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ACUTE AND SUB-ACUTE ORAL TOXICITY ASSESSMENT OF THE HYDROALCOHOLIC EXTRACT OF POLYHERBAL IN WISTAR RATS

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ABSTRACT

Background: Polyherbal extract of different medicinal plants are widely used for several disorders. Toxicity studies on polyherbal extract are not available. Acute and subacute oral toxicities of Polyherbal extract in Wistar rats were evaluated in the present study. **Objectives:** The present investigation explored the protective effect of polyherbal extract of different medicinal plants in rats and no adverse effect ariesd. **Method:** In the acute toxicity study, Polyherbal extract was administered to two rats at 2000mg/kg, once orally and were observed for 14 days. No toxic signs/mortality were observed. In the sub-acute study, WSR extract was administered once daily for 28 days to rats at 500,1000 and 2000 mg/kg, orally. No toxic signs/mortality were observed. **Result and Discussion:** There were no significant changes ($P<0.05$) in the body weights, organ weights and haemato-biochemical parameters in any of the dose levels. No treatment related gross/histopathological lesions were observed. The present investigation demonstrated that the no observed adverse effect level was 2000 mg/kg body weight per day of hydroalcoholic extract of Polyherbal in rats and hence may be considered as non-toxic.

KEYWORDS: Polyherbal extract; toxicity; safety; rats.

INTRODUCTION

Toxicity is the degree at which any substance it may be poison or toxin that can cause the harmful effect on human or animals. In an organism acute toxicity involves the harmful effect through a single or short-term exposure. The ability of a toxic substance to cause effects more than one year but this effect is less than the lifetime of the exposed organism referred as Subchronic toxicity respectively. The ability of any substance or mixture of any substance

which the harmful effect due to the repeated or continuous exposure or due to the extended period, which also be sometime lasting stage for the entire life of the exposed organism and these effect generally referred as Chronic toxicity respectively.[1]

OECD GUIDELINE FOR TESTING OF CHEMICALS

Generally the OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress or changing assessment practices. The original Guideline 420 was adopted in July 1992 as the first alternative to the conventional acute toxicity test, described in Test Guideline 401. Based on the recommendations of several expert meetings, revision was considered timely because:

 International agreement had been reached on harmonised LD50 cut-off values for the classification of chemical substances, which differ from the cut-offs recommended in the 1992 version of the Guideline.

 Testing in one sex (usually females) is now considered sufficient.

 Guidance on the selection of the most appropriate test method for a given purpose can be found in the Guidance Document on Acute Oral Toxicity Testing. This Guidance Document also contains additional information on the conduct and interpretation of Guideline 420.

OECD Guidelines are of following types:

 OECD-420

 OECD-423

 OECD-425

 OECD-407

OECD/OCDE -420

INITIAL CONSIDERATIONS

It is a principle of the method that in the main study only moderately toxic doses are used, and that administration of doses that are expected to be lethal should be avoided. Also, doses that are known to cause marked pain and distress, due to corrosive or severely irritant actions, need not be administered. Animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test.

Criteria

Criteria for making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death, are the subject of a separate Guidance Document respectively. The method provides information on the hazardous properties and allows the substance to be ranked and classified according to the Globally Harmonised System (GHS) for the classification of chemicals which cause acute toxicity. The testing laboratory should consider all available information on the test substance prior to conducting the study. Such information will include the identity and chemical structure of the substance;

- Its physico-chemical properties.
- The results of any other in vitro or in vivo toxicity tests on the substance.
- Toxicological data on structurally related substances.
- The anticipated use of the substance.

This information is necessary to satisfy all concerned that the test is relevant for the protection of human health, and will help in the selection of an appropriate starting dose.

Principle of the test Groups of animals of a single sex are dosed in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg (exceptionally an additional fixed dose of 5000 mg/kg may be considered, see paragraph 19). The initial dose level is selected on the basis of a sighting study as the dose expected to produce some signs of toxicity without causing severe toxic effects or mortality. Clinical signs and conditions associated with pain, suffering, and impending death, are described in detail in a separate OECD Guidance Document. Further groups of animals may be dosed at higher or lower fixed doses, depending on the presence or absence of signs of toxicity or mortality. This procedure continues until the dose causing evident toxicity or no more than one death is identified, or when no effects are seen at the highest dose or when deaths occur at the lowest dose.

DESCRIPTION OF THE METHOD

Selection of animal species

- The preferred rodent species is the rat, and the other rodent species may be used. Normally females are used. This is because literature surveys of conventional LD50 tests show that usually there is little difference in sensitivity between the sexes, but in those cases where differences are observed, females are generally slightly more sensitive.
- However, if knowledge of the toxicological or toxicokinetic properties of structurally related chemicals indicates that males are likely to be more sensitive then this sex should be used. When the test is conducted in males, adequate justification should be provided.

 Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant.

 Each animal, at the commencement of its dosing, should be between 8 and 12 weeks old and its weight should fall in an interval within + 20% of the mean weight of any previously dosed animals.

Housing and feeding conditions

The temperature of the experimental animal room should be 22ºC (+ 3ºC). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal respectively.

Preparation of doses

In general test substances should be administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. Where a liquid end product or mixture is to be tested however, the use of the undiluted test substance, i.e at a constant concentration, may be more relevant to the subsequent risk assessment of that substance, and is a requirement of some regulatory authorities. In either case, the maximum dose volume for administration must not be exceeded. The maximum volume of liquid that can be administered at one time depends on the size of the test animal.

PROCEDURE

Administration of doses

 The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation canula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours.

 Animals should be fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night; with the mouse, food but not water should be withheld for 3-4 hours).

Sighting study

 The purpose of the sighting study is to allow selection of the appropriate starting dose for the main study. The test substance is administered to single animals in a sequential manner

following the flow charts in Annex 2. The sighting study is completed when a decision on the starting dose for the main study can be made (or if a death is seen at the lowest fixed dose).

 The starting dose for the sighting study is selected from the fixed dose levels of 5, 50, 300 and 2000 mg/kg as a dose expected to produce evident toxicity based, when possible, on evidence from *in vivo* and *in vitro* data from the same chemical and from structurally related chemicals. In the absence of such information, the starting dose will be 300 mg/kg.

 A period of at least 24 hours will be allowed between the dosing of each animal. All animals should be observed for at least 14 days.

OBSERVATIONS

 Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation should not be fixed rigidly.

 It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed and also for their body weight and pathology.

 All observations are systematically recorded, with individual records being maintained for each animal.[2]

OECD-423

The acute toxic class method (1) set out in this Guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods (Test Guidelines 420 and 425). The method as adopted in 1996 was extensively validated in vivo against LD50 data obtained from the literature, both nationally and internationally.

Initial Considerations

Test substances, at doses that are known to cause marked pain and distress due to corrosive or severely irritant actions, need not be administered. Moribund animals or animals obviously in

pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. Criteria for making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death, are the subject of a separate Guidance Document.

Principle of the Test

It is the principle of the test that, based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses.

The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

− No further testing is needed.

− Dosing of three additional animals, with the same dose.

− Dosing of three additional animals at the next higher or the next lower dose level.

Description of the Method

Selection of animal species

The preferred rodent species is the rat, although other rodent species may be used. Normally females are used. This is because literature surveys of conventional LD50 tests show that, although there is little difference in sensitivity between the sexes, in those cases where differences are observed females are generally slightly more sensitive. Housing and feeding condition are same as that of oecd -420.

Preparation of doses

In general test substances should be administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. Where a liquid end product or mixture is to be tested however, the use of the undiluted test substance, ie at a constant concentration, may be more relevant to the subsequent risk assessment of that substance and is a requirement of some regulatory authorities. In either case, the maximum dose volume for administration must not be exceeded. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume

should not normally exceed 1mL/100g of body weight: however in the case of aqueous solutions 2 mL/100g body weight can be considered.

Procedure

Number of animals and dose levels

 Generally the three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight.

 The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. The flow charts of Annex 2 describe the procedure that should be followed for each of the starting doses.

 When available information suggests that mortality is unlikely at the highest starting dose level (2000 mg/kg body weight), then a limit test should be conducted. When there is no information on a substance to be tested, for animal welfare reasons it is recommended to use the starting dose of 300 mg/kg body weight.

 The time interval between treatment groups is determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose, should be delayed until one is confident of survival of the previously dosed animals.

Limit test

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e, having toxicity only above regulatory limit doses.

Observations

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days and Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy etc.[3]

OECD-407

OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress. The original guideline 407 was adopted in 1981. In this revised version

changes have been made with the objective of obtaining additional information from the animals used in the study.

Initial Considerations

Generally in the assessment and evaluation of the toxic characteristics of a chemical, the determination of oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained by acute testing. This study provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time. The method comprises the basic repeated dose toxicity study that may be used for chemicals on which a 90 day study is not warranted (e.g. when the production volume does not exceed certain limits) or as a preliminary to a long term study. The duration of exposure should normally be 28 days although a 14-day study may be appropriate in certain circumstances; justification for use of a 14-day exposure period should be provided.

Principle of the Test

The test substance is orally administered daily in graduated doses to several groups of experimental animals, one dose level per group for a period of 28 days. During the period of administration the animals are observed closely, each day for signs of toxicity. Animals which die or are killed during the test are necropsied and at the conclusion of the test surviving animals are killed and necropsied.

