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EVALUATION OF THE ACCEPTANCE OF HERBAL ADAPTOGEN BASED FUNCTIONAL FOOD FOR DOGS

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ABSTRACT

Herbal supplements in diet are one of the Indian traditional practices which are followed since ancient times. *Ocimum sanctum* and *Withaniasomnifera* are well known for their adaptogenic properties to combat stress. But specific documentation on the use of herbal biscuits for dogs was not reported earlier. In this study, the herbal biscuit were prepared using an equal blend of *Ocimum sanctum* and *Withaniasomnifera* powdered extract and acceptance by dogs was assessed. The biscuits were prepared with wheat flour, minced chicken muscle, eggs and minced mutton, an equal blend of *Ocimum sanctum* and *Withaniasomnifera* powdered extract and a pinch of baking soda was added and the mixture was kneaded until the dough became soft. Using dog shape cutter the flour was divided and cut into the required shape and it was baked without using oven at low flame. The colour, crispiness and the flavour of the biscuit was retained and attracted thedogs subjected for analysis. Since the biscuit was made with herbal incorporation the dogs would gain the herbal benefits of both *Ocimum sanctum* and *Withaniasomnifera*, as we all know that these herbspossess analgesic as well as antipyretic and anti-inflammatory activities. The phytoconstituents also can protect lens of the eye; possess radioprotective activity and anti-tumor activities. Before the palatability assessment the herbal extracts were tested for their effect on canine blood mononuclear cells and the designed pet food palatability was measured using a single-bowl and a two-bowl test. While these tests give a general understanding of the liking or preference of one food over another, opportunities exist for further method development.

KEYWORDS: herbs, canine blood mononuclear cells, palatability, acceptance.

INTRODUCTION

*Ocimum sp.*is one of the nature's gifts with proven health benefits. Ocimum belongs to the family Lamiaceae, there are two varieties namely black (Krishna Tulsi) and green (Rama Tulsi). The chemical constituents of both the plants are similar.[1] The leaves of tulsi plants exhibit both insecticidal and antibacterial activities.[2] The holy basil is considered as one of the most important source of medicine and many herbal medicines are derived from this plant. The essential oils are used for the treatment of malaria, diarrhoea, bronchial asthma, dysentery, bronchitis, skin diseases, arthritis, painful eye disease, chronic fever and eye diseases etc.[3,4] *Ocimumsanctum* also shows anticancerous, antifungal, antimicrobial, hepatoprotective, antispasmodic, cardioprotective, antiemetic, antidiabitic, analgesic, adaptogenic, and diaphoretic properties.[5,6,7]

Ashwagandha (*Withaniasomnifera*, fam. Solanaceae) is commonly known as "Indian Winter cherry" or "Indian Ginseng"a plant used in the Indian traditional medicine since ancient period. Withaferin-A, have been shown to have significant anti-stress activity against acute models of experimental stress.[8] Ashwagandha powder can be mixed with water, ghee (clarified butter) or honey. Milk supplemented with ashwagandha has been reported to increase the total proteins and body weight.[9] It enhances the function of the brain and nervous system and improves the memory power. Being a powerful adaptogen, it enhances the body's resilience to stress. It also possesses potent antioxidant properties that help protect against cellular damage caused by free radicals. Ashwagandha is used as a household remedy for old people and children, and as aphrodisiac by young people.

Palatability assessments in Pets

Palatability is the important criteria in determining the success or failure of a pet food in the market. Research is conducted to improve the animal foods to evaluate palatability. It is difficult to evaluate whether the animal liked the product or not. This notion of "liking" is important in animal food industry.[10] Single bowl test and two bowl test was conducted to evaluate the acceptance of the product.[10] The herbal incorporation in animal food wouldenhance their natural immune status and well-being.

The Single-Bowl Test

The benefit to this test is that it mimics in many ways where the animal is served one food for a meal i.e the animal does not have a choice. This test provides a measure or inference regarding "acceptance" but does not yield information about the preference, degree of liking, or any other hedonic aspects of the food. Any breed of animals with varying sizes can be used. This monadic test measures daily intake of the test food, and moreover it is a good indicator of acceptance. This method can be further developed and include other indicators of liking to this test. The indicators include heart rate, pupil dilation, respiration rate, activity level, body movements, eating rate, etc. There were reportson facial indicators of food acceptance and the rejection by the cats.[11]

The Two-Bowl Test

This method is one of the logical methods to develop new product which also improves one product over another. In this method two foods are placed in bowl A and bowl B and the animal is given choice in this test when compared to single bowl test. The animal is allowed to smell the food kept in both the bowls. The animal will be continuously monitored to find which of the foods have been approached first. The amount of food offered for the animal in both the bowls should be same and the animal should consume the food within a given period of time, commonly 15-30 mins. The amount of food consumed by the animal is monitored. Thus the possible results are preference for bowl A, bowl B neither, or a portion from both.

