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Toxicity Study on *Eucalyptus grandis* Bark as Natural Food Colourant in Wistar Albino Rats

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ABSTRACT

The toxicity of the powdered substance of Eucalyptus bark (Eucalyptus grandis) was investigated. The level of feed to be given was optimized in the rats and later in the experimental period of 56 days, same procedure was carried out, where according to the OECD guidelines, oral feed ofaqueous solution with 2ml/100g body weight was fed. The histopathology of the rats showed no alternative changes in liver, kidney, intestine, stomach, brain and spleen proving that E. grandis can be used as natural food colourant. The hematology and biochemical parameters were checked for both the control group and the experimental group. The average values were highly similar to each other proving that there were no biochemical changes that had taken place in the experimental rats. Thus, this study proves that eucalyptus barks can be used as natural food colourants, as an alternative for harmful synthetic food colourants.

Keywords : Eucalyptus grandis, Wistar albino rats, optimization of dosage, fixed dosage, Subchronic toxicity

INTRODUCTION

Traditional medicinal practices are defined to have their origin from plants and plant extracts, precisely known as herbalism. Since 6000-4000 BC, Indians have practiced traditional medicinal therapy that has vital retention in curing various diseases. Currently, natural products especially traditional medicines have gained their significance through their unique therapeutic attributes with phytochemicals, their efficacy and safety.In such case, eucalyptus is considered the best traditional remedy for ailments like arthritis, ulcer, diabetes and bladder diseases. Considering the therapeutic values of *E. grandis,* few researchers and food analysts have recommended *E. grandis* as a substitute for synthetic food colourants (*Abuajah et al., 2015*).

Color additives also known as synthetic coloursare used in a wide variety of foods such as beverages, dairy products, cereals, bakery goods, snack foods and ice creams. Although there are strict guidelines for chemicals to be approved as food additives, the safety of food colorants has not been rigorously proven and acceptable daily intake (ADI) has been used to minimize any possible unfavorable effect of the dyes (*Sarah Kobylewski et al, 2012*).

With proper evidences the artificial food colors (AFCs) have proved to affect

children's behavior, through various studies carried out for around 35 years. Food and Drug Administration Food Advisory Committee convened to evaluate the current status of evidence regarding attention-deficit/hyperactivity disorder (ADHD) caused due to artificial food colours. AFCs appear to be more of a public health problem than an ADHD problem, where it seems to affect children and have an aggregated additive or synergistic effect on most children who suffer from behavioral decrement (*Eugene et al., 2012*).

Naseer (*et al., 2019*) has expressed that "in the investigations carried out to establish the safety and nutritional value of natural dye produced from the bark of *Eucalyptus globules*. The natural brown dye obtained from the *Eucalyptus* bark was applied in the production to access stability of dye in the candies, as *E. grandis bark* poses health benefits. Thus this study is carried out to prove that *E. grandis* can be used in place of harmful synthetic food colours.

MATERIALS AND METHODS

Plant material collection

The bark of *E. grandisbark*were collected from Forest College and Research Institute College in Coimbatore, Tamil Nadu, India. The freshly collected barks were cleared from dust and other particles. The barks were dried in shade, powdered using sterilized mixture jar and stored in zip lock bags. The mixture jar and the zip lock bags were made sure that no moisture content was found in it.

Animals used for Toxicity Testing

Wister Albino rats of 230-250g of male were used for the study. Five rats per group was allocated. Animals were purchased from the Animal House located in Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore*, Tamil Nadu, India*. The University animal ethical committee has approved of animal ethical clearance, providing the clearance number as AIW: IAEC.2023:03 for the study.

The animals were housed under standard environment condition of 25±2°C with sufficient light and dark cycles. The rats were fed with standard commercial feed and normal water. The animals were caged in individual boxes. To help them get accustomed to the separation, the rats were monitored for a week for some behavioural change. After a week the rats settled down and the experimentation was progressed.

Toxicity Testing

According to Organization for Economic Cooperation and Development Guidelines (*OECD, 2018*), the volume of feed should not exceed 1 ml/100g body weight, except in case of aqueous solution 2ml/100g body weight can be fed. The extracts fed to the rats through oral feed in drinking water in parts per million/million per liter of solution in kilogram. In 100 ml of water, the dosage is prepared using the following formula:

$$V_1 N_1 = V_2 N_2$$

$$V_1 = \frac{V_2 N_2}{N_1}$$

Formula used for preparing the dosage, where:

- V_1 indicates the ml (milliliter) of water to be calculated and given,
- N_1 measures the number ml present in 1 (liter) of water,
- V_2 represents the weight of the animal and
- N_2 indicates the number of ppm (parts per million) to be calculated.

The parts per million given for 100ml of water to the rats apart from the normal feeding is in case of 2 ppm:

2 ppm = 100 × 2 ÷ 1000 = 0.2 ml

Likewise, ppm level is increased for every specific dosage fed for 5 days continuously, if there are no changes found in the animals then the dosage is increased gradually with two days wash-out period.

