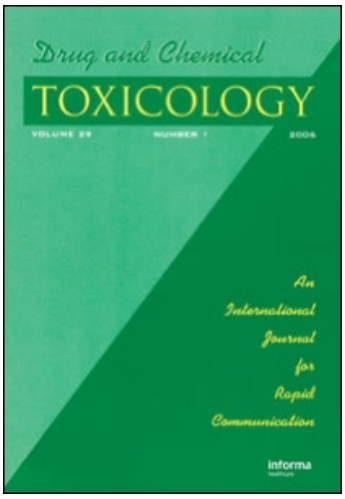

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RESEARCH ARTICLE

Subchronic toxicological evaluation of brea gum (*Parkinsonia preacox*) as a food additive in BALB/c mice

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Abstract

Brea gum is a phloematic exudate from *Parkinsonia praecox*, an autoctonous tree that grows in the arid areas of Argentina. In this work, we propose its potential as a food additive. However, as no toxicological safety evaluation of brea gum has yet been reported, this preliminary study was conducted to evaluate its long-term toxicity over a 120-day period in BALB/c mice fed with brea gum at various levels in the diet. The results showed that animals on diets containing up to 5% brea gum were healthy, exhibiting growth curves similar to controls for both males ($P = 0.9138$) and females ($P = 0.9459$), thereby indicating that feed intake and utilization was not affected. A histopathological examination and weight recording of liver, kidneys, and intestine did not reveal any microscopic abnormalities or adverse toxicological effect (weights respect to control: $P > 0.1$). Moreover, hematological parameters and enzyme activities were within the normal values previously reported for mice. Our findings suggest that feeding brea gum at levels up to 5% to BALB/c mice do not exert any toxicological effects, supporting its potential use as a food additive for human consumption.

Keywords: *Plant gums;* parkinsonia praecox*; tree exudates; safety evaluation; food additive*

Introduction

Brea gum is a phloematic exudate from *Parkinsonia praecox* (Ruiz & Pavon) Hawkins [=*Cercidium praecox* (R. & P.) Harms], an autoctonous tree that profusely grows in the arid areas of Argentina (Hawkins et al., 1999). Wounded plants exudate gum from the bark up to the cambium of the trunk and main branches. This exudate was chemically described by Cerezo et al. (1968), as an acidic nonstarch polysaccharide considered to be a variety of soluble fiber (Annison et al., 1995). The hydrolyzed composition after purification is 34% of galacturonic acid, 29% galactose, 5% arabinose, and 32% xylose (Leon de Pinto et al., 1993). The gum has structural and chemical similarities with other plant gums widely used in the food industry. Hence, brea gum may be a candidate for incorporation as a suitable stabilizing, emulsifying, and thickening additive in food formulations. However, the potential use of brea gum as a food additive requires established toxicological studies in laboratory animals in order to verify its safety before being included in the food codex (Anderson, 1989). Interestingly, brea gum has been traditionally used as a "woodland candy" by countryside people since pre-Colombian times without producing harmful consequences. However, no toxicological safety evaluation of brea gum has yet been reported.

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As recommended by the Joint Expert Committee on Food Additives (FAO, 1987), the criteria to be used for the safety evaluation of food additives involves the use of experimental laboratory animals, such as rodents. The evaluation of feeding protocols should take into account physical appearance and behavior, growth and body weight gain, food consumption, and absorption and utilization of other nutrients. Moreover, the evaluation ought to include plasma hematology and blood chemistry, systematic gross pathological examination on necropsy, and histopathology analysis (WHO, 1973; FAO, 1974). It is worth mentioning that other plant gums, such as arabic gum from *Acacia senegal* and karaya gum from *Sterculia* spp., showed no toxic effect in dietary proportions of up to 10% (w/w) (Anderson et al., 1982; Brown et al., 1982). This preliminary study was conducted in order to evaluate long-term toxicity evaluation over a 120-day period in BALB/c mice fed with brea gum at various levels in the diet.

Materials and methods

Test material

Brea gum samples were collected from several tree specimens of *P. praecox* during summer 2005 in the *Arid Chaco* of Córdoba, Argentina. The gum samples were purified by diluting in deionized water and then filtering the solution to remove all the bark and dust contaminants. Finally, the solution was precipitated by using ethanol to remove the hydrophilic sap contaminants, such as those of phenolic origin. This process was repeated three to four times until reaching a degree of purity, as described by Leon de Pinto et al. 1993 and Ibañez and Ferrero (2003). Then, the purified gum was dehydrated and ground to get an ivory-colored powder and stocked at 4°C in darkness until use.