Description of the Method

Selection of animal species

At the commencement of the study the weight variation of animals used should be minimal and not exceed ± 20% of the mean weight of each sex.

Preparation of doses

The test compound is administered by gavage or via the diet or drinking water. The method of oral administration is dependent on the purpose of the study, and the physical/chemical properties of the test material. Where necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that, wherever possible, the use of an aqueous solution/suspension be considered first, followed by consideration of a solution/emulsion in oil (e.g. corn oil) and then by possible solution in other vehicles. For vehicles other than water the toxic characteristics of the vehicle must be known. The stability of the test substance in the vehicle should be determined.

Number and sex of animals

At least 10 animals (five female and five male) should be used at each dose level. If interim kills are planned, the number should be increased by the number of animals scheduled to be killed before the completion of the study. Consideration should be given to an additional satellite group of ten animals (five per sex) in the control and in the top dose group for observation of reversibility, persistence, or delayed occurrence of toxic effects, for at least 14 days post treatment.

Dose levels

Dose should be selected taking into account any existing toxicity and (toxico-) kinetic data available for the test compound or related materials. The highest dose level should be chosen with the aim of inducing toxic effects but not death or severe suffering. Thereafter, a descending sequence of dose levels should be selected with a view to demonstrating any dosage related response and no-observed-adverse effects at the lowest dose level (NOAEL). Two to four fold intervals are frequently optimal for setting the descending dose levels and addition of a fourth test group is often preferable to using very large intervals (e.g. more than a factor of 10) between dosages.

Limit test

If a test at one dose level of at least 1000 mg/kg body weight/day or, for dietary or drinking water administration, an equivalent percentage in the diet, or drinking water (based upon body weight determinations), using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data from structurally related compounds, then a full study using three dose levels may not be considered necessary. The limit test applies except when human exposure indicates the need for a higher dose level to be used.

Administration of doses

The animals are dosed with the test substance daily seven days each week for a period of 28 days; use of a five-day per week dosing regimen or a 14-day exposure period needs to be justified. When the test substance is administered by gavage, this should be done in a single dose to the animals using a stomach tube or a suitable intubation cannula. The maximum volume of liquid that can be administered at one time depends on the size of the test animal.

The volume should not exceed 1ml/100g body weight, except in the case of aqueous solutions where 2ml/100g body weight may be used.

OBSERVATION

The observation period should be 28 days, unless the study duration is 14 days. Animals in a satellite group scheduled for follow-up observations should be kept for at least a further 14 days without treatment to detect delayed occurrence, or persistence of, or recovery from toxic effects.[4]

History

 Dioscorides, a Greek physician in the court of the Roman emperor Nero, made the first attempt to classify plants according to their toxic and therapeutic effect.

 Ibn Wahshiya wrote the *Book on Poisons* in the 9th or 10th century.

 Paracelsus, a Swiss born in 1493, is often referred to as the father of toxicology. He is known for the famous quote *the dose makes the poison* (Dosis facit venenum).

Basic Toxicology

The goal of toxicity assessment is to identify adverse effects of a substance. Adverse effects depend on two main factors:-

i) Routes of exposure (oral, inhalation, or dermal) and

ii) Dose (duration and concentration of exposure). To explore dose, substances are tested in both acute and chronic models. Generally, different sets of experiments are conducted to determine whether a substance causes cancer and to examine other forms of toxicity.

Type of Toxicant

Three types of chemical, biological, and physical toxic entities are there.

Chemical Toxicants

Chemical toxicants includes inorganic substance such as mercury, lead, hydrofluoric acid, and chlorine gas, and organic compounds such as methyl alcohol, most medications, and poisons from living things. While some of radioactive substances are also for chemical toxicants, many are not radiation poisoning results from exposure to the ionizing radiation produced by a radioactive substance rather than chemical interactions.

1. Biological toxicants

Biological toxicants generally including bacteria and viruses that can induce a disease in living organisms. Biological toxicity can be difficult to measure because of the "threshold dose" may be a single organism. Theoretically one worm, bacterium or virus can reproduce to cause a serious infection. However, in a host with an intact immune system the inherent toxicity of the organism is also balanced by the host's ability to fight back, the effective toxicity is then a combination of both parts of the relationship.

2. Physical toxicants

Physical toxicants are those substances that, due to their physical nature, interfere with biological processes. For Examples include coal dust, asbestos fibers or finely divided silicon dioxide, all of which they can ultimately be fatal if inhaled. Either the corrosive chemicals possess physical toxicity because they also destroy tissues, but they are not directly poisonous unless they interfere directly with biological activity. Similarly the water can act as a physical toxicant if taken in extremely high doses because the concentration of vital ions dramatically decreases if there is too much water in the body. Asphyxiant gases can be considered physical toxicants because they mostly act by displacing oxygen in the environment but they are inert, not a toxic gases.[5]

Factors that influence chemical toxicity

Dosage

- Both large single exposures (acute) and continuous small exposures (chronic) are studied.

Route of exposure

- Ingestion, inhalation or skin absorption

Other factors

- Species
- Age
- Sex
- Health
- Environment
- Individual characteristic

Rout of administration

- Dermal
- Intra-articular
- Intranasal
- Intravaginal and intrapenile
- Ocular
- Oral (gavage, diet and capsule)
- Parenteral (intravenous, subcutaneous, intradermal intraperitoneal)
- Rectal The two basic principles guiding toxicity test in animals To test substances on laboratory animals and its direct toxic effect on human.

Exposure of administration

Intradermal, intramuscular and check the effect of the, laboratory animals to high doses in order to evaluate its possible hazard on human that are exposed to much lower doses.[6]

Acute and Sub-Acute toxicity

Acute toxicity studies in animals are usually necessary for pharmaceutical intended for human use. The information obtained from these studies is useful in choosing the dose for repeate-dose studies which providing the preliminary identification of target organ of toxicity. This study may also aid in the selection of starting dose for Phase 1 human studies and which provide the information to acute overdosing in humans.

Acute Toxicity

Acute toxicity is the toxicity produced by pharmaceuticals, when it is administered in one or more doses during a period which is not exceeding 24 hr. Generally the does are given to a small groups of animals and the animals are observed for mortality and the LD50 dose is then calculated (the dose required to kill the 50% of the population).

MTD Issue

The maximum tolerated dose is define as the highest dose of a chemical or drug that can be administered for the animal's life without causing the excessive toxicity of decreasing survival (except due to tumor induction).

Objective

 To determine the Median Lethal Dose (LD50) after a single dose administered through one or routes.

 To determine MTD (Maximum Tolerated Dose) and No Observable Effect Level (NOEL).

Acute Toxicity Studies:

 Single dose-rat, mouse (5/sex/dose), dog, monkey (1/sex/dose).

 14 day observation.

 Necropsy and histopathology.

 Clinical pathology.

 In life observation (body wt., food consumption, clinical observation. Repeated dose toxicity studies.

Generally the repeated dose are given to animals for 2-12 weeks on the duration of intended treatment in man and the dose are selected on the basis of ED50 as well as LD 50. Animals are observed for their over effects, food intake, haematology, body wt and organ toxicity. Importance of acute toxicity testing.

 To identify the target organ of toxicity.

 To provides safety measures and monitoring guild lines for workers involved in the development and testing of test substances.

 To provides information needed for the dose selection in prolonged toxicity studies.

 To generate data containing the adverse effects of a substance on human, animal health and environment.

 To provides the basis for which other testing program also can be design.

Table No. 1: Repeated dose toxicity along with specific duration of the Repeated Dose Toxicity Study. Objectives.

These studies have three main objectives.

(i) To identify toxicity that develops only after a certain length of continuous exposure to the chemical.

(ii) To identify the organs most affected and

(iii) To determine the doses at which each effect occurs

Sub-Acute Toxicity

This study is conducted to determine organs affected by different dose levels and This study access the nature of toxic dose under more realistic situation than the acute toxicity studies. Three dose levels are normally used.

- Dose that is high enough to elicit definite signs of toxicity but not to kill many of the animals.
- Low dose that is expected to induce no toxic effect.
- Intermediate dose.

Doses are generally selected on the basis of information obtained in acute toxicity studies using both LD50 and the slope of the dose response curve. The duration of subacute toxicity studies depend on intended duration of the test substance.[7]

INTRODUCTION

Toxicity is the degree at which any substance it may be poison or toxin that can cause the harmful effect on human or animals. In an organism acute toxicity involves the harmful effect through a single or short-term exposure. The ability of a toxic substance to cause effects more than one year but this effect is less than the lifetime of the exposed organism referred as Subchronic toxicity respectively. The ability of any substance or mixture of any substance which the harmful effect due to the repeated or continuous exposure or due to the extended period, which also be sometime lasting stage for the entire life of the exposed organism and these effect generally referred as Chronic toxicity respectively.[1]

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- Testing in one sex (usually females) is now considered sufficient.

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Criteria for making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death, are the subject of a separate Guidance Document respectively. The method provides information on the hazardous properties and allows the substance to be ranked and classified according to the Globally Harmonised System (GHS) for the classification of chemicals which cause acute toxicity. The testing laboratory should consider all available information on the test substance prior to conducting the study. Such information will include the identity and chemical structure of the substance;

 Its physico-chemical properties.

