ACUTE ORAL TOXICITY TESTS OF TRANSGENIC SEED IN RAT

ADOPTION : OECD 401

Application and limitation of tests:

Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a test chemical or multiple doses given within 24 hrs. It is the initial step to find out median lethal dose (LD50) value which serve as basis for classification and labelling of the compound. It also forms a basis for selection of dose for subchronic studies. It will provide information on target organs toxicity after single exposure.

Principle:

The test compound is administered orally by gavage in numerical doses to groups of animals, one dose per group. Signs of toxicity and death of animals are observed during 14 days observation period. The dead animals are necropsied during and the surviving animals are sacrificed and necropsied after the 14 days observation period for gross pathology. Vital tissues of moribund and sacrificed animals are put for histopathological studies, clinical biochemistry and haematological examination.

DESCRIPTION OF THE TEST PROCEDURE:

Animals :

Healthy animals kept under standard animal husbandry conditions are used. At least 10 animals (male/female) are dosed. The weight variation of animals does not exceed 5-10g.

Animal Maintenance

Animals are acclimatized to the experimental animal room having temperature 75 ±2°F, humidity 30-70% and 12 : 12 hrs. light dark condition. Animals are caged with maximum of 2 animals in each polypropylene cages. Standard animal diet and water *ad libitum* is given to animals.

Preparation of dose:

Test sample i.e. fine powder of transgenic seed dissolved/suspended in groundnut oil is administered to rats fasted overnight. The volume does not exceed 1 ml/100 g body weight. At least four doses of the test sample spaced in geometrical factor are selected. The treatment schedule is as given below.

Group 1 - Control (normal diet)
Group 2 - Non transgenic seed
Group 3 - Transgenic seed

Limit test dose

If a test sample at 5000 mg/kg body weight produces no mortality, then other doses are not essential.

Observations:

The dosed animals are observed twice daily for 14 days to record the signs of poisoning and death of animals. The signs of poisoning include tremor, convulsion, salivation, diarrhoea, lethargy, sleep, coma, dyspnea, nasal bleeding etc. The time of death of animals is recorded. The body weight, food and water intake is recorded daily and monitored weekly. All the animals (moribund/live) are sacrified after 14 days and examined for gross and histopathological changes, clinical biochemistry and haematological examination.

Pathology

The liver, kidney, gonads, adrenals, spleen and brain are weighed immediately after autopsy. All animals are subjected for gross pathological changes. The vital organs like liver, kidney, brain, gonads, spleen, adrenal, thyroid, lungs, heart, stomach, duodenum, jejunum, colon, uterus, prostate are fixed in formal saline solution and tissues embeded in parafin wax and section cut at 6 um on rotary microtome. The prepared slides are then stained in haematoxylin eosin for microscopic examinations.

Haematology

Haematology is carried out in oxalated blood using standard methods of Wintrobe and Landsberg 1935 and Kolmer et. al. 1951. Blood is analysed for WBC, RBC, Hb differential leucocytes, clotting and prothrombin time and ESR.

Clinical Enzymes

Serum and blood are analysed for:

(i) Glutamic oxaloacetic transaminase (GOT), (ii) Glutamic pyruvic transaminase (GPT), (iii) Alakline phosphatase (Orthophosphoric monoester hydroxylase ALP), (iv) Bilirubin (v) Blood glucose (vi) Blood urea nitrogen, (BUN) (vii) Non protein nitrogen, (NPN) by the method of Wootton (1982), (viii) Acetylcholinesterase (AchE) by the method of Hestrin 1949 and (ix) Protein by the method of Lowry et.al. 1951; (x) Serum histamine level.

Calculations

LD50 values and its range are calculated by the procedure of Weil 1952 and toxicity rating is done by Gleasons et.al. 1969. All observed are recorded and calculated by appropriate methods. The statistical evaluation is done by Fisher's student `t' test. The results are summerised in tabular form.

References

Weil, C.S., tables for convenient calculation of median effective dose (LD or ED) and instruction in their use. Biometrics, 8, 249, 1952.

Gleason, M.N., Gosselin, R.E., Hodge, H.C. and Smith, R.P. Clinical toxicology of commercial products. Acute poisoning 3rd ed. Williams and Williams, Baltimore, Maryland.

Report on Acute oral toxicity

Test Animal.....

Rats..... Sex : Male/Female.....