Animals and diets

A total of 30 weaning BALB/c mice of both sexes, weighing 20–25 g, were randomly distributed into five dietary lots and housed individually, with 12-hour light-dark cycles at a temperature of 20 ± 2°C and a relative humidity of 60 ± 5%. The first group was fed with brea gum 0.2% (GB02), the second with 1% (GB1), and the third received 5% (w/w) (GB5). Additionally, two groups were used as controls, one without gum (C) and the other with arabic gum 5% (w/w) (GA5), a widely used, well-tested food gum (Anderson et al., 1982; Annison et al., 1995). The differences in caloric values among formulae were negligible. Food and water were offered *ad libitum*. At the end of the 120-day period, all the animals were sacrificed under anesthesia, using ether, and then autopsied following a previous protocol (Cremonezzi et al., 2004).

The composition of the basic diet was as follows (%): casein (14.0); corn starch (39.0); sucrose (36.0); lipids (6.0); fiber (2.0); salt mixture (2.5), and vitamin mixture (0.5). The diet was given in pellets, and the mixtures of the different test diets were prepared once a week, using the powder of purified gum (López et al., 1998).

Experimental design

Groups of 6 animals (3 males and 3 females) received the formulae for 120 days. The animals were observed weekly and weighed, and any signs of behavioral abnormalities were recorded. Food intake and water consumption was measured weekly during the study period. Feed conversion efficiency (%) was calculated as follows: weekly body mass gain (g) / weekly food consumption (g) × 100 (Janaki and Sashidhar, 2000).

At necropsy, a macroscopic evaluation of all main organ sizes was carried out by using callipers, and the weights of the liver, kidneys, and intestine were recorded (Anderson et al., 1982). Samples of liver, kidney, heart, spleen, intestine, and lung were taken and fixed in 10% neutral formalin, dehydrated, and embedded in paraffin. Sections were stained with hematoxylin and eosin prior to histological analyses by a trained pathologist (López et al., 1998).

Laboratory analysis

Under anesthesia, 0.3–0.5 mL of blood was obtained from animals by cardiac puncture. Due to the limited amount of blood obtained, it was necessary to pool all the samples in each group. Hematological parameters, namely, hemoglobin concentration, mean cell volume, total erythrocyte count, total and differential leucocyte count, and packed cell volume, were analyzed by standard methods. The levels of glucose, total protein, and albumin were determined in plasma. The plasma activities of the hepatic enzyme markers of hepatic injury, as glutation s-transferase (GOT), γ-glutamine transpeptidase (GPT), and alkaline phosphatase (FAL), were analyzed by using the optimized kinetic technique, ALP 405 (Wiener®, Wiener Laboratories SAIC, Rosario, Argentina, for FAL), while for the GOT and GPT, the Unitest (Wiener) was used.

Statistical analyses

Data were analysed by parametric analyses of variance, using the software package, Infostat®, Facultad de Ciencias Agropecuarias Universidad Nacional de Córdoba, Córdoba, Argentina, and all values were expressed as means ± standard error of the mean. Values of $P < 0.01$ for the Fisher's LSD tests were considered significant.

Results

Animals consumed the formulae containing brea gum at the same rate as both controls did, appeared healthy, and did not show any unusual behavior during the 120-day study period. No significant differences were found in the growth curves between the control and the animals fed different levels of brea gum (Figure 1a and 1b). Neither was there any significant difference in the final body weight in either males ($P = 0.9138$) or females ($P = 0.9459$).

A modest increase in fecal bulk, and its moisture content, was qualitative observed in GB5- and GA5-fed mice of both sexes. No differences in water consumption were observed among treatments in neither males ($P = 0.6471$) nor females ($P = 0.0549$), in comparison to controls. The feed conversion efficiency along the study period for both sexes of controls and experimental groups were similar, indicating that this parameter was not affected (Figure 1c and 1d).

The ratio of the weights of the liver, kidneys, and intestine to the total body mass did not show any changes for either male or female animals at the end of the study period ($P > 0.1$).

A histopathological examination in the control and brea gum–fed group showed no differences between the groups, indicating that feeding brea gum up to a 5% level in the diet did not result in any adverse toxicological effect on the organs. Figures 2 and 3 show

