Research Article

Toxicological Evaluation of Apricot Exudate in Male Albino Rats

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ABSTRACT

The plant exudate is used in many therapeutics' products. There is general belief that all-natural products are safe and can be used without any restriction. So, the present study aimed to investigate the toxic effect of apricot exudate on several biological parameters in male rats. Antimicrobial effect of resin exudate was examined in vitro against some pathogenic bacteria species. For the in vivo biological and the cytogenetic activity of exudate, rats were divided into three groups. Group 1: rats received no supplementations representing the control group. Group 2: rats were supplemented single dose of resin extract (3.6 g/kg) and were dissected after two weeks representing the acute dose. Group 3: rats were supplemented daily with exudate dose (0.12 g/kg) for 45 days. Serum liver and kidney functions in addition to blood picture were evaluated. Gutathione reductase enzyme in liver tissue and acetylcholinesterase in brain tissue were detected. Bone marrow was subjected for cytogenetic examination. At last, an irritation test on the rats skin was preformed to examine the exudate effect on skin for 5 days. Promising results were obtained from the antimicrobial examination ranking the apricot exudate as a highly potent antimicrobial agent. Normal ranges of biochemical studies were observed in both of the exudate supplemented groups compared to control group. The skin irritation test revealed that there was no harmful effect of the resin on the skin layer when observed by the naked eyes. The cytogenetic examination concluded that both doses of the apricot resin exudate were toxic for the cell chromosomes directing us to suggest that the apricot resin may be used as a topical treatment for some skin diseases. Although apricot resin exudate exhibited superior antimicrobial activity in vitro, its cross reactivity in dermal cells must be deeply studied.

Keywords: Apricot exudates, Antimicrobial activity, Toxicological studies.

INTRODUCTION

The growing demand of new therapeutics of natural origin increased the attention of the researchers to evaluate the chemical and physical characters of different parts of a promising plant including roots, bark, leaves fruits or seeds. Gum exudates of different trees were not excluded from these studies. Plant resin was characterized as any sticky plant exudate that becomes hard when exposed to air and is hardly insoluble in water[1]. Gummosis is a phenomenon of exudates gum excretion from various plants. A gum exudate from apricot tree was used in this study. Prunus (apricot) tree is a species of Rosaceae family. Apricot species, a member of Pruna is cultivated in Egypt and many countries. The gum exudates from Prunus species constitute mainly from polysaccharides as arabinose and galactose at different ratio[2].

Most of the studies, up to our knowledge, on the herbal exudates or resins were directed to their chemical compositions. However, the toxicity of these substances in animals is an important issue to consider if an exudate can be applied as therapeutic agent or not. The importance for studying the toxicity of any plant products arises as the general belief of people considering all-natural products are safe and can be used without any restriction especially that these products are available as supplements on the market[3]. Accordingly it is a must for having a proper chemical, toxicological and safety available data to use any plant as a healthy traditional agent. In another word assessing the toxicity profile of a certain herbal product is very essential[4].

After a skin injury occurs, the wound healing process begins immediately to avoid the risk of bacterial contamination[5]. The gram-positive organisms such as *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pyogenes* (*S. pyogenes)* are the dominant organisms involved in the initial stage of the infectious process, On the other hand, gram-negative organisms like *Escherichia coli* (*E. coli)* and *Pseudomonas aeruginosa (P. aeruginosa*) are found only in later stages of the infectious process, i.e. during the development of a chronic wound[6].

New wound dressings are needed nowadays to avoid penetration of bacteria into the wound and avoid microorganisms growth[7]. Based on etiology, the wound healing society classified chronic wounds into four categories: pressure, diabetic, venous and arterial insufficiency ulcers. Mainly the primary cause of chronic inflammation occurs in chronic wounds are caused by bacterial colonization[8].

Although the *in vitro* studies can give us a hint for the toxicity of a product, however the in vivo toxicity data in animals represents the only prediction of the impact of

any agent on human. Toxicity evaluation can be assessed either specific as dermal irritation or general as different biochemical and histological variations imaging a certain harmful effect. In view of the foregoing introduction the different toxicity doses of one species of Prunus gum exudate was evaluated in rats.