 The results of any other in vitro or in vivo toxicity tests on the substance.

 Toxicological data on structurally related substances.

 The anticipated use of the substance.

This information is necessary to satisfy all concerned that the test is relevant for the protection of human health, and will help in the selection of an appropriate starting dose.

Principle of the test Groups of animals of a single sex are dosed in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg (exceptionally an additional fixed dose of 5000 mg/kg may be considered, see paragraph 19). The initial dose level is selected on the basis of a sighting study as the dose expected to produce some signs of toxicity without causing severe toxic effects or mortality. Clinical signs and conditions associated with pain, suffering, and impending death, are described in detail in a separate OECD Guidance Document. Further groups of animals may be dosed at higher or lower fixed doses, depending on the presence or absence of signs of toxicity or mortality. This procedure continues until the dose causing evident toxicity or no more than one death is identified, or when no effects are seen at the highest dose or when deaths occur at the lowest dose.

DESCRIPTION OF THE METHOD

Selection of animal species

 The preferred rodent species is the rat, and the other rodent species may be used. Normally females are used . This is because literature surveys of conventional LD50 tests show that usually there is little difference in sensitivity between the sexes, but in those cases where differences are observed, females are generally slightly more sensitive.

 However, if knowledge of the toxicological or toxicokinetic properties of structurally related chemicals indicates that males are likely to be more sensitive then this sex should be used. When the test is conducted in males, adequate justification should be provided.

 Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant.

 Each animal, at the commencement of its dosing, should be between 8 and 12 weeks old and its weight should fall in an interval within + 20 % of the mean weight of any previously dosed animals Housing and feeding conditions. The temperature of the experimental animal room should be 22ºC (+ 3ºC).

Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal respectively.

Preparation of dosesIn general test substances should be administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. Where a liquid end product or mixture is to be tested however, the use of the undiluted test substance, i.e at a constant concentration, may be more relevant to the subsequent risk assessment of that substance, and is a requirement of some regulatory authorities. In either case, the maximum dose volume for administration must not be exceeded. The maximum volume of liquid that can be administered at one time depends on the size of the test animal.

PROCEDURE

Administration of doses

 The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation canula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours.

 Animals should be fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night; with the mouse, food but not water should be withheld for 3-4 hours).

Sighting study

 The purpose of the sighting study is to allow selection of the appropriate starting dose for the main study. The test substance is administered to single animals in a sequential manner following the flow charts in Annex 2. The sighting study is completed when a decision on the starting dose for the main study can be made (or if a death is seen at the lowest fixed dose).

 The starting dose for the sighting study is selected from the fixed dose levels of 5, 50, 300 and 2000 mg/kg as a dose expected to produce evident toxicity based, when possible, on evidence from *in vivo* and *in vitro* data from the same chemical and from structurally related chemicals. In the absence of such information, the starting dose will be 300 mg/kg.

 A period of at least 24 hours will be allowed between the dosing of each animal. All animals should be observed for at least 14 days.

OBSERVATIONS

 Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation should not be fixed rigidly.

 It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed and also for their body weight and pathology.

 All observations are systematically recorded, with individual records being maintained for each animal.[2]

OECD-423

The acute toxic class method (1) set out in this Guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods (Test Guidelines 420 and 425). The method as adopted in 1996 was extensively validated in vivo against LD50 data obtained from the literature, both nationally and internationally.

Initial Considerations

Test substances, at doses that are known to cause marked pain and distress due to corrosive or severely irritant actions, need not be administered. Moribund animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. Criteria for making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death, are the subject of a separate Guidance Document.

Principle of the Test

It is the principle of the test that, based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

− No further testing is needed.

− Dosing of three additional animals, with the same dose.

− Dosing of three additional animals at the next higher or the next lower dose level.

Description of the Method

Selection of animal species

The preferred rodent species is the rat, although other rodent species may be used. Normally females are used . This is because literature surveys of conventional LD50 tests show that, although there is little difference in sensitivity between the sexes, in those cases where differences are observed females are generally slightly more sensitive . Housing and feeding condition are same as that of oecd -420.

Preparation of doses

In general test substances should be administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. Where a liquid end product or mixture is to be tested however, the use of the undiluted test substance, ie at a constant concentration, may be more relevant to the subsequent risk assessment of that substance, and is a requirement of some regulatory authorities. In either case, the maximum dose volume for administration must not be exceeded. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume should not normally exceed 1mL/100g of body weight: however in the case of aqueous solutions 2 mL/100g body weight can be considered.

Procedure

Number of animals and dose levels

 Generally the three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight.

 The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. The flow charts of Annex 2 describe the procedure that should be followed for each of the starting doses.

 When available information suggests that mortality is unlikely at the highest starting dose level (2000 mg/kg body weight), then a limit test should be conducted. When there is no information on a substance to be tested, for animal welfare reasons it is recommended to use the starting dose of 300 mg/kg body weight.

 The time interval between treatment groups is determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose, should be delayed until one is confident of survival of the previously dosed animals.

Limit Test

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e, having toxicity only above regulatory limit doses.

Observations

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days and Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy etc.[3]

OECD-407

OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress. The original guideline 407 was adopted in 1981. In this revised version changes have been made with the objective of obtaining additional information from the animals used in the study.

Initial Considerations

Generally in the assessment and evaluation of the toxic characteristics of a chemical, the determination of oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained by acute testing. This study provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time. The method comprises the basic repeated dose toxicity study that may be used for chemicals on which a 90 day study is not warranted (e.g. when the production volume does not exceed certain limits) or as a preliminary to a long term study. The duration of exposure should normally be 28 days although a 14-day study may be appropriate in certain circumstances; justification for use of a 14-day exposure period should be provided.

Principle of the Test

The test substance is orally administered daily in graduated doses to several groups of experimental animals, one dose level per group for a period of 28 days. During the period of administration the animals are observed closely, each day for signs of toxicity. Animals

which die or are killed during the test are necropsied and at the conclusion of the test surviving animals are killed and necropsied.

Description of the Method

Selection of animal species

At the commencement of the study the weight variation of animals used should be minimal and not exceed ± 20% of the mean weight of each sex.

Preparation of doses

The test compound is administered by gavage or via the diet or drinking water. The method of oral administration is dependent on the purpose of the study, and the physical/chemical properties of the test material. Where necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that, wherever possible, the use of an aqueous solution/suspension be considered first, followed by consideration of a solution/emulsion in oil (e.g. corn oil) and then by possible solution in other vehicles. For vehicles other than water the toxic characteristics of the vehicle must be known. The stability of the test substance in the vehicle should be determined.

Number and sex of animals

At least 10 animals (five female and five male) should be used at each dose level. If interim kills are planned, the number should be increased by the number of animals scheduled to be killed before the completion of the study. Consideration should be given to an additional satellite group of ten animals (five per sex) in the control and in the top dose group for observation of reversibility, persistence, or delayed occurrence of toxic effects, for at least 14 days post treatment.

Dose levels

Dose should be selected taking into account any existing toxicity and (toxico-) kinetic data available for the test compound or related materials. The highest dose level should be chosen with the aim of inducing toxic effects but not death or severe suffering. Thereafter, a descending sequence of dose levels should be selected with a view to demonstrating any dosage related response and no-observed-adverse effects at the lowest dose level (NOAEL). Two to four fold intervals are frequently optimal for setting the descending dose levels and addition of a fourth test group is often preferable to using very large intervals (e.g. more than a factor of 10) between dosages.

Limit test

If a test at one dose level of at least 1000 mg/kg body weight/day or, for dietary or drinking water administration, an equivalent percentage in the diet, or drinking water (based upon body weight determinations), using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data from structurally related compounds, then a full study using three dose levels may not be considered necessary. The limit test applies except when human exposure indicates the need for a higher dose level to be used.

Administration of doses

The animals are dosed with the test substance daily seven days each week for a period of 28 days; use of a five-day per week dosing regimen or a 14-day exposure period needs to be justified. When the test substance is administered by gavage, this should be done in a single dose to the animals using a stomach tube or a suitable intubation cannula. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. The volume should not exceed 1ml/100g body weight, except in the case of aqueous solutions where 2ml/100g body weight may be used.

Observation

The observation period should be 28 days, unless the study duration is 14 days. Animals in a satellite group scheduled for follow-up observations should be kept for at least a further 14 days without treatment to detect delayed occurrence, or persistence of, or recovery from toxic effects.[4]

History

 Dioscorides, a Greek physician in the court of the Roman emperor Nero, made the first attempt to classify plants according to their toxic and therapeutic effect.

 Ibn Wahshiya wrote the *Book on Poisons* in the 9th or 10th century.

 Paracelsus, a Swiss born in 1493, is often referred to as the father of toxicology. He is known for the famous quote *the dose makes the poison* (Dosis facit venenum)..

Basic Toxicology

The goal of toxicity assessment is to identify adverse effects of a substance. Adverse effects depend on two main factors.

i) Routes of exposure (oral, inhalation, or dermal) and

ii) Dose (duration and concentration of exposure). To explore dose, substances are tested in both acute and chronic models. Generally, different sets of experiments are conducted to determine whether a substance causes cancer and to examine other forms of toxicity.