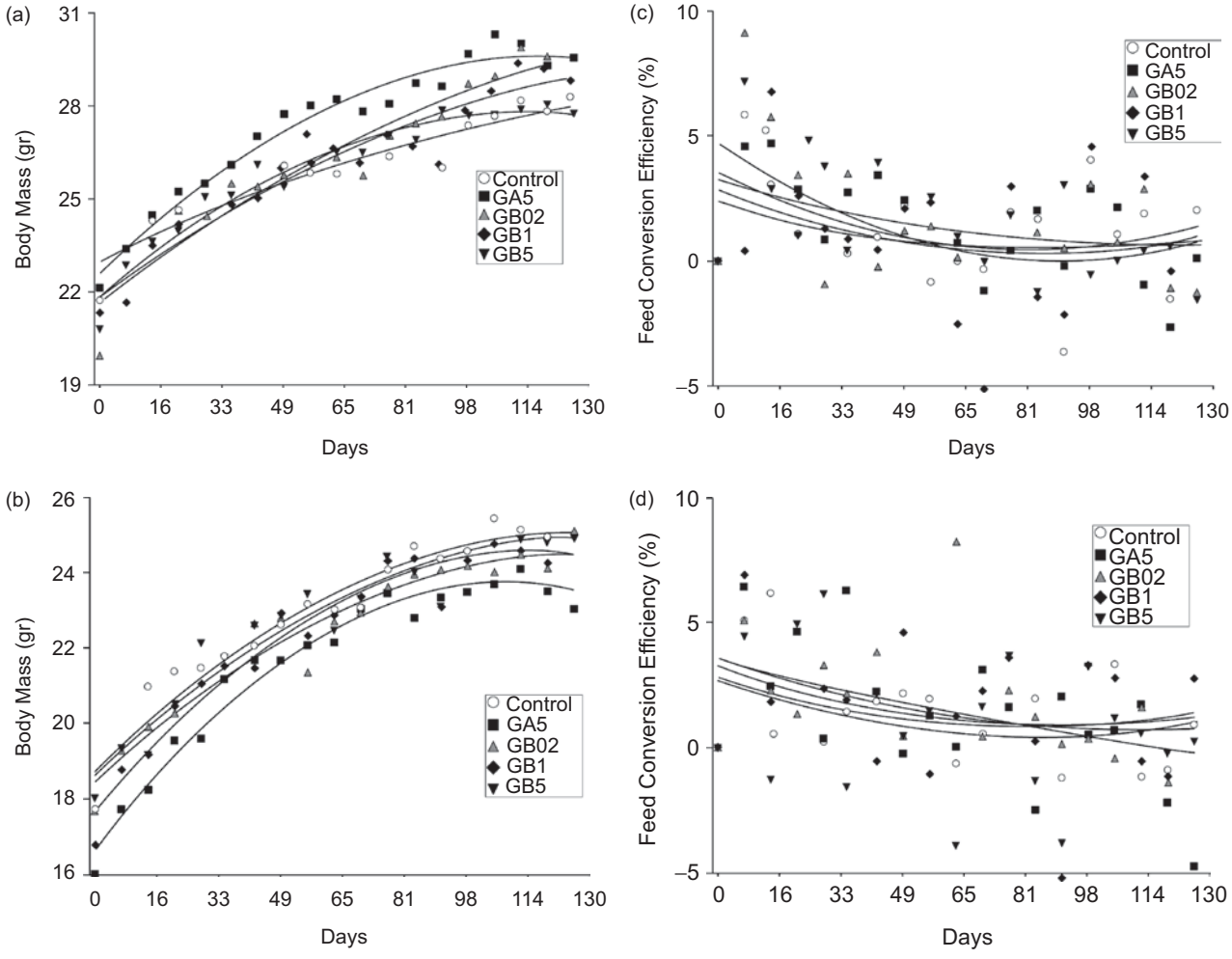

Figure 1. Change in male body mass during the study period for both male (a) and female (b) BALB/c mice. Feed conversion efficiency for males (c) and females (d) during the study period. No significant differences were found among treatments ($P > 0.1$).

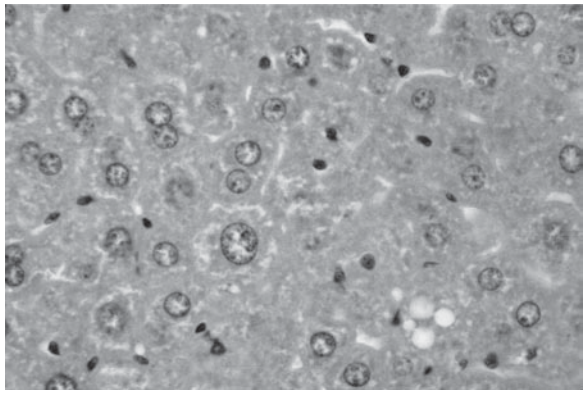

Figure 2. Normal appearance of a liver section from male BALB/c mice fed brea gum 5% (H&E).