The present study was designed to assess the acute and repeated dose toxicity of the mentioned exudates after 45 consecutive days of oral administration in rats. Acute toxicity testing was carried out to determine the effect of a single dose on rats. Blood and different organs were taken for hematological and clinical chemical analysis. The microbiological studies were performed to make use of the exudate if it possesses any anti microbial activity. A dermatological study was applied on rats skin besides the chromosomal aberration investigation of the bone marrow.

MATERIALS AND METHODS

Materials

Chemicals & Kits

All chemicals used in the experiments were of analytical grade. Kits used for the quantitative determination of different parameters were purchased from Biodiagnostic Company, Cairo Egypt.

Methanol, glacial acetic acid (Merk), Colchicine and Giemsa stain Gurr R66 (BDH).

Animals

45 male Sprague Dawley rats weighing 120-150g were obtained from Vacsera Cairo animal house. They were randomly allocated and housed in cages, 5 rats each. The animals had free access to commercial rat chow and drinking water and were allowed 7 days for acclimatization prior to commencement of the experiments.

All the studies were conducted in accordance with the Animal Ethical Committee of the National Research Center under the ethics number (17071).

Methods

Preparation of exudate extract

Bark exudates was collected in summer from Prunus species. 6 gram of exudate was dissolved in 50 ml warm water at 40°C then filtered giving the concentration of 0.12 g/ml.

Biological Activity

The antibacterial and anti- yeast activities were carried out in the Microbial Department, National Research Centre, using the diffusion plate method. A filter paper sterilized disc saturated with measured quantity (25 µl) of the sample (1 mg/ml) was placed on a plate (9 cm diameter) containing a solid bacterial medium (nutrient agar) or a yeast medium (potato dextrose agar) which had been seeded with the spore suspension of the test organism. After incubation at 37°C for 24 h for bacteria (in case of yeast, at 25°C for 72 h), the diameter of the clear zone of inhibition surrounding the sample was taken as a measure of the inhibitory power of the sample against the particular test organism.

(% inhibition = sample inhibition zone (cm) / plate diameter x 100).

All measurements were done in DEMSO as a solvent which has zero inhibition activity. The antimicrobial activity of the tested compound was examined according to El-Samahy *et al*. [9] with gram positive bacteria, *Bacillus cereus*, *Staphylococcus aureus ATCC 6538*, *Salmonella typhimurium ATCC 25566* and gram negative bacteria *Escherichia coli NRRN 3008* , *Pseudomonas aeruginose ATCC 10145* and the yeast *Candida albicans EMCC105*. The obtained results are compared with the reference antibiotic Cephradine that was purchased from Egyptian markets.

Irritation test

Skin irritation test was carried out using occluded dermal irritation test[10] with slight modification. Two groups of rats (each group containing six animals) were employed for this test and two areas on the dorsal side of each rat on each side of the vertebrae (1 cm from the midline of the vertebral column) were shaved and marked before the experiment. One area was for test exudate sample at a dose (1ml of 3.6 g/kg) and the other was left untreated to be used for comparison. The animals were examined for the presence of erythema and oedema at intervals of 1, 24, 48 and 72 h. The affected skin area was followed using a magnificating lens.

The fixed dose procedure

The procedure was used to assess the nonlethal toxicity rather than the lethal dose to reduce the number of consumed animals. The gum exudate extract was administered at five fixed dose levels from 1000 to 3600 mg/ kg. Three rats were used for each dose.

Acute toxicity test

Acute toxicity test was followed according to Gatsing *et al*. [11] with some modifications. Twenty healthy male fasted rats were equally divided into 2 groups and orally received; vehicle (distilled water), or 3.6g/kg as a single dose of exudate extract. Food was withheld for a further 4 hrs. after dosing. The animals were observed for 48h after the administration of the extracts for the onset of clinical or toxicological symptoms. Mortality, if any, was reported over a period of 2 weeks.

Repeated dose toxicity

This test was carried out for 45 days on rats weighing 120-150 gram. The variation in the weight was ± 20 %. Gum extract was daily administrated orally at a dose of 0.12 g/kg. Behavioral and any abnormal signs were daily recorded. At the end of the study blood, livers and brains were collected.

Parallel to this, a group in which the rats received water, was served as negative control one.

Blood and serum separation

At the end of the experiment half the volume of the blood of each rat was collected in sterilized tubes and serum was separated by centrifugation at 3000 r.p.m. for 15 min for biochemical analysis. The other half of the blood was collected in ethylenediaminetetraacetic acid (EDTA) containing tubes for blood count, heamoglobin and hematocrit determination[12]. The measurement of the blood parameters was made in an automatic cell counter Medonic, Stockholm, Sweden.

