1	 1
Adverse effect within 24 hrs	Trembling Breathing difficulty Tearing, Salivating weak	Trembling Tearing, Salivating Breathing difficulty very weak	Forceful trembling Tearing, salivating Breathing difficulty Died after 12:36 hrs
Adverse effect within 48 hrs	Weak Slightly recovered	Breathing difficulty Very weak Trembling	
Adverse effect within 72 hrs	Recovered	Slightly recovered	
Adverse effect within 96 hrs	Completely recovered	Recovered	
Adverse effect within 120 hrs	Weight loss	Weight loss	

Table 8: The effect of different doses of Chlorpyrifos on Balb c mice which were administered orally using interagasteral tube (Belay Y 2018)

Dose in mg/kg	10	50	90
№ of mice treated	 1	 1	 1
Adverse effect within 24 hrs	Distended stomach Bulging eyes Salivating Breathing difficulty	Distended stomach Bulging eyes Salivating Breathing difficulty Tremor	Distended stomach Bulging eyes Salivating Breathing difficulty Tremor & died after 11:36 hrs
Adverse effect within 48 hrs	Slightly improved but with still breathing difficulty	Died after 26:00 hrs	
Adverse effect within 72 hrs	Breathing difficulty		
Adverse effect within 96 hrs	Slightly recovered		
Adverse effect within 120 hrs	Weight loss		

Table 9: The effect of different doses of Cypermethrin on Balb c mice which were administered orally using interagasteral tube (Belay Y 2018)

Cypermethrin Pesticide: The three Balb c mice treated with three different level of doses (10, 50 and 90 mg/kg body weight) prepared from Cypermethrin pesticide developed signs and symptoms of toxicity within about 9 to 23 minutes after treated with single dose depending on the amount of dose administered orally (Table 2). The main signs and symptoms of toxicity significantly developed after treatment with the three different levels of doses were stomach distension, tremor and restlessness, breathing difficulty, salivation and bulging eyes [14]. Salivation, breathing difficulty, tremor and restlessness were the worst in Balb c mice treated with 50 and 90 mg/kg body weight of test chemicals especially about two hours after treatment with single dose acute toxicity [14]. The dose had never limited the toxic property of tested chemical but the magnitude of adverse effect and length of time at which undesired effect manifested in treated Balb c mice. The Balb c mice treated with the highest and second highest doses (90 and 50) mg/kg died at 11:36 and 26:00 hours respectively after treatment (Table 9). The Balb c mouse treated with the lowest dose developed a sign of recovery such as increased activity, eating and drinking in the third day [14]. It was however remained with mild breathing difficulty in the third day after treatment in the oral route [14]. Even though it had shown a sign of complete recovery in the fifth day, the body weight was still less than the weight it had before treatment (Table 7).

Report of other acute Toxicity Studies

Acute toxicity study of 1, 8-cineole in mice was conducted by Jiao Xu *et al.* 2014. Fifty mice from either sex were exposed to 1, 8-cineole with a series of doses of 2969.4, 3374.86, 3847.34, 4385.95 and 5000 mg/kg body weight (10 mice for each dose) [16]. The animals were monitored for 24 hours after treated with test compound and the mortalities were recorded [16]. The LD_{50} value (95% cl) was calculated using the method of Litchfield and Wilcoxon [16]. The study showed that the higher dose magnified the severity of toxicity of test compound more than the lower dose within 24 hour investigation time [16]. Nine out of ten treated mice were died at a single dose of 5000 mg/kg body weight while one out of ten treated mice was died at a single dose of 3374.86 mg/kg body weight within 24 hours after dosing orally [16].

Acute toxicity study of karlotoxins (KmTx 1 and KmTx3) by intraperitoneal injection at doses of 500, 1,000, 2000 and 4000 μg/kg were progressively given to laboratory Balb c mice and monitored for 14 hours after dosing [17]. Pronounced behavioural changes were evident with increasing doses [17]. All had lost a significant amount of weight, however, particularly at the highest dose level [17].

Acute toxicity of aqueous extract of the seeds of *Calycotome villosa* in male and female mice was conducted by Badiaa Lyoussi *et al.* 2018. The laboratory mice were divided into 8 groups of 12 (6 male and 6 female) and acclimatised in cages under standard environmental conditions of light/dark cycles [18]. The laboratory mice had free access to tap water and standard pellet diet, except for a short fasting period of 2 hours before the treatment with the single doses of the lyophilised *C. villosa* C seed-extract [18].