Type of Toxicant

Three types of chemical, biological, and physical toxic entities are there.

Chemical Toxicants

Chemical toxicants includes inorganic substance such as mercury, lead, hydrofluoric acid, and chlorine gas, and organic compounds such as methyl alcohol, most medications, and poisons from living things. While some of radioactive substances are also for chemical toxicants, many are not radiation poisoning results from exposure to the ionizing radiation produced by a radioactive substance rather than chemical interactions.

2. Biological toxicants

Biological toxicants generally including bacteria and viruses that can induce a disease in living organisms. Biological toxicity can be difficult to measure because of the "threshold dose" may be a single organism. Theoretically one worm, bacterium or virus can reproduce to cause a serious infection. However, in a host with an intact immune system the inherent toxicity of the organism is also balanced by the host's ability to fight back, the effective toxicity is then a combination of both parts of the relationship.

2. Physical toxicants

Physical toxicants are those substances that, due to their physical nature, interfere with biological processes. For Examples include coal dust, asbestos fibers or finely divided silicon dioxide, all of which they can ultimately be fatal if inhaled. Either the corrosive chemicals possess physical toxicity because they also destroy tissues, but they are not directly poisonous unless they interfere directly with biological activity. Similarly the water can act as a physical toxicant if taken in extremely high doses because the concentration of vital ions dramatically decreases if there is too much water in the body. Asphyxiant gases can be considered physical toxicants because they mostly act by displacing oxygen in the environment but they are inert, not a toxic gases.[5]

Factors that influence chemical toxicity

- **Dosage**
- Both large single exposures (acute) and continuous small exposures (chronic) are studied.

- **Route of exposure**
- Ingestion, inhalation or skin absorption

- **Other factors**
- Species
- Age
- Sex
- Health
- Environment
- Individual characteristic

Rout of administration

- Dermal
- Intra-articular
- Intranasal
- Intravaginal and intrapenile
- Ocular
- Oral (gavage, diet and capsule)
- Parenteral (intravenous, subcutaneous, intradermal, intramuscular and intraperitoneal)
- Rectal

Figure. 1 .1 Various Adverse Reaction of Drug Administration.

The two basic principles guiding toxicity test in animals To test substances on laboratory animals and its direct toxic effect on human. Exposure of laboratory animals to high doses in order to evaluate its possible hazard on human that are exposed to much lower doses.[6]

Acute and Sub-Acute toxicity

Acute toxicity studies in animals are usually necessary for pharmaceutical intended for human use. The information obtained from these studies is useful in choosing the dose for repeate identification of target organ of toxicity. This study may also aid in the selection of

starting dose for Phase 1 human studies and which provide the information to acute overdosing in humans.

Acute Toxicity

Acute toxicity is the toxicity produced by pharmaceuticals administered in one or more doses during a period which is not exceeding 24 hr. Check the effect of the **te** repeate-dose studies which providing the preliminary on pharmaceuticals, when it is,

Generally the does are given to a small groups of animals and the animals are observed for mortality and the LD50 dose is then calculated (the dose required to kill the 50% of the population).

MTD Issue

The maximum tolerated dose is define as the highest dose of a chemical or drug that can be administered for the animal's life without causing the excessive toxicity of decreasing survival (except due to tumor induction).

Objective

 To determine the Median Lethal Dose (LD50) after a single dose administered through one or routes.

 To determine MTD (Maximum Tolerated Dose) and No Observable Effect Level (NOEL).

Acute Toxicity Studies

 Single dose-rat, mouse (5/sex/dose), dog, monkey (1/sex/dose).

 14 day observation.

 Necropsy and histopathology.

 Clinical pathology.

 In life observation (body wt., food consumption, clinical observation.

Repeated dose toxicity studies

Generally the repeated dose are given to animals for 2-12 weeks on the duration of intended treatment in man and the dose are selected on the basis of ED50 as well as LD 50. Animals are observed for their over effects, food intake, haematology, body wt and organ toxicity.

Importance of acute toxicity testing

 To identify the target organ of toxicity.

 To provides safety measures and monitoring guild lines for workers involved in the development and testing of test substances.

 To provides information needed for the dose selection in prolonged toxicity studies.

 To generate data containing the adverse effects of a substance on human, animal health and environment.

 To provides the basis for which other testing program also can be design.

Table No. 1: Repeated dose toxicity along with specific duration of the Repeated Dose Toxicity Study.

Duration of clinical trials Rodents N o n Rodents

Single Dose 2-4 weeks*** 2 weeks

Up to 2 weeks 2-4 weeks*** 2 weeks

Up to 1 month 1month 1 month

Up to 3 Months 3 month 3 month

Up to 6 months 6 month 6 month***

> 6 months 6 month chronic***

Objectives

These studies have three main objectives-

(iv) To identify toxicity that develops only after a certain length of continuous exposure to the chemical.

(v) To identify the organs most affected and

(vi) To determine the doses at which each effect occurs

Sub-Acute Toxicity

This study is conducted to determine organs affected by different dose levels and This study access the nature of toxic dose under more realistic situation than the acute toxicity studies. Three dose levels are normally used.

 Dose that is high enough to elicit definite signs of toxicity but not to kill many of the animals.

 Low dose that is expected to induce no toxic effect.

 Intermediate dose.

Doses are generally selected on the basis of information obtained in acute toxicity studies using both LD50 and the slope of the dose response curve. The duration of subacute toxicity studies depend on intended duration of the test substance.[7]

MATERIAL AND METHOD

Plant material and their extraction

The dried roots of *Withania somnifera* (Bath number: EBD-18), dried whole plant of *Centella asiatica* (Bath number: ERD-040), *Bacopa monnieri* (Bath number: ERD- 92) and dried leaves of *Ocimum sanctum* (Bath number: RHD 283), *Camellia sinensis* (Bath number: ERM-23) has been purchased from Natural Remedies, Bangalore. The aerial part of *Hypericum perforatum* L. have been collected from herbal garden of Jaypee University of Information Technology, Waknaghat, Dist. Solan, Himachal Pradesh. The identification of the plant *Hypericum perforatum* L. was done by Dr. Bhupendra Dutt, Research Officer, Department of Forest Products, College of Forestry, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. A voucher specimen of the said plant has been deposited to UHFHerbarium (Field book number: 13420). The individual plant was coarsely powdered with a mechanical grinder and passed through sieve number 60 and stored in an air tight container. The coarsely powdered individual plant was subjected to defatting with petroleum ether (60°-80°C). The defatted air dried individual plant powder have been extracted by Soxhlet apparatus using 70% v/v of ethanol (hydroalcoholic) at 50°C for 48 hours. The solvent is recovered by evaporation under reduced pressure using rotary evaporator (Heidolph, Germany). The semisolid mass have been further lyophilized (New Brunswick) at -80°C for 24 hours. The obtained dry powder of individual plant is being stored at -20°C till further use. The percentages of different extractive values were also calculated in terms of air-dried weight of plant material. The percentage yield of hydroalcoholic *Withania somnifera, Centella asiatica, Bacopa monnieri, Ocimum sanctum, Camellia sinensis* and *Hypericum perforatum* L were found to be 9.28% w/w, 24% w/w, 8% w/w, 10.8% w/w, 15.25% w/w and 12.75% w/w respectively.

A. Soxhlet Apparatus B. Lyophilizer

Fig. Extraction procedure of different plants Experimental animals

Animal studies were conducted at Jaypee University after the due approval (Institutional Animal Ethics Committee (IAEC). Wistar rats procured from the Central Animal Facility, Jaypee University, Waknaghat. Toxicity study was conducted on Wistar rats i.e Wistar male

rats (200-260g) and Wistar female rats (150-185g), both sexes of Wistar rats were used respectively and divided into different groups for the toxicity purpose study. They were grouped housed in Autoclave polypropylene cages (640 x 410x 250 mm high) with sterile paddy husk as the bedding material. For the experimental animals, their housing temperature, relative humidity, light : dark cycle and air changes per hour were maintained at 22±3 0C, 30 to70%, 12-12 hr, 12-15 air changes per hour, respectively. All the animals had access to standard rodent feed (ad libitum) and RO water (ad libitum). Rats were randomly housed in group of three cages, 5 day before the commencement of experimental study for the acclimatization period respectively.

Habituation to animals

Wistar Rats were habituated to handling by holding them and injecting vehicle through oral routes to minimize non-specific stress and to simulate the actual protocol conditions. Handling also consisted of weighing and restraining animals on platform for 1 min, and gently massaging on dorsal site as was done in the actual protocols. The same platform was used during drug administration. Moreover, the animals were familiarized with the diet in their home cage-environment and laboratory environments, and also familiar with the person who conducted the study before subjecting them to the tests. All the experiments commenced 24 hours following the final habituation and were conducted according to the protocols mentioned below.