Preparation of tissue homogenates for biochemical assays

1 gram of tissue (liver or brain) from each rat was homogenized in 10 ml distilled water using an electrical homogenizer with a teflon rod and then centrifuged at 3000 r.p.m for 15 minutes. The supernatant was collected and stored at - 20°C for enzymatic assays.

Biochemical assays

Serum alkaline phasphatase (ALP) was determined according to Belfield and Goldberg[13], transaminases (AST& ALT) were determined by the method of Reitman and Frankel[14], creatinine was determined as described by Bartles *et al.* [15]. Liver glutahione reductase was measured by the method of Calberg and Mannervik [16].

Brain acetylcholinesterase (AChE) was determined as described by Gorun *et al.* [17]. Tissue total protein content was detected according to the method of Gornal *et al.* [18].

Chromosomal Aberration Assay in Rat Bone Marrow Cells

Bone marrow was excited and the chromosomal abberations were detected for both control and treated group as described by Sharma [19]. Somatic chromosomal aberrations in rat bone marrow cells were scored after preparing metaphases according to Yosida and Amano [20] with some modifications. Metaphase were stained with Giemsa in phosphate buffer, five rats were used for each treatment. About 50 well-spread metaphases were analyzed per animal.

Statistical Analysis

A possible difference between control and treated animals in the mean percentage of bone marrow was analyzed by Student t-test. The biochemical parameters and the biological activity data were analyzed by the one way ANOVA test.

RESULTS

Antimicrobial Activity

As shown in table (1) the tested resin exhibited superior antimicrobial activity against, gram negative bacteria *E.coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. In addition, gram positive bacteria *Bacillus cereus* and *Staphylococcus aureus* were also inhibited to a certain limit compared to the reference antibiotic drug. On the other hand, the tested resin showed non significant activity against the yeast pathogen *Candida albicans* with respect to the reference antibiotic.

Table 1: Antimicrobial activity of apricot resin exudate.

Microorganism	Gram Stain Reaction	Mean Clear Zone (mm) by treated exudate	Mean Clear Zone (mm) by Reference antibiotic Cephradine
Bacillus cereus	Positive	14±0.57[a]	30±0.57
Staphylococcus aureus	Positive	20±0.88[a]	29±0.88
Escherichia coli	Negative	40±0.57[a]	14±0.88
Pseudomonas aeruginosa	Negative	25±0.57[a]	20±0.57
Salmonella typhimurium	Negative	44±0.33[a]	15±0.57
Candida albicans	-----	35±1.1[b]	34±0.50

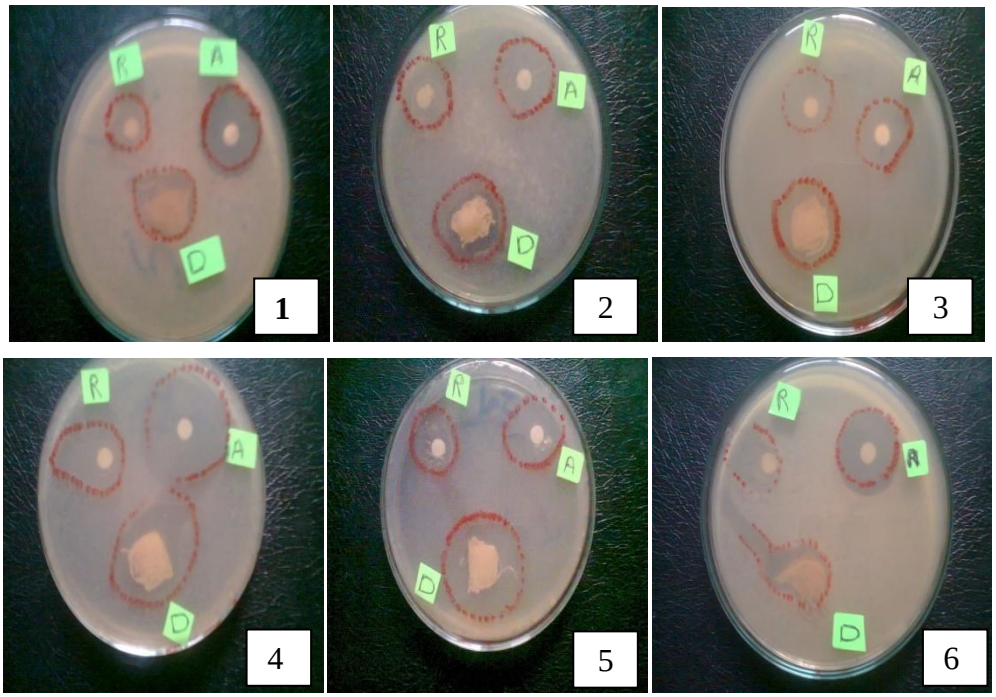
Data are represented as Mean ±SE of triplicate.