Table 1 : Level of Water Given with Natural Food Colourants

Powdered Forms	Name of the Rat	Calculation of Level of Water to be given	Determined Level of Water (in ml)
Eucalyptus barks (*Eucalyptus grandis)*	Test rat – 1 (TR1)	376 × 2 ÷ 100	7.5

According to the *table 1,* the determined level of water in which the natural food colourant are to be optimized are 7.5 ml for the test groups of rats with powdered forms of *E. grandis.*

Pilot Study

A pilot study was carried out with two Wistar Albino rats, where the consumption level of the colourant was finalized by monitoring the animals for eight weeks with two days of wash out period. Around 100 g of weight gain was observed in both the rats. But the sensitivity of thresh hold level of wistar albino rats for the eucalyptus bark powder was higher than expected. After determining the maximum threshold level, the experimentation was carried out with the other rats.

Table 2 : Profile of the Rat used for Pilot Study

Profile of the Rat	Eucalyptus Bark (*Eucalyptus grandis*)	
Type of Group	Test Group	
Name	Eu 1	
Age (months)	8-12 months	
Sex	Male	
	During Purchase	Eighth Week
Weight (g)	344 g	452 g
Water Consumption (in ml)	20-25 ml from 120 ml of water	10 ml + 8 ml of Sample
Food Consumption (g)	25 g	25 g
Body Parts		
	During Purchase	Eighth Week
Fur/Skin	Normal White	Normal White
Eyes	Normal Red	Normal Red
Ears	Normal Pink	Normal Pink
Nose and Mouth	Normal Pink	Normal Pink

Profile of the Rat		Eucalyptus Bark (*Eucalyptus grandis*)
Limbs and Nails	Normal	Normal
Tail	Normal Pink	Normal Pink
	During Purchase	Eighth Week
Active	9	9
Sluggish	X	X
Aggressive	X	X
Irritated	X	X
Sleepy	X	X

The test groups for the powdered form of *E. grandis* barkswere named as test rat – 1. The Wistar Albino rats' level of water to be given was calculated with the body weight of the rats during the purchase. The calculation for the feeding of rats with natural food colourant is as follows for *E. grandis*barks, where the rat weighed 376 g. The level of natural food colourant that was added to the oral feed was optimized in the pilot study for eight weeks. The optimized level was orally fed continuously for 56 days during the experimentation period.

Table 3 : Optimization of *Eucalyptus grandis*

No of Weeks	Water (ml)	ppm	Calculation of ppm	ppm of Natural Food Colourants added in water (mg)
1	7.5	2	7.5 × 2 ÷ 1000	0.01
	7.5	12	7.5 × 12 ÷ 1000	0.08
2	7.5	14	7.5 × 14 ÷ 1000	0.10
3	7.5	28	7.5 × 28 ÷ 1000	0.20
4	7.5	41	7.5 × 41 ÷ 1000	0.30
5	7.5	54	7.5 × 54 ÷ 1000	0.40
6	7.5	67	7.5 × 67 ÷ 1000	0.50
7	7.5	80	7.5 × 80 ÷ 1000	0.60
8	7.5	94	7.5 × 94 ÷ 1000	0.70

2.3.1 Experimentation

The animals were divided into two groups with five animals in experimental group and two animals in control group. Group - 1 is the control group and Group - 2 is experimental group were the animals are fed with *E. grandis*for 56 days.

Table 4 : Experimentation Plan for Toxicity Testing

Groups	No. of animals	Food and dosage given during experimentation
Control Group	2	Commercial Feed (25 g) + Normal Drinking water
Experimental Group – 1	5	Commercial Feed (25 g) + Normal Drinking water + Specific Dosage of Food Colourants Eucalyptus barks (*Eucalyptus grandis)* (calculated according to the weight of the rat)

From the scheduled feed plan, the animals are weighed every week and checked for finding any physical changes indicating the weight gain or loss. Level of water consumption and intake of feed will also be checked every week, to verify if the animals are under certain abrupt meal pattern due to the intake of natural food colourants.

After 56 days, the animals were euthanasia was carried out for histopathology study in all the animals, to identify the effects of the natural food colourant in the experimental group. The animals were harvested and organs (the heart, liver, kidney, intestine, stomach and spleen) were collected in 1:10 formalin solution.

The blood was used for hematology screening such as - hemoglobin, red blood cells and white blood cells. Other part of blood was centrifuged at 3000 rpm for five minutes and serum was separated to estimate the biochemical parameters like - SGOT, SGPT, Glucose, Total Protein, Albumin, Creatinine, Sodium, Potassium and Chloride.

In the estimation of biochemical parameters - SGOT and SGPT also known as Serum glutamic-pyruvic transaminase and Serum glutamic-oxaloacetic transaminase are the markers of liver functions. The analysis for albumin, creatinine, sodium, potassium and chloride are carried out to analyze if the functioning of kidney is normal. If there is an elevation in the values of biochemical parameters, it indicates that there has been malfunctioning of kidney in the tested organism. A decrease in total protein and albumin is a sign of the reduced synthetic functioning of the liver or due to impaired hepatocellular function.