Test Sample
Solid, Liquid, any other

Nature of vehicle
dist. water, peanut oil, corn oil, any other

Date of experiment started .....

Date of experiment terminated .....

LD50.....mg/kg; Range..... to .....mg/kg

Dosage (mg/kg)	Animals Died/Dosed	Death	Symptoms of toxicity
1. Control			
2.			
3.			
4.			

Statistical Method

Gross Pathology

Observations

Conclusions

Toxicity Rating

Report on Acute Oral Toxicity

Test animal : Rat.

Test Chemical : Solid, Liquid, any other

Nature of vehicle: dist. water, peanut oil, corn oil, any other

Date of expt. started....., date of expt. terminated.....

FOOD (G) WATER (ML) INTAKE OF MALE OR FEMALE ANIMALS EXPOSED

TO.....FOR 14 DAYS

Dosage (mg/kg/day)	**Days**													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
MALE														
1. Control														
2.														
3.														
4.														
FEMALE														
1.														
2.														
3.														
4.														

Report on Acute Oral Toxicity

Test Animal : Rat

Test Chemical : Solid, Liquid, any other

Nature of vehicle : Dist, water, peanut oil, corn oil, any other

Date of expt. started :..... date of expt. terminated : :.....

RELATIVE ORGAN WEIGHT OF MALE OR FEMALE ANIMALS EXOSED TO ..... FOR 14 DAYS

Dosage (mg/kg/day)	Liver	Kidney	Adrenalm	Heart	Spleen	Brain	Pituitary	Test is Ovary	Epididymis Uterus	Cervix	Vagina
MALE											
1. Control											
2.											
3.											
4.											
FEMALE											
1.											
2.											
3.											
4.											

*Organ weight / Body weight x 100

Report on Acute Oral Toxicity

Test Animal : Rat

Test Chemical : Solid, Liquid, any other

Nature of vehicle : Dist, water, peanut oil, corn oil, any other

Date of expt. started :..... date of expt. terminated :.....

BLOOD PICTURE OF MALE OR FEMALE ANIMALS EXPOSED TO:..... FOR 14 DAYS

Dosage (mg/kg/day)	RBC (x 10 mm)	WBC (x 10 mm)	Hb	PVC	platelet	Differential Leucocyte count (%)			
						Neutrophils	Lymphocytes	Monocytes	Eosinophils
MALE									
1. Control									
2.									
3.									
4.									
FEMALE									
1.									
2.									
3.									
4.									

Report on Acute Oral Toxicity

Test Animal : Rat

Test Chemical : Solid, Liquid, any other

Nature of vehicle : Dist, water, peanut oil, corn oil, any other

Date of expt. started :..... Date of expt. terminated:.....

BIOCHEMICAL CHANGES IN MALE OR FEMALE ANIMALS EXPOSED TO ..... FOR 14 DAYS

Dosage (mg/kg/day)	Blood Sugar	Alk. Phos.		Protein		GOT		GPT	
		Liver	Serum	Liver	Serum	Liver	Serum	Liver	Serum
MALE									
1. Control									
2.									
3.									
4.									
FEMALE									
1. Control									
2.									
3.									
4									

Title : SUBCHRONIC (90 DAYS) ORAL TOXICITY TEST OF TRANSGENIC SEED IN RAT

Adoption OECD 408

Application and Limitation of Test

Subchronic oral toxicity is the adverse effect occuring as a result of repeated daily oral dosing of a chemical to the animals. In the evaluation of toxic characteristics of a chemical the subchronic oral toxicity provides information on possible health hazards due to repeated exposure over a limited period of time. It will provide the information on target organ and the possibility of cumulation and for the selection of dose level for chronic studies.

Principle

The test sample is orally administered in three doses to animals for a period of 90 days. The animals are observed for any signs of toxicity and death during the period of exposure. Vital tissues of moribund and sacrificed animals are put for histopathological studies. Clinical biochemistry and haematological examinations are also made.

Description of the test Procedure

Rat is the preferred rodent model for subchronic oral toxicity studies. Healthy animals kept under standard animal husbandry conditions are used. At least 20 animals of 6-8 weeks old are used per group for three dose levels. The weight of the animals does not vary + 20 g.

Animal Maintenance

Animals are acclimatized to the experimental animal room having temperature (75 + 2 F), humidity (30-70%) and 12 : 12 hr light : dark conditions. Animals are given commercial feed and water *ad libitum*.