The lyophilised extract was diluted with distilled water (1 g/mL) on the day of the experiment, and administered intraperitoneally [18].The treated laboratory mice, which had been monitored for signs of toxicity and mortality, were observed continuously for 1 hour after the treatment, and then intermittently for 4 hours and thereafter over a period of 24 hours [18].The mice were further observed once a day for up to 14 days following treatment for behavioural changes and signs of toxicity and/or death [18].

The study revealed that the mortality rate as well as the acute toxicity of intraperitoneally administered *C. villosa* seed-extract increased progressively as the dose increased from 3 g/kg to 5 g/kg, respectively [18].The undesired effect of the higher dose of test substance was manifested at a length of time which is shorter than the lower dose. The adverse effect of the lower dose was manifested within 60 hours whereas the adverse effect of the higher dose was manifested within 36 hours [18]. The main behavioural signs of toxicity observed in this study were atypical locomotion, asthenia, trembling, piloerection, and urination [18]. Asthenia, hypo-activity and urination were noticed immediately after intraperitoneal administration of plant extract and were more pronounced at the higher doses and persisted until death [18]. The study also revealed that there was a gender difference in terms of the acute toxicity of *C. villosa* seeds extract (adverse effects, mortality and death latency) given by the intraperitoneal route, the females being more sensitive than the males [18]. Acute, toxicity study with Felbamate, 2-phenyl-1,3-propanediol dicarbamate was conducted by J.H McGee *et al.* 1998 on four to five week old mice [Crl:CD-l(ICR)BR], and rats (Crl:CD(SD)BR] [19]. After intraperitoneal administration of 375 to 775 mg/kg to the mouse, clinical signs included rough hair coat, decreased activity, tremors (when handled), decreased muscle tone, ataxia, ptosis, prostration, and death [19]. The study showed that the number of mice affected at each dose level and the severity and duration of the clinical signs were increased in a dose-related manner [19].

Ptosis, occurring within 15 min and lasting less than 1 h, was the only clinical sign observed in rats after oral administration of 3000 or 5000 mg/kg of felbamate [19]. After intraperitoneal administration of 3000 to 5000 mg/kg in the male and 1000 to 3000 mg/kg in the female, clinical signs included diarrhoea, decreased activity, decreased muscle tone, ataxia, ptosis, prostration, and death [19]. The number of rats affected at each dose level and the severity and duration of the clinical signs were increased in a dose-related manner [19].

The acute toxicity study of novel biogenic selenium nanoparticles was conducted by Moitaba *et al.* 2012 on ninety six male NMRI mice, which were randomly divided in to 12 groups with 8 mice each. A bolus of selenium dioxide and Se NPs dispersed in 0.9% apyrogenic sterile NaCl was administered orally by gavage at different level of doses (2-24) mg/kg and (120–320) mg/kg body

weight respectively [20]. In a similar trial, the control group received 0.9% apyrogenic sterile NaCl. The animals were monitored for viability and clinical signs of toxicity on the day of dosing and then daily for 14 days. Cumulative mortality within 24h after the treatment was used for the calculation of median lethal dose (LD_{50}) [20]. The study showed that SeO_2 caused complete mortality at the dose of 24 mg kg^{-1} while biogenic Se NPs showed no acute toxicity at this dose [20]. However, the body weight of male mice receiving Se NPs at daily dose of 20 mg kg^{-1} for 14 days were significantly decreased ($p < 0.05$). Although the laboratory mice treated with the smaller doses of SeNPs (2.5, 5, and 10) mg/kg had shown increased body weight, the increment in body weight was not as significant as the control mice This is clear evidence that the adverse effect of smaller doses remained without being noticed which can be recognised as the adverse effect of the higher dose in the later life of the animal. It is an assumption to determine the toxic nature of a test substance by determining the effective dose (ED_{50}) in laboratory animal within the period of the experiment.

The study revealed that the recognizable adverse effect of Selenium dioxide (SeO_2) manifested much more quickly than SeNPs at the same dose administered to NMRI mice orally and hence different length of time is required to evaluate the acute toxicity of both test compounds. It could not be possible to evaluate the acute toxicity of biogenic SeNPs within 24 hours to proceed with adequately validated data to the next stage of trial.