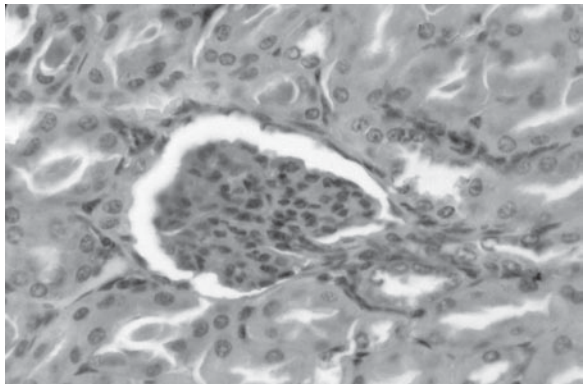

Figure 3. Normal appearance of a kidney section from male BALB/c mice fed brea gum 5% (H&E).

the liver and kidney sections of the 5% brea gum–fed group. Even at 5%, brea gum–fed mice did not show any macro- or microscopic abnormalities.

All hematological and plasmatic parameters evaluated were within the normal range, as reported for mice; moreover, no significant differences were observed in enzyme activities between the control and brea gum–fed animals, with all values being within the normal limits reported for mice (Festing, 1979).

Discussion

P. praecox is a native species widely distributed in the arid areas of Argentina. Countryside people have been using it as a type of natural chewing gum for many centuries without any apparent harmful effects. However, the gum exudate of this plant has never been used as a food additive, because it is not included in local publications related to food products, such as *Código Alimentario Argentino* (2007). Considering its potential as a food additive, the toxicological evaluation of brea gum seems to be of interest in order to assay its safety for eventual human consumption (Anderson, 1989) by using an experimental design and a number of animals similar to that of other studies done in other plant gums (Anderson et al., 1982; Taupin and Anderson, 1982; Dikshith et al., 1984; Janaki and Sashidhar, 2000; Kang et al., 2007)

In the present work, the metabolism and growth of the animals were normal, in agreement with similar toxicity studies carried out into the safety assessment of other plant gums now widely used in the food industry (Anderson et al., 1982; Brown et al., 1982; Anderson, 1989). Up to 5% of gum in the diet was included in the study, because a higher concentration of nonstarch polysaccharides in the diet and its hygroscopic properties could produce an increase of fecal bulk and its moisture content, accelerating digestion processes with the consequent decrease of nutrient assimilation (Blackburn and Johnson, 1981). This evidence must not be taken as a toxicological effect of the gum consumption. For long-term toxicity assays, a 120-day study period is considered as acceptable for these varieties of food additives (FAO, 1987). Feeding brea gum up to the 5% level did not appear to retard growth or affect the food consumption or its utilization in both sexes of BALB/c mice. The feed conversion efficiency of the control and experimental group animals of both sexes was similar, which indicates that food intake and the utilization of protein and other nutrients was not adversely affected by brea gum intake (Janaki and Sashidhar, 2000).

Fecal bulk and moisture content of the feces, qualitatively evaluated in both the brea gum and arabic gum 5% groups, were slightly greater than in the controls without gum in the diet. Eastwood et al., (1983) compared the effects of different hydrocolloids on fecal bulking and reported that hydrocolloids increased fecal bulk due to their hygroscopic capability. As reported by Cerezo et al. (1968), brea gum had a high soluble fiber content, and in consequence, this may be a desirable side effect (Blackburn and Johnson, 1981; Hara et al., 1994).

The hematological parameters of control and experimental groups fed brea gum were within the normal values reported for BALB/c mice (Festing, 1979). These results suggest that brea gum is not toxic, since it does not effect the circulating blood cells. Our results are in agreement with those of Taupin and Anderson (1982), who reported that karaya gum fed animals did not show any abnormalities regarding hematological parameters. Eastwood et al. (1983) and Anderson (1989) have also reported that ingestion of karaya and tragacanth gum in humans did not affect these parameters.

As a key role is played by the liver and kidney in the detoxifying processes, a histological survey of these organs was performed. However, no significant alterations were found (Kang et al., 2007). In addition, feeding brea gum did not alter the serum levels of

glucose, protein, or hemoglobin, indicating the normal functioning of the liver and kidneys (Kang et al., 2007). Moreover, our results are in agreement with a previous study that used karaya gum at levels up to 7% (Dikshith et al., 1984).

Conclusions

The observations of this preliminary investigation are in agreement with earlier toxicological studies on several varieties of gums, such as arabic, tragacanth, and karaya gum in experimental animals. In the present study, feeding brea gum from *Pakinsonia praecox* in BALB/c mice did not result in any adverse effects on feed consumption and utilization or clinical conditions, as revealed by hematology, blood chemistry, liver enzymatic profile, organ mass, and histopathological examination, suggesting that the addition of brea gum to foods, up to a percentage of 5%, may be a potential food additive for human consumption, once further studies are completed.

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