Preparation of dose

Test sample i.e. fine powder of transgenic seed dissolved/suspended in peanut oil is orally administered by gavage to animals consequently (5 days/week) for 90 days. The selection of the dose is made on the basis of acute toxicity studies of the test sample. At least three dose level, one maximum, one minimum and one intermediate are used. Consideration is given that the highest dose may result toxic effects without causing excessive lethality and lowest dose may not produce any toxic effects. A group of vehicle control is also used.

Limit test dose

If a test at one dose level of at least 1000 mg/kg body weight (but expected human exposure may indicate the need for a higher dose level), using the procedures described for this study, produces no observable toxic effects, then a full study using three dose levels may not be considered necessary. The treatment schedule is given below:

Group 1 - Control
Group 2 - Non transgenic seed
Group 3 - Transgenic seed

Observations

Animals are observed once daily to record the signs of poisoning, like tremor, convulsion, diarrhoea, lethargy, dyspnea and nasal bleeding etc. The time of death is also recorded. The body weight, food and water intake is recorded daily and monitored weekly. At the end of 90 days animals are weighed and sacrificed.

Pathology

The liver, kidney, gonad, adrenals, spleen and brain are weighed immediately after autopsy. All animals are subjected for gross pathological changes. The vital organs like liver, kidney, brain, gonads, spleen, adrenal, thyroid, lungs, heart, stomach, duodenum, jejunum, colon, uterus, prostate are fixed in formal saline solution and tissues embeded in parafin wax and section cut at 6 um on rotary microtome. The prepared slides are then stained in haematoxylin eosin for microscopic examinations.

Haematology

Haematology is carried out in oxalated blood using standard methods of Wintrobe and Landsberg 1935 and Kolmer et.al. 1951. Blood is analysed for WBC, RBC, Hb differential leucocytes, clotting and prothrombin time and ESR. Immunoglobulin profile (IGM, IGA, IGE).

Clinical Enzymes

Serum and blood are analysed for

(i) Glutamic oxaloacetic transaminase (GOT), (ii) Glutamic pyruvic transaminase (GPT), (iii) Alakline phosphatase (Orthophosphoric monoester hydroxylase ALP), (iv) Bilirubin (v) Blood glucose (vi) Blood urea nitrogen (BUN), (vii) Non protein nitrogen (NPN), by the method of Wootton (1982), (viii) Acetylcholinesterase (AchE) by the method of Hestrin 1949 and (ix) Protein by the method of Lowry et.al. 1951. (x) Serum histamine level.

Calculation and Evaluation of Data

All observed data are recorded and calculated by appropriate methods. The statistical evaluation in done by Fisher's student `t' test 1950. The results are summerised in tabular form.

References

Wintrobe, M. and Landsberg, J.W. A standard technique for blood sedimentation test. American J. Med. Sci. 189, 102, 1935.

Kolmer, K.A. Spaulding, E.H. and Robinson, H.W. Approved laboratory techniques Ves Scientific Book Agency Calcutta, India, 1951.

Wootton, I.D.P. microanalysis in Medical Biochemistry Sixth Edition, Churchill Ltd. London, 1982.

Hestrin, S.H. The reaction of Acetylcholine and other carboxylic acid derivatives with hydroxyl amine and its analytical applications J. Biol. Chem. 180, 249, 1949.

Lowry, O.H. Rosenburgh, N.J. Farr, A.L. and Randall, R.J. Protein measurement with the Folin Phenol reagent J. Biol. Chem. 193, 265, 1951.

Fisher, R.A. Statistical methods for research workers 11th edition Edinburgh Oliver and Boyd 1950.

Report on Subchronic Oral Toxicity

Test Animal : Rat.

Test Chemical : Solid, Liquid, any other

Nature of vehicle : dist. water, peanut oil, corn oil, any other

Date of expt. started :..... , Date of expt. terminated :.....

FOOD (G) WATER (ML) INTAKE OF MALE OR FEMALE ANIMALS EXPOSED TO ..... FOR 13 WEEKS

Dosage (mg/kg/day)	**Weeks**												
	1	2	3	4	5	6	7	8	9	10	11	12	13
MALE													
1. Control													
2.													
3.													
4.													
FEMALE													
1.													
2.													
3.													
4.													