Histopathological Evaluation of the viscera

The effect of test extracts from the dried seed of *A.elegans* on the kidney, liver, stomach and intestines of Balb-c mice were investigated by histopathological examination [6]. All the sections from the kidney of the mice given a single dose of 5000mg/kg of both test extracts revealed marked degeneration and necrosis of tubular epithelial cells [6]. Hyaline casts were observed in the lumen of the tubules [6]. Focal parenchymal hemorrhages were also observed [6]. The lesions were all similar with minor variations in their severity [6]. Sections from the liver showed mild to moderate hepatocellular degeneration and vacuolar to fatty degeneration with hepatocellular necrosis of individual cells [6]. In general, the liver damage was less severe than the kidney damage [6]. Hemorrhages in the stomach were seen in four out of ten mice given a single dose of 5000mg/kg of this crude extracts [6]. Examination of the intestine of all the ten mice given a single dose of 5000mg/kg of these crude extracts revealed no significant lesions [6].

Quantitative Immunoassay

Immunoglobulins (IgG and IgM) quantification test has been conducted to evaluate the immune response against tested chemicals which were administered to Balb c mice orally. Except Balb c mice treated with the highest dose (90mg/kg) of Dichlorvos test chemical which was died at 1:36 hours after dosing orally, about 1 ml of blood sample had been collected from each treated Balb c mice using micro test tubes before treatment as reference test and four hours after treatment for comparison (Table 5). The quantitative immunoassay revealed that the lethal doses prepared from Chlorpyrifos and Cypermethrin suppressed immunoglobulin M (IgM) [14]. However, specific quantitative result was unable to get for immunoglobulin G (IgG). It was rather reported each serums sampled for IgG quantification test as less than 1.1 g/L (<1.1g/L) (Table 5).

The negative result of toxic severity and toxic reaction rate in (Table 3 and 4) respectively indicated the magnitude of toxic effect of test chemicals which was negligible in the biology of treated Balb c mice [14]. This means that the dose of test chemical which is corresponding to the negative value of toxic reaction rate and toxic severity has negligible adverse effect at the organismal level [14]. This doesn't mean that the test chemical is safe at the cellular level. The toxic effect of test chemicals at the lowest level of doses is only limited at the cellular level which could not be significantly manifested at the organismal level [14]. This implies that the toxic effect begins with biochemical changes that leads to cellular changes which eventually leads to physiological changes in the organ system which could be detected as gross biological response at the organismal level depending on the amount of dose administered to study subject. This indicates that the dose is only to determine the magnitude of adverse effect and length of time at which the adverse effect could be manifested in treated organism [14].

'The immune system is an essential part of the biological system of an organism that detects and react to any abnormality caused by any etiologic agent within the body. The magnitude of immune response could be measured in terms of immunoglobulins concentration in the biochemical component (plasma or serum) of the body as presented in (Table 5). The strength of immune response predicts the resistance of an organism to the harmful effect of any etiologic agent. Evaluation of the immunological state of an organism after exposure is therefore essential to determine whether or not any xenobiotic is harmoniously incorporated within the body mass of an organism in the course of metabolism"

Discussion

The Dose as Determinant of Lifespan: Biological and Clinical Analyses

Ethanol and ether test extracts from the dried seed of *A. elgans* were bio-assayed on 30 Balb-c mice (aged about 4 months) at different doses per body weight for a maximum period of 10 days [6]. Even if the sampled mice did not die within 72 hours after

the administration of prepared dosage form in the oral route, they started dying as if they were sleeping peacefully on the 4th day at the highest dose per body weight (5000mg/kg) [6]. All treated Balb c mice with different doses died within 4-9 days depending on the amount of dose administered orally (Table 1). A single dose of Dichlorvos, Chlorpyrifos and Cypermethrin were also bio-assayed on 9 Balb c mice at different amounts (10, 50 and 90) mg/kg body weight which were administered in the oral route and an integrated biological approach was employed in the study to evaluate the acute toxicological property of test chemicals for a maximum period of 5 days. The approach used mathematical formulations mentioned earlier to compute biological responses as toxic severity and toxic reaction rate which were used to analyse the cause of toxicity of each doses administered to study animals. The result of computed biological responses showed that undesired effect of test chemicals was because of its toxic reaction rate in the biology of treated Balb c mice.