Acute toxicity study of polyherbal extract in rats

The acute toxicity study was performed according to the Organisation for Economic Cooperation and Development (OECD) test guidelines - 425 for the conduct of acute oral toxicity with slight modifications (OECD, 2001) respectively. In this study healthy female Wistar rats (nulliparous and non-pregnant) of 8–12 weeks of age and their individual body weights falling within ±20% of the mean initial body weights of each sex which were used. Hydroalcoholic polyherbal extract dissolved in distilled water which containing CMC 1% respectively, adjusted to 1 ml/100 g body weight was administered by oral gavage at a limit dose of 2000 mg/kg body weight. The animals were observed individually during the first 30 min after the dosing and also observe periodically during the first 24 hr and also observe daily thereafter for -:

- general behavioral changes
- signs of toxicity

- mortality and
- latency of death.

Table 2. Acute toxicity studies Group No. No. of rats Dose Day of Sacrifice Day of Blood collection

G1 3 2000 mg/kg 14th day 1 4 t h d a y

3 females

Sub acute toxicity study

The sub-acute toxicity study was generally performed according to the OECD test guidelines-407 for the conduct of repeated dose 28 day, oral toxicity study in rodents with slight modifications (OECD, 1995) respectively. The male Wistar rats and female Wistar rats of 6–8 weeks of age were used. During the whole study, the weight variation of animals did not exceed + 20% of the mean weight of each sex. The Wistar rats divided into six groups which were treated as follows.

Group No. No. of rats Dose Day of sacrifice Day of blood Collection G1 10 Control 28th day 28th day.

G2 10 500 mg/kg b.w 28th day 28th Day (low dose).

G3 10 1000 mg/kg b.w 28th day 28th da.y

(Intermediate dose)

G4 10 2000 mg/kg b.w 28th day 28th day (High dose).

G5 10 Control (satellite) 42nd day 28thand 42ndday

G6 10 2000 mg/kg b.w 42nd day 28th and 42nd day (High dose satellite) Five males + five females.

Body weight of each rat was recorded prior to the administration of the test substance (i.e from day 0) and weekly thereafter respectively. Their cage- wise feed consumption was recorded once in a week. All the animals were observed twice daily for;

- morbidity/mortality
- clinical sign of toxicity.

Preparation of the test substance

The polyherbal formulation was firstly prepared and the doses prepared by dissolving the hydroalcoholic polyherbal extract in distilled water which containing the 1% CMC, which were administered orally by using the stainless steel cannula and the total dose volume was adjusted to 1ml/100g body weight of the rats respectively.

Preparation of polyherbal extract

Polyherbal ratio Ratio 100 g

Centella asiatica 220 gm

Bacopa monniera 220 gm

Hypericum perforatum 110 gm

Withania somnifera 220 gm

Oscimum sanctum. 220 gm

Camellia sinensis 110 gm

Collection of blood.

The blood was collected from the retro-orbital sinus of the rats in ethylene-diamine tetraacetate(EDTA)-coated vials and heparinised vials for the assessment of haematological and biochemical parameters respectively. Whole the blood which collected in the EDTA vials was used for the determination of haematological parameters and the blood which collected in Heparinised vial was used for the biochemical parameters.

Haemato-biochemical observations

Haematological parameters which were observed included, white blood cell (WBC) count, relative leukocyte counts Neutrophil (NEU-R), Eosinophil (EOS-R), Basophil (BAS-R), Monocytes (MON-R) and Lymphocytes (LYM-R)), Red blood cell (RBC), hemoglobin (HGB), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), Packed cell volume (PCV) and Erythrocyte rate (ESR). The biochemical parameters which were observed included, Blood urea, Serum creatinine (S.CRE),

Serum uric acid, Total billirubin, Billirubin direct, Billirubin indirect. SGOT, SGPT, cholesterol (CHOL), Blood sugar,Serum alkaline phosphatase (S.ALP), Serum protein, albumin (ALB) ,Globulin, A/G Ratio, Gamma-glutamyl transpeptidase (GGT).

Pathology

Wistar rats were sacrificed by using cervical dislocation method and were subjected to complete and detailed gross necroscopy. The weights of different organ like liver, adrenals, spleen, heart, kidneys, brain, thymus, testes/ovaries, epididymis and uterus were recorded and then these were expressed in absolute and relative terms (g and g/100 g of body weight, respectively).Brain, pituitary, thyroid, parathyroid, thymus, trachea, lungs, kidneys, heart, aorta, liver, spleen, adrenals, pancreas, ovaries, uterus, esophagus, stomach, small intestine, large intestine, urinary bladder, mesenteric lymph node, peripheral nerve (sciatic nerve) and sternum with bone marrow were preserved in 10% Neutral buffered formalin (NBF) and then processed for histopathological examination.

Statistical evaluation

In the statistical evaluation all the observed values are expressed as mean ± standard error mean. Variance in data for body weights, feed and water consumption,

hematology, serum biochemistry and organ weights (both absolute and relative weights) was checked for homogeneity by Bartlett's procedure. If the variance was homogeneous, the data were assessed by one-way analysis of variance followed by the Tukey test. If not, the Kruskal –Wallis test was applied, and when statistically significant differences were indicated, the Dunn test was employed for comparison between the control and treatment groups. A probability level lower than 5% ($p<0.05$). Was considered significant. Statistical analyses were performed using Graph Pad Prism 6 software.

RESULT

Acute toxicity

Acute toxicity of Polyherbal extract in rats.Nobehavioural signs of toxicity were observed in therats after the single oral administration of 2000 mg/kg of Polyherbalextract. All the five rats survived untilday 14, and the necropsy examination of the carcasseson day 14 revealed no treatment related gross pathologicalchanges.Following four table showing all the result of acute toxicity.

Table 1. Effect of oral administration of hydroalcoholic extract of Polyherbaloverthe body weight and feed intake of Wistar rats.

Polyherbal extract (mg/kg) Day 2000

Body weight(g) Female

0 176.2±0.83

7194.0±0.51

Feed intake (g) Male

016.33±0.55.

7 19.00±0.36.

Means bearing * vary significantly between groups (p ≤0.05).

Table 2.Effect of oral administration of hydroalcoholic extract of Polyherbal over the haematological parameters of Wistar rats.

Parameter Units 2000 mg/kg

WBC 103µL 4.73±1.38

LYM –R R% 78.50±1.17

MON-R R% 3.00±0.51

NEU-R R% 17.83±1.30

EOS-R R% 0.50±0.34

BAS-R R% 0.00±0.00

RBC 106/µL 6.73±0.01

HGB g/dL 12.42±0.00

MCV µm3 59.73±2.81

MCH Pg 18.52±0.10

PLT 103/µL 3.64±0.25

MCHC g/dL 34.67±1.38

ESR mm/1st hr 1.50±0.22

PCV % 50.08±0.58

Means bearing * vary significantly between groups (p ≤0.05).

Table: 3. Effects of oral administration of hydroalcoholic extract of polyherbal overthe biochemical parameters of Wistar rats. Parameter Units 2000 mg/kg

ALB g/L 3.63±0.08

CHO mg/dL 83.82±0.68

Serum protein g/L 8.06±0.16

ALP U/L 121.1±0.67

CRE mg/dL 0.52±0.21

Urea mg/dL 30.88±1.01

Total billirubin mg/dL 0.52±0.00

Billirubin direct mg/dL 0.03±0.01

Billirubin indirect mg/dL 0.03±0.01

SGOT U/L 51.23±1.06

SGPT U/L 24.43±1.24

Globulin g/L 4.06±0.09

A/G ratio Ratio 0.80±0.00

Blood sugar mg/dL 136.7±0.84

Means bearing * vary significantly between groups ($p \leq 0.05$).

Table 4.Effect of oral administration of hydro alcoholic extract of Polyherbal over the organ weights and relative organ weights of Wistar rats. Organs weight 2000 mg/kg

Brain 1.82±0.00

Heart 0.76±0.01

Liver 7.36±0.00

Spleen 0.42±0.00

Thymus 0.32±0.01

Kidney 1.75±0.00

Adrenals 0.08±0.03

Ovaries 0.10±0.00

Uterus 0.45±0.00

Organ to body weight ratio 2000 mg/kg

Brain 0.97±0.00

Heart 0.43±0.01

Liver 4.07±0.00

Spleen 0.27±0.00

Thymus 0.20±0.00

Kidney 0.97±0.00

Adrenals 0.04±0.00

Ovaries 0.06±0.00

Uterus 0.27±0.00

Means bearing * vary significantly between groups ($p \leq 0.05$).

Sub-acute toxicity study

Clinical observations

Clinically, no toxic signs such aspiloerection, alteration in the locomotor activity orsignificant changes in feed and water consumptionwere observed in the treated groups. No mortalitywas observed in the treated groups. No significantchanges ($P<0.05$) in the body weights and feed intake (Table 5) were observed in the treatment groups ascompared to those of the control. **Haemato-biochemical parameters**:-Administration ofPolyherbalextract did not cause any significant changein the haematological and biochemical parameters (Tables 6 and 7, respectively) of the rats at any of thedose levels tested ($P<0.05$).

Table 5. Effect of oral administration of hydroalcoholic extract of Polyherbaloverthe body weight and feed intake of Wistar rats. Day Body weight.