Report on Subchronic Oral Toxicity

Test Animal : Rat

Test Chemical : Solid, Liquid, any other

Nature of vehicle : Dist, water, peanut oil, corn oil, any other

Date of expt. started :..... Date of expt. terminated :.....

RELATIVE ORGAN WEIGHT OF MALE OR FEMALE ANIMALS EXPOSED TO ..... FOR 13 WEEKS

Dosage (mg/kg/day)	Liver	Kidney	Adrenalm	Heart	Spleen	Brain	Pituitary	Testes	Epididymis	Cervix	Vagina	Ovary	Uterus
MALE													
1. Control													
2.													
3.													
4.													
FEMALE													
1.													
2.													
3.													
4.													

*$\frac{\text{Organ weight}}{\text{Body weight}} \times 100$

Report on Subchronic Oral Toxicity

Test Animal : Rat

Test Chemical : Solid, Liquid, any other

Nature of vehicle : Dist, water, peanut oil, corn oil, any other

Date of expt. started :..... Date of expt. terminated :.....

Blood Picture of Male or Female Animals Exposed to..... for 13 Weeks

Dosage (mg/kg/day)	RBC (x 10 mm)	WBC (x 10 mm)	Hb	PVC	platelet	Differential Leucocyte count (%)			
						Neutrophils	Lymphocytes	Monocytes	Eosinophils
MALE									
1. Control									
2.									
3.									
4.									
FEMALE									
1.									
2.									
3.									
4.									

Report on Subchronic Oral Toxicity

Test Animal : Rat

Test Chemical : Solid, Liquid, any other

Nature of vehicle : Dist, water, peanut oil, corn oil, any other

Date of expt. started :..... Date of expt. terminated :.....

BIOCHEMICAL CHANGES IN MALE OR FEMALE ANIMALS EXPOSED TO ..... FOR 13 WEEKS

Dosage (mg/kg/day)	Blood Sugar	Alk. Phos.		Protein		GOT		GPT	
		Liver	Serum	Liver	Serum	Liver	Serum	Liver	Serum
MALE									
1. Control									
2.									
3.									
4.									
FEMALE									
1. Control									
2.									
3.									
4									

Title : PRIMARY SKIN IRRITATION TEST OF TRANSGENIC SEED IN RABBIT

Adoption : OECD 404

Application and Limitation of Test

The assessment and evaluation of the toxic characterestics of a substances', determination of the irritant effects on the skin of mammals is an important initial step. Information derived from the test serves to indicate the existance of possible hazard likly to arise from exposure of the skin to the test substance.

Principle

The substances to be tested is applied in a single dose to the skin of several experimental animals, each animal serving as its own control. The degree of irritation is read and scored at specified intervals and is further described to provide a complete evaluation of the effects. The duration of the study should be sufficient to evaluate fully the reversibility or irreversibility of the effects observed.

Description of the Test Procedure

Animals

At least three adult rabbit should be used. Additional animals may be required to clarify equivocal responses.

Animal Maintenance

Animals are acclimatized to the experimental animal room having temperature 75 + 2°F, humidity 30-70% and 12 : 12 hrs light dark cycle. Animals are caged with maximum of two animals in each cage. Standard animal diet and water *at libitum*.

Preperation of Dose and Limit Test Dose

Test sample i.e. transgenic seed at a dose of 0.5 ml. of liquid or 0.5 g. of solid is applied to the test side. The treatment schedule is given below:

Group 1 - Control
Group 2 - Non transgenic seed
Group 3 - Transgenic seed

Observations

Animals are ovserved for signs of erythema and oedema and the responses scored at 30-60 minutes, and then at 24, 48, 72 hours and 7 and 14 days after patch removal. Dermal irritation is scored and recorded as per the grades given in the table below.

References

Draise, J.H. The Appraisal of Chemicals in Foods, Drugs, and cosmetics pp. 46-48. Association of Food and Drug Officials of United States, Austin, Texas 1959.

Draise, J.H. Appraisal of the Safety of chemicals in Foods, Drugs and Cosmetics; pp. 46-59. Association of Food and Drugs official of the United States, Topeka, Kanasas 1965.

Evaluation of Skin Reaction

Erythema and Eschar Formation	***Value***
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Maximum possible - 4

Oedema Formation	
No Oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre) and extending beyond area of exposure)	4

Maximum Possible - 4

Title : IRRITATION TO MUCOUS MEMBRANE TEST OF TRANSGENIC SEED IN FEMALE RABBIT

Adoption : OECD 405

Application and Limitation of Test

In the assessment and evaluation of the toxic characteristics of a substance, determination of the irritant effects on the mucous membrane of the rabbit is an important step. Information derived from this study serves to indicate the existence of possible hazards likely to arise from exposure on the mucous membrane to the test substance.