The different structure of the body of living things (humans, animals, microorganisms and plants) is the metabolic by-product of ingested doses of substances from the environment which involves different metabolic pathways in which one substance is bio-transformed into another substance with multiple bio-transformation mechanisms [21]. This biologically transformed substance within an organism always causes disproportioned desirable and undesirable effect to the natural process of living tissue depending on the nature of its chemical component. Of the undesired effects, a notable example to mention is its cause to a living thing getting old, an ageing process with unknown mechanism yet. If we don't eat a dose of food substance frequently, we don't get old but we die. If we eat, we get old. If we get old, we eventually die. Death is unavoidable one way another. Metabolism is therefore the means of life sustaining chemical transformation within the living organism if it is supplied with desirable dose of substance which is biological friendly. These biologically activated chemical transformations allow living things to grow, and reproduce, helps to maintain their structures and normal physiology in the environment for limited period of time.

The metabolic system of an organism reveals the poisonous or nutritious or medicinal nature of a substance either at the cellular or organismal level depending on the amount of substance ingested. Thus, the manifestation of harmful effect of a substance within the biology of an organism determined by the nature of its chemical component rather than by the amount of substance ingested. The amount of a substance ingested by the living organism could however speed up the time at which biochemical and physio-pathological changes could be manifested in the biological system of an organism. Since the higher dose could manifest its adverse effect within a short period of time and the lower dose after a longer period of time in the treated organism, the lethal dose (LD_{50}) and effective does (ED_{50}) of test substance has no scientific ground to declare at a point of time that the lower dose is safe and the higher dose is unsafe for life. Even if the adverse effect of the lower dose is not significantly manifested at the organismal level within a short period of time, its adverse effect is significantly manifested at the cellular level which could cause impact on the life of an organism in the long run. In the first study by Belay Y 2011, for instance, the test extract at 500 mg/kg body weight killed treated Balb c mice in the 9th day and the highest dose (5000 mg/kg body weight) killed them in the 4th day after dosing orally [6]. In the previous study also 50 mg/kg of test chemical prepared from Cypermethrin pesticide killed treated Balb c mice in the second day whereas 90 mg/kg body weight of tested chemical killed treated Balb c mice in the first day. The study proved that the dose determines the length of time at which significant adverse effect could be manifested in treated organism. The adverse effect of test substance is however determined by the nature of its toxic component which ultimately determines the toxic reaction rate in the biology of an organism which in turn determines the intensity of toxic severity of a test substance. The main concept of toxicology is therefore the effect of a dose in the natural process of an organism that could be measured in terms of toxic reaction rate and toxic severity in the course of metabolism.

Toxic Reaction Rate: The toxic reaction rate of a chemical substance refers to the computed amount of administered dose that has elicited undesired biological reaction in the natural process of living organism over a period of time. It could also defined as the undesired biological response caused by known proportion of administered dose during the biological process of living organism. The toxic reaction rate has been computed by mathematical formulation expressed below. The computed toxic reaction rate of administered doses at 50 and 90 mg/kg body weight prepared from Cypermethrin, for instance, was 10 and 30 mg/sec in which only one fifth and one third of the administered doses respectively cause toxic reaction rate in the biology of treated Balb c mice depending on the strength of immune response (Table 4 and 5). In other words, there was only 20% ($\frac{r}{d}\times100=(10)/(50)\times100=20\%$) and 33.3% ($\frac{r}{d}\times100=(30)/(90)\times100=33.3\%$) of the administered doses (50 and 90 mg/kg body weight) that has caused undesired biological response in the natural process of treated Balb c mice respectively where r is toxic reaction rate, and d is the administered dose. The dose at 10 mg/kg prepared from the same chemical compound had, however, negligible toxic reaction rate (toxic component) in the natural process of treated Balb c mice (Table 4). This means that the components of a chemical compound could be both toxic and nontoxic elements at a certain proportion which leads to the consensus that all chemical substances are poisons with different intensity. This implies that the biology of living things are in both desirable and undesirable biological phenomenon in a daily basis depending on the proportion of toxic and nontoxic components of ingested substances. This proportion could be very much affected by the metabolic route of a chemical substance in the natural process of living thing because different cellular metabolic pathways have different metabolic by-product depending on the type of essential enzyme involved in the reaction [22]. In other words, the rate of production of toxic and nontoxic metabolites in the natural process of an organism could depend on the metabolic pathway involved for biotransformation. However, the pathways of cellular metabolism is often flexible in which it is not always simple to determine the metabolic route or to decide the enzyme that leads toxic chemical component

into nontoxic one [22]. Once the dose administered into living organism, it has to pass multiple steps of metabolic routes in which every steps could affect the toxic and nontoxic proportion of a chemical compound that has to be distributed to the body mass of an organism. This distribution could be intra or extracellular both of which could cause biological response at different level of an organism depending on the amount of administered dose.