(g) Male

0 mg/kg 500 mg/kg 1000 mg/kg 2000 mg/kg

0 2.14±1.01 254±0.88 263.5±0.76 250.0±0.57

7 216.0±0.57 253.5±0.76 261.0±0.60 250.2±0.70

14 216.5±0.76 251.5±0.76 261.5±0.76 251.0±0.57

21 219.2±0.70 253.5±0.76 262.5±0.76 254.2±1.07

28 220.5±0.76 253.0±0.57 262.0±0.57 253.5±0.76

Female

0 1.70±0.60 183.5±0.76 174.5±0.76 175.2±1.35

7 173.3±0.76 184±0.60 174.5±0.76 175.2±1.44

14 175.5±0.76 185.5±0.61 175.3±1.05 176.5±0.76

21 178.5±0.99 185.5±0.99 177.2±0.79 179.3±1.83

28 179.2±0.70 185.0±0.57 178.3±0.49 181.8±0.83

Feed intake

(g) Male

0 mg/kg 500 mg/kg 1000 mg/kg 2000 mg/kg

0 16.50±1.17 15.83±0.94 20.67±0.88 17.67±0.76

7 21.50±0.76 20.67±1.05 19.83±1.35 18.67±0.71

14 21.00±0.73 20.17±0.70 18.00±0.73 19.17±0.47

21 21.33±0.71 19.67±1.11 19.17±1.55 20.50±1.05

28 22.17±0.60 22.00±0.81 20.33±1.11 21.17±0.79

Female

0 12.50±0.42 12.17±0.60 13.67±0.71 10.17±0.47

7 14.50±0.76 12.50±0.76 13.50±0.76 14.17±1.07

14 14.83±0.79 13.83±1.53 13.17±0.70 15.50±0.56

21 14.83±1.40 14.83±1.57 14.83±1.24 15.17±1.19

28 16.00±0.96 14.50±1.40 14.17±0.94 16.17±87

Means bearing * vary significantly between groups ($p \leq 0.05$).

Table 6. Effect of oral administration of hydroalcoholic extract of Polyherbal over the haematological parameters of Wistar rats.

Parameter

Male

0 mg/kg 500 mg/kg 1000 mg/kg 2000 mg/kg WBC(103µL) 9.02±2.17 7.08±60.0 453±133.3± 535±76.3.

LYM –R% 78.83±1.07 75.0±1.52 73.33±0.91 74.33±1.02

MON-R% 1.02±0.16 1.16±0.16 1.66±0.21 1.33±0.21

NEU-R% 17.50±0.61 20.17±0.60 21.50±0.42 22.17±0.30

EOS-R% 1.00±0.00 1.16±0.16 1.00±0.00 1.33±0.21

BAS-R% 0.00±0.00 0.00±0.00 0.00±0.00 0.00±0.00

RBC(106µL) 5.11±0.00 4.78±0.00 4.88±0.00 4.95±0.00

HGB(g/dL) 13.32±0.04 12.22±0.04 12.30±0.00 12.46±0.00

MCV(µm3) 88.07±0.52 89.23±0.41 86.42±0.06 89.13±0.04

MCH(pg) 24.70±0.53 25.42±0.07 24.00±0.29 23.35±0.47

PLT(103µL) 29.02±0.10 28.07±0.07 28.68±0.06 27.30±0.11

MCHC(g/dL) 2.24±0.00 2.37±0.00 3.94±0.00 2.36±0.00

ESR(mm/1st hr)
1.33±0.21 1.16±0.16 1.33±0.21 1.16±0.16
sPCV(%) 42.58±0.90 39.30±0.78 42.07±0.98 41.07±0.98

Female
WBC(103µL) 4.41±9.45 4.50±9.66 45.8±1.13 4.56±1.20
LYM –R% 57.14±0.73 62.57±0.64 66.29±0.68 38.71±0.96
MON-R% 2.00±0.36 2.50±0.67 1.83±0.30 2.00±0.44
NEU-R% 36.83±1.07 29.00±1.06 27.67±1.14 31.67±0.88
EOS-R% 0.83±0.30 1.33±0.30 0.33±1.21 0.33±1.21
BAS-R% 0.00±0.00 0.00±0.00 0.00±0.00 0.00±0.00
RBC(106µL) 4.34±0.00 4.69±0.00 5.11±0.01 4.66±0.01
HGB(g/dL) 12.70±0.05 11.62±0.10 13.20±0.05 11.50±0.09
MCV(µm3) 76.63±5.61 62.93±5.82 70.32±6.77 65.85±6.02
MCH(pg) 21.88±1.02 21.32±0.76 22.13±0.94 22.12±0.65
PLT(103µL) 32.65±0.59 31.57±0.59 31.60±0.60 30.12±2.12
MCHC(g/dL) 4.06±0.02 4.06±0.01 4.04±0.01 2.36±0.03
ESR(mm/1st hr)
1.50±0.34 1.33±0.21 1.16±0.16 1.16±0.16 PCV(%) 42.32±0.84 38.47±0.65 44.03±1.15 39.83±0.84

Means bearing * vary significantly between groups ($p \leq 0.05$).

Table 7. Effect of oral administration of hydroalcoholic extract of Polyherbal over the biochemical parameters of Wistar rats. Parameter Male
0 mg/kg 500 mg/kg 1000 mg/kg 2000 mg/kg
ALB(g/L) 30.31±0.02 30.24±0.01 30.17±0.01 30.18±0.01
CHO(mg/dL) 90.03±0.00 90.37±0.20 90.69±0.42 90.52±0.50
Serum protein (g/L)
6.55±0.07 6.68±0.06 6.51±0.08 6.61±0.07
ALP(U/L) 266.5±0.76 239.5±0.76 207.5±0.76 244.5±0.76
CRE(mg/dL) 0.50±0.01 0.52±0.005 0.50±0.00 0.51±0.02
Urea(mg/dL) 21.86±0.01 24.66±0.00 22.27±0.00 19.28±0.00

Total
billirubin(mg/dL)
0.50±0.00 0.49±0.00 0.51±0.00 0.51±0.00
Billirubin
direct(mg/dL)
0.21±0.00 0.23±0.00 0.21±0.00 0.22±0.00

Parameter 0 mg/kg 500 mg/kg 1000 mg/kg 2000 mg/kg
Billirubin
indirect(mg/dL)
0.27±0.00 0.28±0.00 0.28±0.01 0.26±0.01
SGOT(U/L) 87.50±0.76 74.33±0.66 75.00±1.39 91.33±0.42
SGPT(U/L) 46.50±0.76 47.50±0.76 45.66±1.29 45.00±0.93
Globulin(g/L) 3.46±0.11 3.54±0.08 3.65±0.07 3.65±0.04
A/G Ratio 0.81±0.00 0.79±0.01 0.78±0.00 0.74±0.01
Blood sugar 15.49±0.13 15.65±0.07 15.67±0.09 15.43±0.15

Female
ALB(g/L) 30.19±0.01 30.25±0.00 30.17±0.01 30.17±0.16
CHO(mg/dL) 110.6±0.00 120.0±0.00 127±0.01 122.9±0.01
Serum protein (g/L) 6.65±0.04 6.40±0.07 6.40±0.07 6.38±0.08
ALP(U/L) 239.9±0.00 222.2±0.00 208.0±0.16 245.3±0.00
CRE(mg/dL) 0.51±0.00 0.50±0.00 0.51±0.00 0.52±0.00
Urea(mg/dL) 21.82±0.00 22.30±0.01 21.89±0.01 25.42±0.00
Total 0.51±0.00 0.52±0.00 0.51±0.00 0.51±0.00
Means bearing * vary significantly between groups ($p \leq 0.05$).

Table 8.Effect of oral administration of hydroalcoholic extract of Polyherbal over the organ weights and relative organ weight of Wistar rats.

Organ weight Male
0 mg/kg 500 mg/kg 1000 mg/kg 2000 mg/kg
Brain 1.35±0.00 1.94±0.01 1.88±0.01 1.85±0.01
Heart 0.93±0.00 1.02±0.01 1.02±0.01 0.97±0.01
Liver 10.33±0.01 10.82±0.01 10.43±0.01 10.12±0.01

Spleen 0.57±0.02 0.61±1 0.61±0.01 0.47±0.05

Thymus 0.36±0.01 0.33±0.01 0.32±0.00 0.32±0.00

Kidney 2.66±0.01 2.90±0.00 2.77±0.00 2.72±0.01

Adrenals 0.02±0.00 0.04±0.00 0.03±0.00 0.03±0.00

Testes 2.65±0.01 2.63±0.00 2.83±0.01 2.71±0.00

Epididymis 0.34±0.00 0.84±0.01 0.84±0.00 0.83±0.00

Organ to body weight ratio

0 mg/kg 500 mg/kg 1000 mg/kg 2000 mg/kg

Brain 0.63±00 0.63±0.00 0.65±0.01 0.66±0.00

Heart 0.33±0.00 0.35±0.00 0.36±0.00 0.35±0.00

billirubin(mg/dL)