Principle

The substance is tested is applied in a single dose to the mucous membrane of the experimental animals. Simultaneous animals in the control group are also taken. The degree of irritation is read and scored at specific intervals. The complte evaluation is then described. The duration of the study is sufficient to evaluate fully the dermal irritation.

Description of the Test Procedure

Healthy adult animals at least 3 in number are used in both experimental and control groups. Animals are kept in the experimental animal room having temperature (75 ± 2°F), humidity (30-70%), and 12 : 12 hrs light: dark condition. Animals are fed conventional laboratory diet and water *ad libitum.*

A dose of 0.1 ml of liquid or 0.1 gm of solid or semisolid is applied to the upper vault of the vagina. Exposure duration is 4 hrs. Longer exposure may be indicated under certain conditions. At the end of the exposure period residual substance is removed where practicable using water or appropriate solvent without disturbing the epidermis. The treatment schedule is given below :

Group 1	-	Control
Group 2	-	Non transgenic seed
Group 3	-	Transgenic seed

Observation

Observation period is not fixed but is sufficient to evaluate fully the effects of the test substance. Normally it need not exceed 14 days after application. Animals are examined for signs of erythema and oedema and responses scored at 30-60 minutes, 24, 48, 72 hrs and then at 7 and 14 days. Mucous memberane irritation is scored and recorded as per the grades given in table below:

References

Draise, J.H. The approval of chemical in Food, Drug and Cosmetics pp. 46-48. Association of Food and Drug Officials of United States, Austin Texas 1959.

Draise, J.H. Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics; pp. 46-59. Association of Food and Drugs Official of the United States, Topeka, Kanasas 1965.

Evaluation of Skin Reaction

Erythema and Eschar Formation	***Value***
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Maximum possible - 4

Oedema Formation	
No Oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre) and extending beyond area of exposure)	4

Maximum Possible - 4

Title : SKIN SENSITIZATION TEST OF TRANSGENIC SEED IN GUINEA PIGS

Adoption : OECD 406

Application and Limitation of Test

In the assessment and evaluation of the toxic characteristics of a substance, determination of its potential to provoke skin sensitization reaction (allergic dermatitis) is important. Information derived from skin sensitization serves to identify the possible hazards to a population exposed to the substance.

Principle

After initial exposure to a test substance the animals are subsequently subjected for 9 injections, then a challenge exposure to establish a hypersensitive state. Sensitization is determined by examining the reaction to the challenge exposure.

Desicription of Test Procedure

The guinea pigs are the generally recommended species. A sufficient number of animals are used. Animals are kept in experimental animal room having temperature (75 ± 2°F), humidity 30-70% and 12 : 12 light: dark condition. Animals are fed on conventional laboratory diet and water *ad libitum*. It is essential that guinea pigs receive an adequate amount of ascorbic acid. A treatment and a control groups are simultaneously taken. Animals are clipped off at dorsal side for the area 6 x 6 cm. The test substance 0.5ml is administered intradermally as a initial dose. There after nine subsequent injections are given intradermally on every alternate days. After giving a rest period of 15 days a booster dose of 0.05 ml is injected. The treatment schedule is given below :

Group 1 - Control
Group 2 - Non transgenic seed
Group 3 - Transgenic seed

Observation

Scoring of Skin reaction was performed on day 2 and then 24 hours after each injection. On day 36 and 37 animals are shaved again to check the intensity of erythema or edaema. With administration of booster dose, skin sensitization reaction was observed. The subsquently spreaded to longer area of the skin and resulted in necrosis at site of injection. Scored reaction are recorded in form of table.

Refereces

Draize, J.H., Food Drug Cosmets. Law J. 10, 722, 1955.

Evaluation of Skin Reaction

Erythema and Eschar Formation	***Value***
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Maximum possible - 4

Oedema Formation	
No Oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre) and extending beyond area of exposure)	4

Maximum Possible - 4

SUBCHRONIC ORAL TOXICITY – GOATS – 90 DAYS STUDY FOR GENETICALLY ENGINEERED SEEDS

OBJECTIVE

The objective of this study is to compare the whole someness of engineered seeds with control seeds and control seeds lines will be administered to the goats through the diet for 90 days.