A dose of chemical substance which elicits undesired biological response in a shorter period of time has higher toxic reaction rate (toxic component) than the same dose from another chemical substance which elicits undesired biological response at a longer period of time in the natural process of exposed organism. The toxic reaction rate of Cypermethrin at 50mg/kg, for instance, was greater than the toxic reaction rate of Dichlorvos at the same dose administered into Balb c mice which was elicited undesired biological effect after a longer period of time (Table 2, 3 and 4). The toxic reaction rate in the natural process of an organism determines the intensity of toxic severity of test chemical which in turn determines the lifespan of exposed organism. The toxic reaction rate (r) is therefore the administered dose (d) over the length of time (t) at which the signs and symptoms of adverse effect is manifested minus plasma immunoglobulins change (ΔIg) as counter response to the toxic effect of test chemical in the treated Balb c mice which is expressed in milligram per second [15]. It could be defined mathematically as ($r=\frac{d}{t}$ - *ΔIg)mg/sec* where r is the toxic reaction rate of test chemical, d is the administered dose, t is the time at which clinical signs and symptoms manifested in treated Balb c mice and ΔIg is immunoglobulins change after dosing [15]. The toxic reaction rate of test chemical is higher at the higher administered dose and the shorter the length of time at which adverse effects manifested in treated Balb c mice and Vic versa [14]. Time is an important factor in the measurement of toxic reaction rate and toxic severity of a dose. The longer the period of time at which undesired effect of test substance manifested in treated organism, the smaller the dose and its toxic reaction rate (Table 4).

Toxic Severity of a Dose: The toxic severity of a dose refers to the magnitude of adverse effect caused by exposure of living organism to a single amount of chemical substance over a period of time. The toxic severity of tested doses were computed by mathematical formulation written below. The toxic severity of 90mg/kg body mass of Balb c mice prepared from Chlorpyrifos and Cypermethrin was 11.1 and 33.3 %/sec which implies that a dose of 90mg/kg prepared from Chlorpyrifos and Cypermethrin could cause 1110 times and 3330 times biological injury in every second respectively (Table 3). This means that a dose of 90mg/kg prepared from Chlorpyrifos and Cypermethrin could manifest detectable biological response in the natural process of living organism which has up to 12.3 kg body mass ($\frac{s}{d}=\frac{\text{üüüü}\quad e}{90mg/kg}=12.3kg$) and 37 kg body mass ($\frac{s}{d}=\frac{3330/\sec}{90mg/kg}=37kg$) respectively depending on the strength of immune response of exposed organism where s is toxic severity and d is the dose of a chemical compound. The toxic severity of a dose determines the longevity of exposed living organism. The toxic severity of a chemical substance is therefore the toxic reaction rate over the administered dose multiplied by one hundred which is expressed in percent per second [15]. It could also be defined mathematically as ($s=\frac{r}{d}\times 100$)%/sec [15]. The higher the toxic severity of test substance, the higher the toxic reaction rate and the smaller the administered dose which caused undesired effect in the biology of treated lab animal within a short period of time and Vic versa. The toxic severity and toxic reaction rate of 50mg/kg prepared from Cypermethrin was, for instance, higher than the toxic severity and toxic reaction rate of the same dose prepared from Dichlorvos and Chlorpyrifos within a shorter period of time (Table 3 & 4)

During the previous study where the toxicological effect of three test chemicals (Dichlorvos, Chlorpyrifos and Cypermethrin) were evaluated on Balb c mice aged more than two months, the toxic severity and toxic reaction rate of each test substances was calculated and recorded in (Table 3 and 4) respectively. The value of toxic reaction rate (r) indicated the safety limit of test chemicals whereas the value of toxic severity (s) of test chemicals predicted the length of time at which lethal effect of test materials could probably manifest on treated organism. The Balb c mice which were treated with the amount of dose whose toxic reaction rate was less than zero survived from death whereas those sampled Balb c mice treated with the amount of dose which had toxic reaction rate more than zero died at different length of time after treatment depending on the toxic severity of tested chemicals (Table 2 and 4).