P^a Significant at $P \leq 0.05$ compared to the reference drug.

P^b Non Significant at $P \leq 0.05$ compared to the reference drug.

Application of the antimicrobial activity

The above results were applied *in vitro* by using sterile wound dressing immersed in the prepared resin, Figure (1). Excellent inhibition effect was obtained by using these sterile pieces of wound dressing confirming the data obtained in Table (1) and considering this natural resin as a novel antimicrobial agent.



R: resin, A: antibiotic, D: dressing.

Figure 1: Application of the resin antimicrobial effect on wound dressing in vitro (1-*Bacillus cereus*, 2- *Staphylococcus aureus,* 3- *Echerichia coli*, 4- *Pseudomonasaeruginosa*, 5- *Salmonella typhimurium* 6-*Candida albicans*)

Irritation response

The irritation preliminary test revealed that exudates didn't induced neither erythema nor edema when applied on the skin of the rats at any interval time.

Acute toxicity study (Clinical Observations).

Dose levels of 3600 mg/kg body weight of the extract did not cause any signs of toxicity or deaths in the 2 weeks of treatment. Rats of the treated group gained weight progressively as in the control one.

Blood analysis

Although the results in table (2) show slight significant changes in the measured parameters, however no great differences were found comparing the treated group with the control one. The decrease in WBCs was nonsignificant.

Table 2: Effect of Apricot exudate on blood Picture

Parameters Groups	RBCs count x 10^6/µl	WBCs count x10^3/µl	Heamoglobin (g/dl)	Heamatocrit %
Control	6.41± 0.27	16.44± 0.45	11.16± 0.49	31.70± 1.29
Apricot exudate	7.44±0.035*	14.07±1.18	13.32 ±0.24*	8.97±1.10*

Data are represented as mean ±S.E of 5 rats;

*P**significant at $p< 0.05$ compared to control group.

Serum parameters.

Creatinine as well as all liver enzymes measured in sera showed no significant changes in treated group when compared to the control one. Also, a good sign for the nontoxicity of the exudates is that no significant change was detected in the AST/ALT ratio as shown in table (3).

Liver and brain homogenates parameters.

Glutathione reductase in liver tissue homogenate, as a measure for antioxidant activity, shows no significant change. The only significant elevation was detected in liver total protein content.

Exudate supplementation did not induce any significant change in acetylcholinesterase activity which represent one of the most important parameters measuring the toxicity as shown in table (3).

Table 3: Effect of Apricot exudate on some enzyme activities.

Group/parameters	Control	Exudate
Ceriatinine (mg/dl)	0.72±0.09	0.98±0.14
AST (U/ml)	30.19±0.50	29.60±0.81
ALT (U/ml)	12.27±0.63	13.38±0.46
AST/ALT	2.46±0.4	2.22±0.1
ALP(IU/L)	232.00±19.14	210.52±13.33
Glutathione reductase enzyme (nmol/min./ mg protein)	34.91± 4.11	42.59± 1.37
Liver total protein content (mg protein/g. tissue)	126.60± 3.48	161.60± 9.19*
Acetylcholinesterase enzyme. (µgSH/min/mgprotein)	2.14± 0.13	1.99± 0.23

Data are represented as mean ±S.E of 5 rats.

*significant at $p< 0.05$ compared to control group.

Chromosomal aberrations

After administration of exudate (3.6 g/kg b. wt.) to rats, the percentage of chromosomal aberrations in bone marrow cells was recorded in table (4). 24 hrs post administration of the acute dose, the frequency of chromosomal aberration recorded highly significant effect (14.8%) and after 48 hrs recorded a significant effect (10.4%), according to the control value (1.2%), with many types of aberrations as deletions, fragments, centromeric attenuations and chromatid gabs.