Billirubin

direct(mg/dL) 0.21±0.00 0.22±0.00 0.22±0.01 0.21±0.00

Billirubin

indirect(mg/dL) 0.27±0.00 0.26±0.00 0.29±0.00 0.26±0.00

SGOT(U/L) 55.77±0.94 59.70±0.16 61.53±0.00 63.81±0.04

SGPT(U/L) 41.51±0.00 42.11±0.00 41.11±0.00 42.20±0.00

Globulin(g/L) 3.15±0.04 3.23±0.04 3.13±0.03 3.23±.04

A/G Ratio 0.82±0.00 0.83±0.00 0.84±0.00 0.83±0.00

Blood sugar 2.10±0.00 2.11±0.00 2.12±0.00 2.11±0.00

Liver 3.60±0.01 3.57±0.00 3.56±0.01 3.52±0.00

Spleen 0.20±0.00 0.21±0.00 0.22±0.00 0.20±0.00

Thymus 0.13±0.00 0.12±0.00 0.12±0.00 0.12±0.00

Kidney 0.93±0.00 0.96±0.00 0.96±0.00 0.97±0.00

Adrenals 0.01±0.00 0.01±0.00 0.01±0.00 0.02±0.00

Testes 0.94±0.00 0.37±0.00 0.12±0.00 0.97±0.00

Epididymis 0.28±0.00 0.28±0.00 0.28±0.00 0.30±0.00s

Organ weight Female

0 mg/kg 500 mg/kg 1000 mg/kg 2000 mg/kg

Brain 1.77±0.00 1.77±0.00 1.82±0.00 1.76±0.01

Heart 0.72±0.00 0.67±0.00 0.74±0.00 0.71±0.00

Liver 7.42±0.07 6.57±0.01 7.14±0.00 6.76±0.00

Spleen 0.41±0.00 0.36±0.00 0.38±0.00 0.36±0.00

Thymus 0.34±0.00 0.29±0.00 0.35±0.00 0.30±0.00

Kidney 1.73±0.00 1.61±0.00 1.73±0.00 1.87±0.00

Adrenals 0.04±0.00 0.04±0.00 0.06±0.00 0.06±0.00

Ovaries 0.10±0.00 0.10±0.00 0.12±0.00 0.12±0.00

Uterus 0.42±0.00 0.43±0.00 0.42±0.00 0.60±0.00

Organ to body weight ratio

Brain 0.97±0.00 1.07±0.00 1.02±0.00 1.03±0.00

Heart 0.39±0.00 0.42±0.00 0.42±0.00 0.41±0.00

Liver 4.00±0.00 3.94±0.00 4.06±0.00 3.88±0.00

Spleen 0.23±0.00 0.21±0.00 0.21±0.00 0.20±0.00

Thymus 0.18±0.00 0.18±0.00 0.22±0.00 0.19±0.00

Kidney 0.94±0.00 0.97±0.00 0.96±0.00 1.07±0.00

Adrenals 0.03±0.00 0.04±0.00 0.03±0.00 0.03±0.00

Ovaries 0.06±0.00 0.03±0.00 0.05±0.00 0.06±0.00

Uterus 0.23±0.01 0.25±0.01 0.23±0.00 0.34±0.00

Means bearing*vary significantly between groups($p \leq 0.05$)

Satellite group animals

No significantchanges ($P<0.05$) in the body weights and feed intakewere observed in the treatment groups ascompared to those of the control.

Haemato-biochemical parameters.

Administration ofPolyherbalextract did not cause any significant changein the haematological and biochemical parameters (Tables 9 and 10 respectively) of the rats at any of the dose levels tested ($P<0.05$).

Table 9.Effect of oral administration of hydroalcoholic extract of Polyherbalroots over the haematological parameters ofWistar rats.

PARAMETERS

Male

0 mg/kg 2000 mg/kg

WBC(103µL) 9.02±0.00 8.94±0.00

LYM-R% 78.70±0.00 77.30±0.00

MON-R% 0.37±0.00 0.32±0.00

NEU-R% 20.51±0.00 23.38±0.00

EOS-R% 0.42±0.00 0.94±0.00

BAS-R% 0.00±0.00 0.00±0.00

RBC(106/µL) 7.75±0.00 7.84±0.00

HGB(g/dL) 13.52±0.00 13.50±0.00

MCV(µm3) 49.83±0.00 17.42±0.00

MCH(pg) 34.24±0.42 34.33±0.01

PLT(103µL) 675.2±0.73 744.6±0.00

MCHC(g/dL) 1.50±0.54 1.16±0.16

ESR(mm/1st hr) 45.50±0.00 45.51±0.00

PCV(%) 42.32±0.84 38.47±0.65

Female

WBC(103µL) 9.03±0.00 8.94±0.00

LYM-R% 78.70±0.00 77.30±0.00

MON-R% 0.37±0.00 0.33±0.00

NEU-R% 20.51±0.00 23.28±0.01

EOS-R% 0.42±0.00 0.94±0.00

BAS-R% 0.00±0.00 0.00±0.00

SRBC(106/µL) 7.76±0.00 7.84±0.00

HGB(g/dL) 13.52±0.00 13.50±0.00

MCV(µm3) 49.83±0.00 49.29±0.00

MCH(pg) 17.42±0.00 17.16±0.00

PLT(103µL) 34.25±0.41 34.34±0.01

MCHC(g/dL) 675.2±0.73 744.6±0.00

ESR(mm/1st hr) 32.24±0.42 34.33±0.01

PCV(%) 45.49±0.00 45.49±0.01

Means bearing*vary significantly between groups($p \leq 0.05$).

Table 10.Effect of oral administration of hydroalcoholic extract of Polyherbalroots over the biochemical parameters of Wistar rats.

PARAMETER

Male

0 mg/kg 2000 mg/kg

ALB(g/L) 30.16±0.00 30.27±0.00

CHO(mg/dL) 90.03±0.00 92.02±0.00

Serum protein (g/L) 7.50±0.13 8.21±0.04

ALP(U/L) 239.3±0.01 245.2±0.01

CRE(mg/dL) 0.49±0.00 0.52±0.00

Urea(mg/dL) 21.85±0.01 19.29±0.01

Total billirubin(mg/dL) 0.46±0.00 0.50±0.00

Billirubin direct(mg/dL) 0.24±0.00 0.25±0.00

Billirubin indirect(mg/dL) 0.23±0.22 0.22±0.00

SGOT(U/L) 91.5±0.04 0.23±0.00

SGPT(U/L) 39.22±0.04 92.03±0.07

Globulin(g/L) 1.02±0.00 39.17±0.04

A/G Ratio 4.13±0.03 1.02±0.00

Blood sugar 2.37±0.02 4.46±0.07

Female O mg/kg 2000 mg/kg

ALB(g/L) 30.16±.05 30.26±0.01

CHO(mg/dL) 90.03±0.00 92.02±0.00

Serum protein (g/L) 7.50±0.14 8.30±0.17

ALP(U/L) 239.8±0.00 245.2±0.00

CRE(mg/dL) 0.49±0.00 0.52±0.00

Urea(mg/dL) 21.85±0.01 19.29±.01

Total billirubin(mg/dL) 0.46±0.00 0.50±0.00

Billirubin direct(mg/dL) 0.24±0.00 0.26±0.00

Billirubin indirect(mg/dL) 91.58±0.04 92.42±0.60

SGOT(U/L) 39.27±0.06 39.22±0.05

SGPT(U/L) 0.22±0.00 1.03±0.00

Globulin(g/L) 1.02±0.00 4.43±0.09

A/G Ratio 4.16±0.02 2.38±0.00

Blood sugar 2.37±0.02 2.38±0.00

Means bearing*vary significantly between groups($p \leq 0.05$)

Table 11. Effect of oral administration of hydroalcoholic extract of Polyherbalover the organ weights and relative organ weight of Wistar rats.

Organ weight (g)

Male

0 mg/kg 2000 mg/kg

Brain 1.66±0.14 1.82±0.00

Heart 0.95±0.00 1.02±0.00

Liver 1.38±0.01 10.15±0.00

Spleen 0.58±0.00 0.58±0.01

Thymus 0.36±.01 0.35±0.01

Kidney 2.66±0.01 2.75±0.01

Adrenals 0.03±0.00 0.03±0.00

Testes 2.67±0.00 2.72±0.00

Epididymis 0.83±0.0 0.83±0.00

Organ to Body weight ratio

Brain 0.64±0.70 0.66±0.05

Heart 0.33±0.03 0.36±.03

Liver 0.60±0.31 3.56±0.16

Spleen 0.20±0.04 0.20±0.02

Thymus 0.13±0.04 0.12±0.03

Kidney 0.92±0.08 0.97±0.09

Adrenals 0.01±0.00 0.02±0.00

Testes 0.04±0.10 0.97±0.10

Epididymis 0.29±0.03 0.30±0.03

Organ weight(g) Female

0 mg/kg 2000 mg/kg

Brain 1.66±14 1.80±0.02

Heart 0.71±0.00 0.71±0.00

Liver 0.34±0.00 6.73±0.00

Spleen 0.42±0.01 0.35±0.01

Thymus 0.36±0.01 0.35±0.00

Kidney 1.36±0.01 1.79±0.02

Adrenals 0.02±0.00 0.02±0.00

Ovaries 0.10±0.02 0.12±0.02

Uterus 0.43±0.12 0.60±0.41

Organ to body weight ratio

Brain 0.98±0.07 1.03±0.10

Heart 0.39±0.04 0.41±0.05

Liver 4.00±0.39 3.89±0.25

Spleen 0.23±0.02 0.21±0.02

Thymus 0.18±0.06 0.19±0.02

Kidney 0.94±0.07 1.07±0.22

Adrenals 1.03±0.01 0.03±0.00

Ovaries 0.06±0.01 0.07±0.02

Uterus 0.24±0.06 0.35±0.25

Means bearing*vary significantly between groups($p \leq 0.05$).