It could be a scientific fact to declare that a dose is safe when the value of toxic reaction rate (r) is less than or equal to zero. This means that the administered dose is successfully neutralized and harmonised with the biology of treated organism. The result of toxic severity (s) of each administered dose showed that treated Balb c mice with different doses had no equal opportunity to exist in life but equal fate for death at different lifespan depending on the amount of test chemical administered orally. This means that the higher the toxic severity of test substance the shorter the lifespan of treated organism and Vice versa. At the same time, the higher the toxic reaction rate of test substance in the biology of treated organism, the higher toxic severity of test material will be which shortens the lifespan of an organism.

The study by Belay Y. 2011 and 2018 showed that the dose had only limited the magnitude of adverse effect and length of time at which the adverse effect in treated Balb c mice was manifested at different doses administered orally (Table 1 and 2). This implies that the adverse effect of test substance was a result of its toxic component rather than the dose which determined the probable length of time at which the undesired effect of test material was manifested in the biology of treated laboratory animal. In the first study, the toxic reaction rate (toxic component) and toxic severity of each test substance was calculated without having the

quantitative data on immunoglobulins change in treated Balb c mice. The toxic reaction rate (***r***) was (0.0145+, 0.0116+, 0.0069+, 0.0039+, and 0.0017+) mg/sec for the administered doses (5, 4, 3, 2 and 1)gm respectively. The extra result of (***r***) was because of the dual effect (toxicity and immune suppression) of test materials in which quantitative data for immunological changes had never been computed Since the value of toxic reaction rate (r) for each tested material was greater than zero, it was clearly unsafe for the wellbeing of Balb c mice which died at different length of time after treatment depending on the amount of dose administered orally (Figure 3a and b), (Table 1) [15].

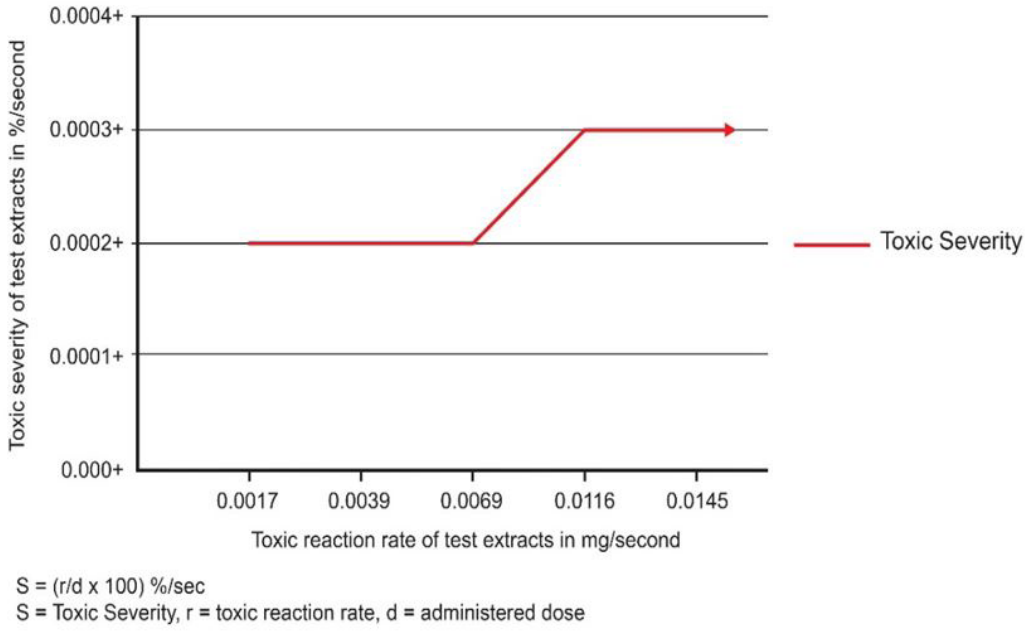


Figure 3a: The toxic severity (s) of test extract at different doses administered to lab. Balb c mice (Belay Y 2017)