After 45 days of exudates administration, (0.12 g/kg) the frequency recorded a significant lower value (7.2%) as a subchronic dose effect. It was noticed that the administration of the chronic dose affected the same types of aberration excluding the chromatid gaps.

Table 4: Effect of Apricot exudate on chromosomal aberrations in rat bone marrow cells.

Treatment	Scored metaphase	Abnormal metaphases		Types of aberrations				
		No.	Mean % ± S.E.	Del.	Cht.g.	C.A.	Frag.	Ch. b.
Control (non-treated)	250	3	1.2± 0.33	1	1	-	1	-
Acute dose (24 hrs)	250	37	14.8 ±0.76**	11	3	12	6	5
(48 hrs)	250	26	10.4±1.34*	9	3	7	4	3
Subchronic dose	250	18	7.2± 0.89*	4	3	-	3	8

* Significant at (P < 0.05) t-test.

** Significant at (P < 0.01) t-test

Del.: deletion; Cht.g. : Chromatid gab; C.A. Chromosomal attenuation; Frag. : fragments; Ch.b. : Chromosomal breaks.

DISCUSSION

Gum exudates represent a rich natural source of food biopolymers due to their amphiphilic moieties and water-holding capacities. They are applied in food, cosmetic, and paper industries [21, 22, 23]. Some natural gums, exuded by plants as a result of mechanical injury or microbial invasion[24], are used in medicine.

Prunus gums are applied in some countries in the confectionery industry. In India, the prunus gums in combination with other gums such as Arabic gum are used in several applications[25]. In the present study we tried to investigate the toxicity of apricot gum exudates (Prunus tree) which is cultivated in Egypt on different toxic axes in order to highlights their possible medicinal use.

As found in the present study the resin displayed a broad spectrum antibacterial activity *in vitro* investigation. Its activity against Gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus* is lower than its effect towards Gram- negative ones *E.coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. As explained by Patrone and Stein[26], Gram – negative bacteria are more resistant towards antimicrobial agents. They attributed this to the fact that they are coated with a phospholipid membrane, carrying the structural lipopolysaccharide component. This cover reduces the permeability of antimicrobial substances. So as we noticed, the apricot resin could penetrate the resistant membrane in the gram negative bacteria and increased its permeability more than that of the reference drug. Also the apricot resin antimicrobial activity compete with the reference drug in inhibition of the fungus *Candida albicans*. High rates of morbidity and mortality are caused by bacterial contamination of skin wounds. Antibiotics, natural products and nanoparticles represent most of the antimicrobial agents nowadays[27].

Simoes *et al*, [7] reviewed the relation between the antimicrobial agents incorporated in wound dressings and their mode of action. They demonstrated that in order to improve the healing process and reduce wound bacterial colonization, wound dressings must be loaded with antimicrobial agents.

These results are in accordance with that of Gigante *et al*. [28], who tested 15 different resin acid derivatives against some human pathogenic microorganisms *Trichophyton mentagrophytes*, several yeasts species, and *S. aureus*. They found that these acids separated from the resin possessed antifungal and antibacterial activity. They supposed that the naturally occurred plant resins can have future applications in pharmacology. A similar result on the antibacterial effect of different plant resins was found by several authors. Bouaziz *et al*. [29] studied antibacterial activities of water-soluble polysaccharides and hemicelluloses from almond gum. They found that both polymeric compounds isolated from almond gum showed growth inhibition of pathogenic strains Yao *et al*.,[30] found similar effect of, peach gum derived oligosaccharides as antibacterial agents with activities against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* at the concentration of 100 µg/mL.

The high activities of the apricot gum exudates against some pathogenic microorganisms as well as its anti-fungus activity encourage us to study its toxic effect on the skin as well as its oral toxicity.

The skin is the largest organ of the human body. Its main role is to protect the body against chemical and physical and microorganisms injuries from the external environment. However, many of the drugs provided used for topical administration are not only expensive but also can produce some allergical and drug resistance problems[31, 32].