Water intake

There was an increase in the amount of water intake while treated with these different doses.

Day 0 Day 7 Day 14 Day 21 Day 28

0

10

20

30

40

50

Feed intake

The graph showed the increase the feed intake with days during whole study.

Water intake

There was no significant change in water intake. They showed the increase in water intake during whole study.

Feed intake

There was increase the feed intake of animals from day 0 to 7 respectively.

EVALUATION OF TOXICITY OF POLYHERBAL EXTRACT IN RATS

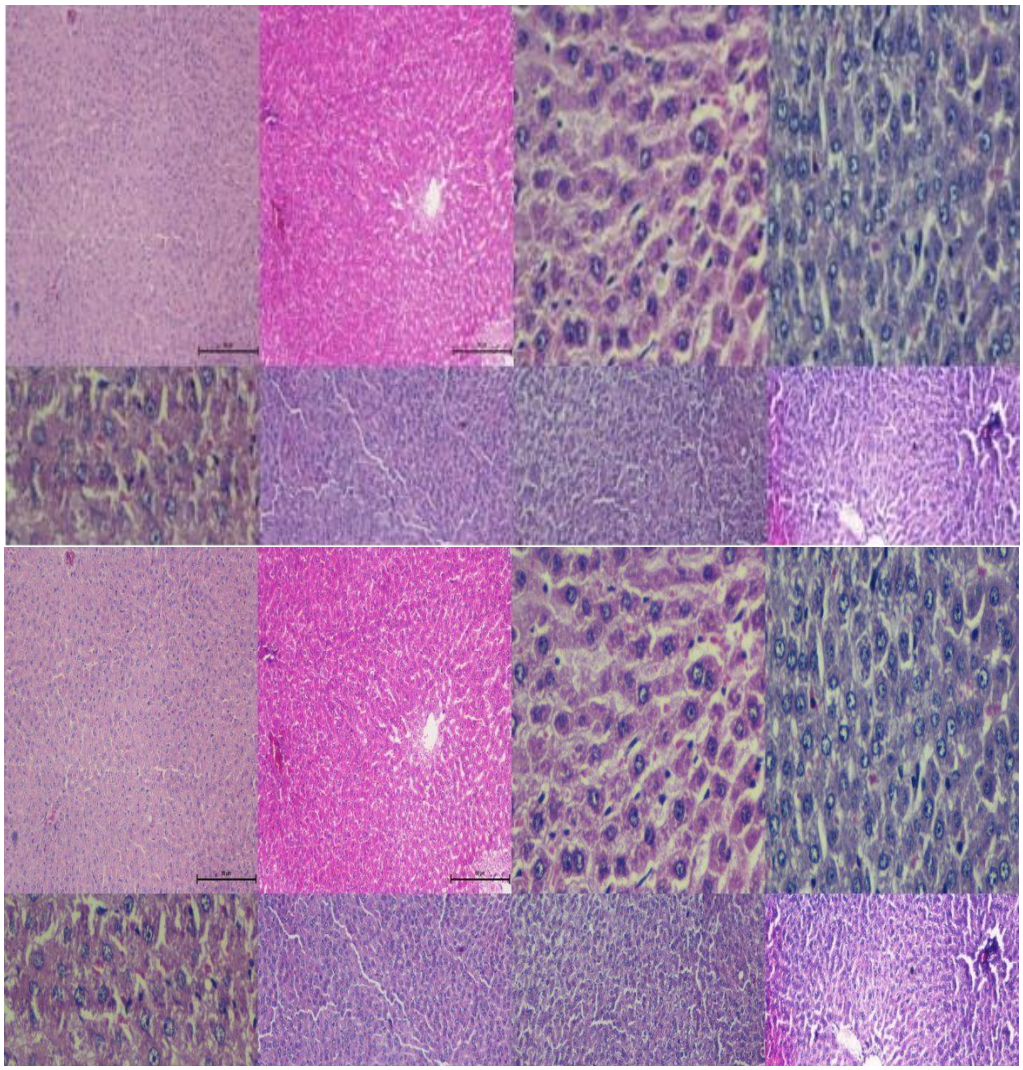

Figure: Histopathology of liver organ of treated and control rats.

(A) & (B) Liver section of rats with Polyherbal treated with 2000 mg/kg showing liver normal (females) HE ×100. (C) Liver section of rats treated with 1% CMC showing Liver normal cord of hepatocyte, a central vein on the right normal(PH-Normal) HE×100 Male.(D) Liver Normal(PH-Normal) HE×100 Female. (E) Liver normal (PH-500) HE×400 Male. (F) Liver Normal (PH-500) HE×100 Female. (G) Liver (PH-1000) HE×100 Male Portal tract with vein & bile duct right. (H) Liver (PH-1000) HE×100 Female showing the Central vein in centre. (I) Liver (PH- 2000) HE×100 Male. (J). Liver (PH-2000) HE×100 Female, Portal tract has an artery (lower). (K) Liver normal (PH-2000) HE×100 Male.(L) Liver normal(PH-2000) HE×100 Male.

A B C D

E F G H

I J K L

DISCUSSION

Efficacy of medicinal plant in the management of diseases is indubitable and the World Health Organisation has recognised its use in primary health care delivery system. Even when efficient, the toxicity of the used preparations is usually unknown and the population does not care, believing that if the preparation has been used so far, it should be devoid of toxicity (Albert et al., 2011). However, animal toxicity testing have shown many plants currently used, to name a few Momordica charantia (Raman and Lau, 1996; Basch et al., 2003), Urtica dioica (Tahri et al., 2000), Crocus sativus (Hosseinzadeh and Younesi, 2002) and Erythrophleum guineese (Adeoye and Oyedapa, 2004), as highly toxic when given either acutely or sub-chronically. Acute oral toxicity test is conducted at a limit dose (2000mg/kg) primarily in situations where the experimenter has information indicating that the test material is likely to be non-toxic (OECD, 1998). The acute study was hence, conducted at the limit dose of 2000 mg/kg and was found to be well tolerated. This maximum tolerated dose was taken as the highest dose for the sub-acute toxicity study. Two fold decreases were made from the highest dose to arrive the medium and low doses of the study, i.e. 1000 and 500 mg/kg body weight, respectively (OECD, 1995). In the sub-acute toxicity study, Polyherbal extract did not cause any significant change in general behavior, body weight and feed intake at any of the dose levels. Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Tofovic and Jackson, 1999; Raza et al., 2002; Teo et al., 2002). The results of haematology and clinical chemistry provide information regarding the overall health status of the animals as well as the general metabolic, adaptive or toxic processes and target organs associated with the exposure to toxic agents. In particular, they are necessary to evaluate the Efficacy of medicinal plant in the management of diseases is indubitable, and the World Health Organisation has recognised its use in primary health care delivery syst m. Even when efficient, the toxicity of the used preparations is usually unknown, an the population does not care, believing that if the preparation has been used so far, it should be devoid of toxicity (Albert et al., 2011). However, animal toxicity testing have shown many plants currently used, to name a few Momordicacharantia (Raman and Lau, 1996; Basch et al., 2003), Urtica dioica (Tahri et al., 2000), Crocus ativus (Hosseinzadeh and Younesi, 2002) and Erythrophleum guineese (Adeoye and Oyedapa, 2004), as highly toxic when given either acutely or subchronically. Acute oral toxicity test is conducted at a limit dose (2000mg/kg).

Primarily in situations where the experimenter has information indicating that the test material is likely to be non-toxic (OECD, 1998). The acute study was hence, conducted at the limit dose of 2000mg/kg and was found to be well tolerated. This maximum tolerated dose was taken as the highest dose for the sub-acute toxicity study. Two fold decreases were made from the highest dose to arrive the medium and low doses of the study, i.e. 1000 and 500 mg/kg body weight, respectively (OECD, 1995). In the sub-acute toxicity study, Polyherbal extract did not cause any significant change in general behavior, body weight and feed intake at any of the dose levels. Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Tofovic and Jackson, 1999; Raza et al., 2002; Teo et al., 2002).

The results of haematology and clinical chemistry provide information regarding the overall health status of the animals as well as the general metabolic, adaptive or toxic processes and target organs associated with the exposure to toxic agents. In particular, they are necessary to evaluate the attributable to the treatment of Polyherbal extract, indicating the non-toxic nature in Wistar rats.

CONCLUSION

The present investigation demonstrated that the hydroalcoholic extract of Polyherbal to be safe without significant toxicity, as it neither caused mortality nor produced any significant haematological, biochemical, gross and histopathological changes in Wistar rats. Since, the oral dose of 2000 mg/kg/day of Polyherbal extract was the highest dose (limit dose) used in sub-acute studies and that it did not cause any adverse effects, it is concluded as the no observed- adverse-effect level for Wistar rats of both sexes under the experimental conditions used. Further studies have to be undertaken to assess the other aspects of toxicities like long term toxicity (90 days/1 year), reproductive toxicity, teratogenicity and carcinogenicity

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