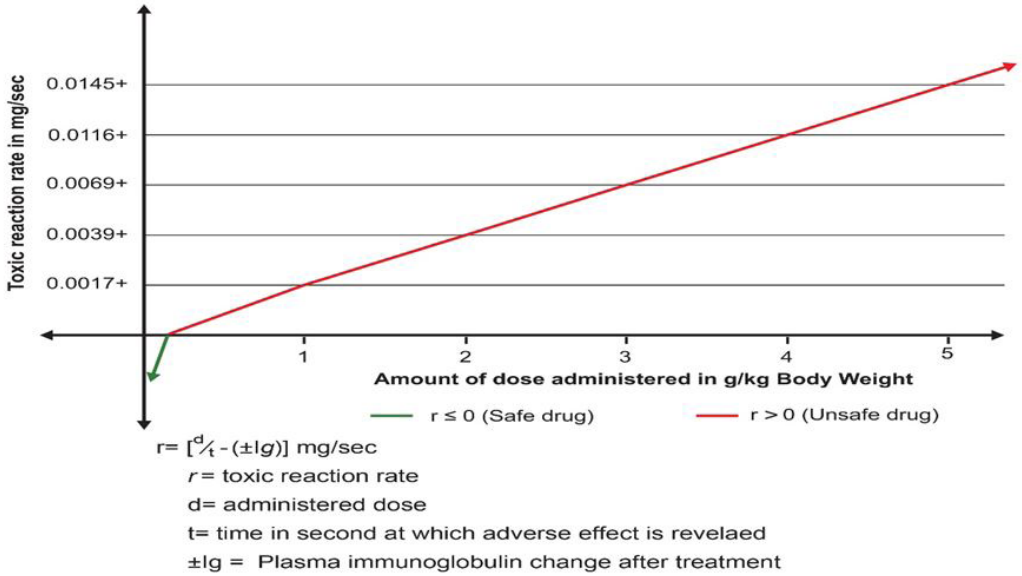


Figure 3b: The toxic reaction rate (r) of different doses of test extract in lab. Balb c mice (Belay Y 2017)

A test substance said to be toxic not only when it causes death but also pharmacological mechanism against the biology of treated organism. Death refers to a living thing that has lost complete bio-physiological interaction with its environment due to impaired systemic network which is probably caused either by an etiologic agent or a disaster. Death usually happens when the impaired part outweighs the viable part of a systemic network in the diseased organism. It is a complete bio-physiological discontinuation of life from its environment where it has been rooted for years and decades. The amount of an etiologic agent therefore determines the magnitude of adverse effect which in turn determines the length of time at which death could probably happen to a living thing.

All the different level of doses prepared from different test materials mentioned earlier manifested the toxic effect on treated Balb c mice with different magnitude at different length of time depending on the amount of dose administered orally. If the higher dose

is lethal to the study subject, the lower dose is most likely to have undesired effect in the long run. There is no scientific ground to categorise the different doses of a test substance as safe dose (ED_{50}) and lethal dose (LD_{50}) to the treated laboratory animals within the period of the experiment. The lower dose could not be safe for the wellbeing of an organism when the higher dose is lethal. It is most likely to be a waste of time and resources to categorise a single test substance as effective dose (ED_{50}) and lethal dose (LD_{50}) and proceed to the next phase of preclinical trial with inadequately validated data.

Other Studies on Dose and It's Effect

Other studies by Muhammad *et al.* 2011 and Regina *et al.* 2015 also showed that the dose determined the length of time at which significant adverse effect was observed in acute toxicity trial [23,24]. Significant reduction in the body weight of laboratory rats treated with distilled water test extracts from the fruits of *Sclerocarya birrea* was observed in the 3rd week after oral administration of 3000mg/kg and in the 1st week after administration of 4000mg/kg in the same route. Even if the smaller orally administered doses (1000 and 2000mg/kg) to laboratory rats showed increased body weights throughout investigation period, the increment in body weight was not as significant as the control rats [23].