The current therapeutic agents available to treat skin inflammation and infection are mainly glucocorticoids, which often exhibit adverse reactions that affect many organs related to the immune system, lipids, carbohydrates, proteins and bone metabolisms[33, 34, 35]. As a result natural substances are the alternative for new safer and more effective topical anti-inflammatory drugs[4]. Accordingly, parallel to any oral toxicity study, it was important to investigate the effect of Prunus (apricot) gum exudates on the skin. The topical administration of the exudates (1 ml of 3.6 g/kg for 5 days) on rat skin revealed no signs of inflammation. This is in accordance with several studies on different resin extracted from plants. Simpson *et al*. [36] recorded a significant reduce in ear oedema, inflammatory cell infiltration and production of pro-inflammatory cytokine IL-1β in the ear tissue when using formulated cream containing 5% w/w of leaf resin from *D. polyandra*. Also the peach gum oligosaccharides exhibited antioxidant and antibacterial activity as found by Yao *et al* [30].

The acute oral toxicity investigations on Prunus gum exudates extract revealed high LD50 dose of the extract (> 3g/kg). No significant modification in the general behavior of the rats and no death after 48 hrs were recorded at the high dose administrated. These results encouraged us to undergo further toxicity studies. Repeated dose toxicity test was designed to study the effect of the extract on rats after 45 days of daily administration. 45_days of resin administration did not induced changes in clinical signs nor in animals' growth.

No great variation was detected in heamatological parameters. As reported in previous work the hematopoietic system represents a sensitive target for toxic substances[37]. The non significant change in WBC count can be attributed to normal response to stress associated with the chronic toxicity studies as explained by Kelly[38].

The repeated dose toxicity test represents an important issue for the evaluation of the safety of any substance to be used[39]. At the end of the study, biochemical and chromosomal aberration data were compared.

Kidney and liver are the primary organs prone to the toxic substances. AST, found in several organs such as muscle, heart, brain and liver, it is key enzyme in amino acid metabolism. Also ALT and AST as cytosolic enzymes and ALP as a membrane bound enzyme are highly concentrated in liver and kidney. In addition AST/ALT ratio in serum is considered as a good clinical biomarker for any liver leakages or rupture[40]. On base of the present results no significant changes were found in any of the three enzymes nor in AST/ALT ratio.

Serum creatinine level is a good indicator of renal function. Accordingly any increase in its level is associated with kidney failure[41]. Oral supplementation of Prunus gum exudates did not induced any significant changes in creatinine and in all the measured enzymes which indicate the safety of the resin. Similar results were found by Goudah *et al*. [42] when studying the toxicity of Ferula assa- foetida gum in rodents.

Many neurons in the human body secrete the neurotransmitter acetylcholine in the synaptic space which binds to the post-synaptic membrane and then diffusing the post-synaptic membranes to transmit the information. Acetylcholinesterase enzyme (AChE) terminates this neurotransmitter, acetylcholine, by decomposing it into choline and acetate[43]. This enzyme plays an essential role in nerve conduction at the neuromuscular junction and motor function[44]. Accordingly any inhibition in the enzyme activity reflects a harmful effect of the treatment under discussion. Brain AChE activity is essential for normal brain physiological function[45]. Since no significant changes in AChE activity was measured in treated animals, this is an indication for the non toxic effect of the resin on the rat's brain.

Accidental exposure to chemicals or foreign particles induces Reactive Oxygen species (ROS). Oxidative stress is

an imbalance between ROS and antioxidants[46]. Under normal circumstances, body neutralized ROS by the antioxidant enzymes. Glutathione reductase GR is one of the enzymes of glutathione oxidation reduction cycle[47]. The non significant change in GR activity shows another evidence for the non toxic effect of the resin.

The chromosomal aberrations studies showed a significant effect especially with the acute dose of the exudates as found in the present study. Exposing the rats to oral supplementation for 45 days with the exudates showed significant effect on the rat bone marrow cells. The centromeric attenuation and chromosomal breaks were recorded in the bone marrow cells of different rats, as a remarkable degree of danger with that acute dose. The frequency of chromosomal aberrations induced with the subchronic dose of the exudate was less significantly than acute dose, indicating a many types of aberrations. Subjecting the animals to any harm compound, either chemical or natural one, can induce chromosomal aberrations[48].

CONCLUSION

Apricot gum exudate is one of the herbal origins that can be used in therapeutic aspects. As it shows antimicrobial and anti fungus activity it can be applied in dermatological aspects. However more studies must be deeply conducted, especially with the non-promising results of the chromosomal studies.

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