From the *table 2,* it is interpreted that *E. grandis*of 6 mg as natural food colourantwas mixed in 7.5 ml of water was given to the rats orally from week one to week eight.

RESULTS AND DISCUSSION

Clinical Signs

The rats fed with *E. grandis*did not show any symptoms of presence of toxicity andno clinical changes were found in the rats throughout the experimental period. The physical observation in their skin, fur, eyes, mucus, eating pattern, behavioural change and sleep were all normal.

Table 5 : Mean weight gain in the rats

Groups	Mean weight on Day zero (g)	Mean weight on Day 56 (g)	Difference in weight (g)
Control	201	292	92
Experimental Group 1	272	354	82

Table 5 represents the mean weight gain that has taken place in the animals. All the animals in both the control group and the experimentation group have gained weight.

The difference in the weight gain of the control group after the eighth week is 92 g, whereas the difference of weight gain in the group that consumed *E. grandis*is 82 g.

The increase in weight is due to increase in intake of water and food. Likewise, loss of appetite will be due to some metabolic change in intake of either carbohydrates, protein or fat. The change in metabolism may lead to loss of appetite and loss of appetite will lead to loss of weight gradually. But, in this experimentation there is no loss of weight observed in the rats.

Hematology and Biochemical Analysis

Table 6 represents the hematology and biochemical parameter of the rats to find if there has been any alteration in the renal functioning that was influenced by the powder mixed in water. Liver and kidney analysis is very important as the toxicity evaluation of the plant substance will be noted through the tissues, which is responsible for the survival of the organism.

The hematology value of hemoglobin is 15.9 g/dl, RBCs and WBCs are 8.35 ten million per cubic meter and 4.2 thousand per cubic meter. Whereas the SGOT and SGPT obtained are 27U/L and 25U/L respectively. The other parameters like glucose and cholesterol are 45 g/dL and 40 g/dL, which showed the same level of absorption of glucose and cholesterol in blood.

Table 6 : Mean hematology and biochemical parameter values

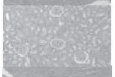
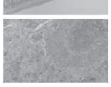
Biochemical Parameters	Estimated Values	
	Control Group	Experimental Group
Hemoglobin (g/dl)	14.53	15.9
Red blood cells (10^6/mm^3)	7.56	8.35
Total White blood cells (10^3/mm^3)	4.93	4.2
SGOT (U/L)	27.17	27
SGPT (U/L)	20.18	25
ALP (U/L)	72.45	72.45
Glucose (g/dL)	46	45
Cholesterol (g/dL)	42	40
Total Protein (g/dL)	6.5	6.5
Albumin (g/dL)	4.2	3.5
Creatinine (mg/dL)	0.9	0.65
Sodium (mg/dL)	137.3	107.9
Potassium (mg/dL)	4.1	6.5
Chloride (mg/dL)	88	89

Serum concentration of total protein 6.5 g/dL and albumin 3.5 g/dL in the treated and control group confirms that the substance has not damaged the hepatocellular or secretory functions of the liver. Renal dysfunction is assessed through the concurrent values of creatinine valuing 0.65 mg/dL and their normal value reflects the normal renal functioning in the rats. The proper functioning of kidney is again proved through the standard values of sodium 107.9 mg/dL, potassium 6.5 mg/dL and chloride 89 mg/dL.The values have proved that the *E. grandis* substance did not affect the hepatocytic functions of the rats.

Histopathological Study of the Organs

The microscopic examination of the organs of the rats treated with *E. grandis*powder did not show any change in colour compared with the control group rats' organs. Hypertrophy of organ indicates the toxicity of chemical and biological substance. In addition, the microscopic examination revealed that none of the organs from the treated rats have shown any alteration in cell structure or any unfavourable effects under the light microscope using multiple magnification powers

Table 7 : Microscopic view of the harvested organs from the rats

Organs	Control Group	Experimental Group
Brain		
Liver		
Kidney		
Heart		
Stomach		
Intestine		
Spleen		

No pathologies were recorded in the histological sections of the vital organs (brain, liver, kidney, heart, stomach, intestine and spleen) of the control group. Equally, there was no change in the creatinine after administration of *E. grandis*powder when compared to the control group. Any alteration found in the level of creatinine show that there has been damage in the functioning of nephrons. The observations in the histopathology study of the kidney tissue have been recorded no changes. Thus, this study proves that *E. grandis*is non toxic in nature and can be used as natural food colourant.

CONCLUSION

The results demonstrate that natural form of powders substance of *E. grandis* barks is practically proven to be non toxic in nature when administered orally and is safe for consumption. So, the study has proved that in place of synthetic food colourants, eucalyptus barks can be powdered and used as it is in foods making the food therapeutically valuable.

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