An *In vitro* acute cytotoxic study conducted by Regina V and Umarajan Km in 2015 showed that the amount of administered test compound was directly proportional to the significant toxic effect it had caused to the immortalized liver cancer cell lines (viable cells) which were plated in 100µl of well [24].The viability of the cell was significantly affected by the higher dose than the smaller dose during the course of investigation period. Even if the severity of adverse effect was high in the viable cell treated with the higher dose, significant adverse effect was also revealed in the viable cell treated with smaller doses. This implies that the amount of dose accelerates the period of time at which the undesired effect of test substance could be significantly manifested in experimental organism. The study showed that the dose could not determine the adverse effect but the severity of toxic effect of test substance which determines the length of time at which the undesired effect could be manifested in treated laboratory organism. The undesired effect of test substance refers to the pharmacological effect which is unacceptably deviated not only negatively but also positively from the study control. Acute toxicity study of Zerumbone-Loaded Nanostructured Lipid Carrier which was conducted by Heshu Sulaiman Rahman *et al* 2014 showed that there is disproportioned weight gain either positively or negatively as compared to control Balb c mice depending on the amount of dose administered in the oral route [25]. In this case, it is hard to declare that a test substance is safe to the treated organism. The experiments were done in triplicate, and the results were expressed as mean ± standard deviation (SD) and analysed statistically using SPSS version 20.0 (SPSS Inc., Chicago, USA) [25]. Post hoc comparison test one way ANOVA was done using the Tukey's *b* test. Probability values of less than alpha 0.05 ($P < 0.05$) were considered statistically significant [25]. Test substances are molecules that interact with molecular component of treated organism by means of biological mechanism through which it causes biochemical and physio-pathological changes. Evaluation of the mechanisms of these changes within an organism helps to determine the toxic nature and pharmacological property of test substance. When the toxicity of test compound causes pharmacological mechanism against the well-being of treated study subject, the immune system of the organism generates counter response in form of inflammatory reaction as defense mechanism against the toxic component of test material [26]. It becomes a threat to the life of treated organism when the biological system has failed to neutralize and harmonize the chemical component of test substance with the molecular counter part of an organism.

The Immunoglobulins: An Indicator of Health Status

The term immunoglobulin refers to any of a class of proteins present in the serum and cells of the immune system which function as antibodies [27]. Immunoglobulins play an essential role in the body's defence mechanism against antigens [27]. The immune system is made up of a network of cells, tissues and organs that work together to provide protection to the organism from environmental agents such as microbes or chemicals, thereby preserving the integrity of the organism. The specific immunity is further divided into humoral immunity, the one involved with antibody, and cellular immunity, which is orchestrated by T cells [27]. Through a series of steps which is called the immune response, the immune system attacks organisms and substances that invade the biological system of the body and cause disease. When antigens are detected in the body, several types of cells work together to recognize them and respond. These cells trigger the B lymphocytes to produce antibodies which are specialized proteins that lock onto specific antigens [28].

The normal biological component of the immune system is therefore the overall indicator of the wellbeing of an organism whereas the abnormality in the immune system is, in general, the indicator of the ill health of an organism. What is going wrong in the biology of an organism is ultimately reflected as a quick counter response by the immune system that could be detected as abnormal temperature, supressed appetite, and abnormal immunoglobulin concentration in the plasma, physical and biological disintegration and many more to mention depending on the antigen detected by the immune system[28].

When the administered test substance is absorbed and interacted with the natural processes of study animal, it is not only the subjective effect but also other multiple effects that could be triggered in the body which is ultimately detected by the immune system. The immune system is highly complex and sensitive organ, with many facets poorly understood [12] in which assessment of harmful effects of chemicals against the functionality of this organ is not a simple task. Measurement of a variety of components of the immune system and their functionality is required to gain information of toxicity from chemical exposure [12]. The

administered test chemical substance becomes harmful to treated organism when the biological systems of the body has failed to neutralize and harmonise the chemical component of test substance with the molecular counter part of an organism. Thus, an integrated biological analyses in the earliest stage of preclinical trial is crucial to make adequate assessment about the general safety of test material which could help to avoid progressive trail of harmful test substance.

Conclusions

Information from different experimental studies showed that the dose had never limited the toxicity of doses prepared from different test materials but the magnitude of adverse effect and length of time at which significant biological responses were manifested in study subject. The undesired effect of test materials was, however, determined by the biological response called toxic reaction rate of a dose in the natural process of study subject. One day was inadequate to evaluate the toxic nature of most administered doses with acute toxicity trial. Adequate investigation time for acute toxicity study was important:

- To minimize expenditure of resources and time
- To avoid infiltration of harmful products for public consumption
- To reveal relevant information about the toxic nature of test substance
- To determine the toxic severity $s = \frac{r}{d} \times 100$ and toxic reaction rate $(r = \frac{d}{t} - (\Delta lg)$.

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