The animals which are selected for the tests should be healthy. Aggressive animals should be separated from the other animals from the environment. Large or small animal from a popular or rare breed can be used for the palatability. For eg: Labrodor, Retrievers may have the ability to differentiate the food in bowl so they can be effective in a two-bowl palatability test. There is no evidence exist to suggest that a breed has the ability to differentiate one food over other, but it stands to the type of breed to which the food is served.[12] Human sensory analysis technique can provide some insight into the taste, aroma, and textural context of pet foods.[13,14,15]

MATERIAL AND METHODS

Materials

Wheat, minced chicken muscle, minced goat meat, eggs, baking soda were purchased from the local market of Chennai (Tamil Nadu), India. Dog cutters were used to cut the dough in varying shapes and sizes.

Medicinal herb

Dried powder of *Ocimum sanctum* and *Withania somnifera* were purchased from ISO certified herbal supplier and herbal drug authentication was done by Dr. S. Tamilalagan. They were stored in air-tight polythene packets for further use and analysis.

Preparation of herbal water extract

5g of each powdered sample was extracted using spent water after boiling chicken meatby a 0.44µmmillipore filter followed by centrifugation (1600 rpm; 15 min; 40°C). The supernatant was harvested and refrigerated and used in the preparation of herbal treats. The lyophilized herbal extract (25, 50, 75 and 100µg/ml) was checked for their effect on viabilityof canine blood mononuclear cells.[17] Briefly, Peripheral blood (12 mL) was obtained from a total of six healthy domesticated dogs with consent from their owners that were selected for one / two bowl test analysis. The blood was collected in 5 mL tubes with sodium heparin. Immediately after collection blood was diluted in 1 volume of PBS and mixed thoroughly. 3 mL of Ficoll-Paque media was added to the centrifuge tube. Diluted blood sample (4 mL) was layered onto the Ficoll-Paque solution carefully without mixing. The monolayer fraction was harvested after a density gradient centrifugation of 20 min at 1600 g and rinsed twice. The cells pellet was re-suspended by gently drawing them in and out of a pipette and centrifuged at 500 × g for 10 min at 20°C. The cell pellet was re-suspended in culture medium (8 mL RPMI 1640, 5% FBS with L- glutamine, 1% Antibiotic) and incubated for 48 hours with intermittent shaking under 5% CO_2 at 37°C. The cells ($1x10^6$ cells) were subjected to a synergistic herbal concentration of 100 µg/mL upto 96 hours.

Preparation of the herbal dog treats

The dough was prepared by adding sifted wheat flour, minced chicken muscle, minced goat meat a pair of eggs, a pinch of baking soda, Herbal extracts were added with required amount of water and mixed together in a planetary mixer and kneaded well. The dough was cut using a dog cutter into the required shapes and was kept in a greased plate. The dog treats were prepared without oven in gas stove. It was prepared by using a wide mouthed saute pan or skillet with a slightly deeper centre allowing easy evaporation of the moisture. Sand was filled and heated. Agreased perforated plate was kept above the sand. The prepared plate of treats was kept above it and closed with the lid. It was cooked for 40 mins under low flame. The treats were cooked well untilturns golden brown colour on both sides. They were cooled and packed in polythene containers and were stored.

RESULT AND DISCUSSION

The dogs were given herbal treats with a combination of adaptogenic herbs such as *Ocimum sanctum* and *Withania somnifera* which are proved potent anti-stressors. The dogs were evaluated on the basis of standard assay techniques namely, single bowl test and two bowl test shown in (Fig 1) which resulted that dog accepted two pan or force choice preference test. The herbal incorporation did not show any deleterious effect on viability (Table 1).

The process of assessing the safety of pet food ingredients is complex. *In vitro* screening methods are consistent to reduce, refine, and replace animal testing with *in vitro* alternative approaches that are scientificallyproven and provide solid signals for selecting pet food ingredients that are safe to target species. *In vitro* methods are the most obvious choice, especially when multiple target-organ cell lines are selected to produce an integrated panel that uses a range of cells possessing different physiological functions. *In vitro* studies provide a basis for comparing cytotoxicity data across species. They can be used to predict *in vivo* toxicity only if independent exposure and pharmacokinetic disposition and metabolism studies are also available.[16, 17] A recent National Research Council[18] study explored how the safety of dietary substances should be assessed in horses, dogs, and cats. A common misconception is that products widely found in human food (e.g., garlic and chocolate) are assumed to be safe for pets. In contrast, the major conclusion of the NRC report was that safety in humans or any one species does not guarantee safety in animals.[18] Such studies serve as a bridge to similar *in vitro* cytotoxicity evaluations conducted in other laboratory species or humans. In the present study, it has been shown that the blood mononuclear cells treated with the selected adaptogenic herbs were not toxic and there were no significant adverse effects on the liveability of cultured cells during the observation period. Supporting our results the administration of a 75 per cent methanolic extract of the *Withania somnifera* was found to significantly increase the total white blood cell (WBC) count in normal Balb/c mice and reduce the leucopoenia induced by sublethal dose of gamma radiation (γ-GR).[19] Major activity of *Withania somnifera* seemed to be in the stimulation of stem cell proliferation.