MATERIAL AND METHODS

The methods, species of animals and the route of administration described in this protocol are based up on the standard OECD guidelines No. 408 (1993). This procedure deals with handling, maintaining and other procedures to be followed while dealing with feeding studies with goats. In order to maintain even distribution, the goats will be provided a number, based on random selection.

The test material will be administered in the diet. This route of administration was selected because it represents the most likely route of exposure of goat species in their natural habitat.

The test substance will be properly identified as per the detailed specification provided by the sponsor.

Treatment Groups

A group of 12 goats (6 males and 6 females) will be assigned to each group by the indiscriminate draw to each of the treatment and control group. All goats will be uniquely identifiable with an identification mark on the body and/or with a number plate around their neck.

The test will comprise feeding of the goats for 90 days regularly with concentrate of which 12.5% will be test seed and the concentrate itself will be 10% of the total feed i.e. concentrate and green grass. The consumption range of the feed will be pre-determined.

Each group is fed for 90 days and observed. An additional control group will be fed normal diet which will not contain cotton seeds throughout the test period.

Duration of the study.

All animals in the treatment groups will get Indian hybrid control cotton seeds in diet during acclimation.

Analysis will be initiated during this period itself viz., feed consumption, weight gain etc. This will facilitate statistical analysis.

Pilot study will be done before acclimation to assess the consumption of cotton seeds. Parameters like feed consumption, weight gain etc. will also be assessed for this group.

This study will be divided as under.

1. Acclimation: From receipt of the animals till the initiation of the study (a minimum duration of 15 days)

2. Exposure : 90 day

Test animals

Goat husbandry is generally associated with agriculture in Indian rural set ups. The availability of standard genetically defined goats and dietary and husbandry conditions, also make goats ideal in the Indian context and safety data on this ruminant model will be appropriate.

All goats will be 12 months old and healthy at the initiation of the study. The body weight will range between 15 and 18kg. Each treatment and control group will have 12 animals. The Barbari goats will be obtained from the State Animals Husbandary Departments. All the animals will be acclimated to their pens and facilities from the time of receipt until the initiation of the study.

ANIMAL CARE AND FACILITY

Animal Species

Goat - The Indian Barberi breed

Source

State Animal Husbandary Departments.

Number of animals

Twelve animal (6 males and 6 females) per group

Age and weight

Age of the animals will be 12 months and the weight between 15 and 18 kg.

Acclimation

The animals will undergo an acclimation for a period of not less than 15 days prior to the actual studies. The goats will be given anti-healminth drugs and also drugs for treatments for ectoparasites before the initiation of the study. All animals in the treatment groups will get Indian hybrid control cotton seeds control in diet during acclimation and group will not be given any cotton but will have groundnut cake instead in its diet.

Animals identification

Each animal will be numbered accordingly with the help of a tag around the neck.

Housing the animal care

Goats will be housed individually in a well constructed, cemented pens and maintained under strict hygienic conditions of veterinary care.

Food and water

Each animal will be allowed access to food for the whole day. Clean drinking water will be provided *ad libitum*. Feed consisting of wheat bran, gram, salt, minerals, cotton seeds and grass will form the daily diet of the goats.

The test will comprise feeding of the goats for 90 days regularly with concentrate of which 12.5% will be cotton seed and the concentrate itself will be 10% of the total feed i.e. concentrate and green grass. The consumption range of the feed will be predetermined.

Bedding

No bedding will be used; instead the floor will be made of rough cement/concrete to avoid slipping of goats while walking or standing.

Exercise

Though the goats do not need any strenuous exercise, they will however, be allowed to go out of their pens in an open field for about 2-3 hours each day but ensuring that they do not eat any other foliage. The area of their movement would be devoid of any vegetation but water will be provided during this period of their routine.

Animal diet

The test diet will be prepared by blending the test substance directly with the ration. Blending is normally done with a blender. Unless otherwise specified, the diets will be prepared every day. The diets will be provided to the goats from day 0 of the 90 day exposure period. Every batch of concentrate will be analysed and relevant record will be maintained. Cotton seed will be added to the concentrate everyday to avoid the concentrate going rancid because of the presence of cotton oil, if the concentrate is blended with cotton seed and stored. The ingredients will be purchased in bulk and made available for mixing; but the mixing and blending of the constituents will be done daily. The feed ingredients will be maintained in a dry and clean room to avoid attack by fungus. The test material will be crushed and mixed with the feed. The analysis of the feed will be for the following parameters: Crude protein, fat, acid detergent fiber, neutral detergent fiber, Calcium, phosphrus, Magnesium, Sodium, Potassium, Copper, Zinc, Manganese, Iron, Vitamin A, Vitamin D, Vitamin E. The analysis will be done on the mix and the raw ingredients. Also the mix will be randomly analysed once a week.

Housing and environmental conditions

Goals will be housed in properly constructed pens. Each pen measuring 1.5 sq. mt. per goat, allowing proper movement to the animals. The floor of the pen would be constructed of concrete and the walls of bricks. The roof will be made of corrugated sheet. At initiation of the study, each pen will hold a single goat and goat will be identifiable by a number. During the test, the temperature in the housing will be 25-30°C approximately. If necessary, air cooler will be provided to maintain the specified temperature. Relative humidity will be recorded at 24 hour interval. The goats will be provided a 16 hour light and 8 hour dark photoperiods during the test. Housing and animal husbandry practices will be followed as mentioned by Devendra and McLeroy 1982.

EXPERIMENTAL DESIGNS

Design

The study will be conducted as a randomised block design in which goats wil be distributed randomly in different treatment groups evenly consisting of a single goat as a replcate.

The study would have at least three following groups

1. Geneticaly engineered cotton line
2. Indian hybrid cotton line
3. Control group-Normal diet without cotton seeds but ground nut, instead.

Observations

All the animals will be observed daily for morbidity, mortality and clinical signs.

Daily observations

The general health of all the animals will be monitored daily and relevant records will be maintained. Any adverse observation will be documented. Animals found moribund or dead during the study period will be necropsied to the extent necessary to determine the probable cause.

Body weight and temperature

Body weights will be measured weekly at a predetermined time along with their health status. A chart of weekly temperature will also be maintained.

Body weight/feed consumption

Individual body weights will be taken at the initiation of the experiment, during the exposure period and at the end of the exposure period. Average feed consumption for individual animal will be maintained for the entire period. Determination of feed consumption and body weight will continue, if the study period is extended. Daily feed offered and refused will be measure for the concentrate and grass.

Feed intake

Goats will have access to the experimental feed (concentrate) from 9 a.m. to 12 p.m. each day.

Necropsy and Pathological examinations

Goats found moribund or dead during the study period will be necropsied to the extent necessary to determine the probable reason. Any gross lesions observed at necropsy will be processed for histopathological examinations.

Hematological observations

Following parameters would be assessed:

- Total RBC count
- Total WBC count
- Differential leucocytic count
- Haemoglobin concentration
- Clotting time
- ESR immunoglobulin profile

Clinical biochemistry

The following parameters will be analysed.

- Total Serum protein
- Glucose
- Blood urea
- Nitrogen
- Non-protein
- Nitorgen
- Bilirubin
- Histamine
- Got
- Gpt
- Alkaline phosphatase
- LDh

Necropsy

All the animals are sacrified on day 91. Goats are sacrified by administration of a saturated solution of magnesium sulphate intravenously and the autopsy is carried out as the standard procedure by the venterinary pathologist of the study.

Organ weights

The gross lesions in the organ are noted and weights of the following organs are recorded:

- Adrenals,
- Heart,
- Liver,
- Gonads (testes and ovaries),
- Brain,
- Kidneys,
- Spleen

Histopathological examinations

Following organs are preserved in 10% buffered formalin:

- Adrenals
- Kidneys
- Testes
- Liver
- Thymus
- Lungs
- Colon
- Spleen
- Ovaries
- Stomach (all 4 compartments)
- Heart
- Small intestine

Histopathological examinations of these organs will only be conducted if gross lesions are noted.

The tissues are subjected to dehydration procedure and processed in a tissue processor through different grades of alcohol and cleared in chloroform. They are embedded in paraffin wax, sectioned at 7 to 10 microns and stained with Haematoxylin-Eosin.

Disposal

The carcass will be mutilated by using Calcium hydroxide and buried deep ensuring that these are not removed by men or other animals like dogs and jackals.

References

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3. Devendra C. and McLeroy, G.B. (1982). Goat and Sheep Production in the tropics. Intermediate Tropical Agricultural Series, Longman, London.