Supporting our findings, herbs were checked for the cytotoxicity assessment in 4 canine cell types to 20 different food components provided a baseline that begin to illustrate how such an *in vitro* panel could be used for hazard assessment.[16]

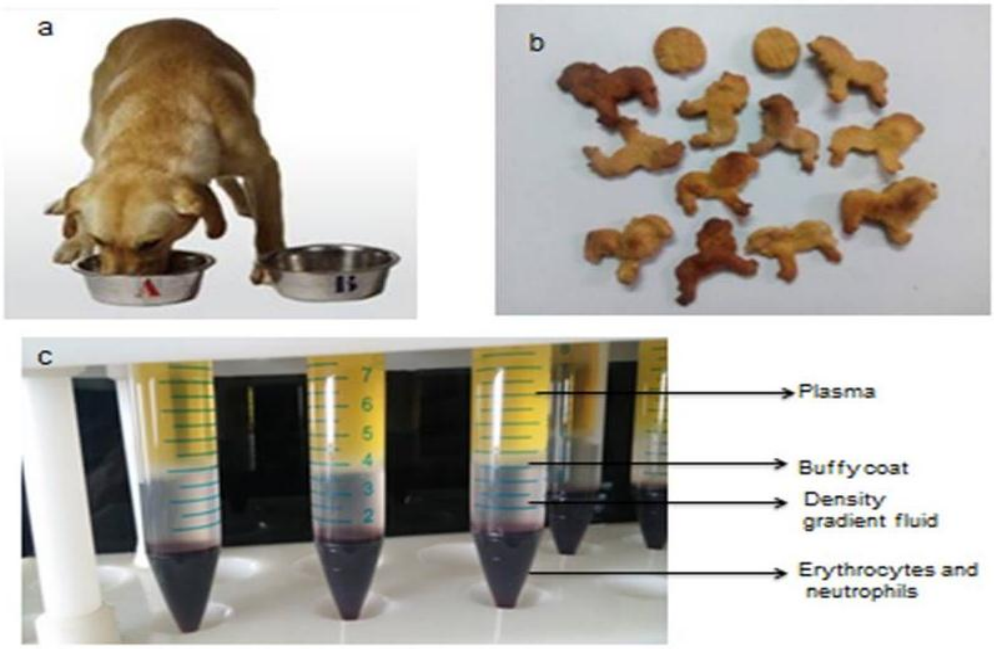

Fig 1: Dog treats with the combination of the herbs and palatability assessment.

Table 1: Cell viability of herbal treatment in dog blood derived cells.

Dog blood progenitors	Cells without herbal treatment			Cells with herbal treatment *(Ocimum sanctum and Withaniasomnifera)* at 100µg/ml		
Hours	**Total cell count**	**Live cell concentration**	**Cell viability**	**Total cell count**	**Live cell concentration**	**Cell viability**
0	$105\pm0.05x10^6$	$45\pm0.01\ x10^6$	87.14%	$105\pm0.01x10^6$	$88.25\pm0.05\ x10^6$	88.92%
24	$94.5\pm0.01x10^6$	$39.75\pm0.04\ x10^6$	83.27%	$100\pm0.05x10^6$	$72.75\pm0.04\ x10^6$	85.45%
48	$50.5\pm0.03x10^6$	$20.75\pm0.07\ x10^6$	79.14%	$88.4\pm0.03x10^6$	$65.25\pm0.01\ x10^6$	82.58%
72	$52\pm0.00x10^6$	$20.25\pm0.02\ x10^6$	74.87%	$75.5\pm0.05x10^6$	$58.75\pm0.01\ x10^6$	79.66%
96	$44.5\pm0.06x10^6$	$15.75\pm0.01\ x10^6$	70.16%	$70.2\pm0.02x10^6$	$50.25\pm0.05\ x10^6$	75.29%

CONCLUSION

In the present palatability assesement of dog treat revealed the acceptance of herbal incorporation by dogs and that the selected adaptogenic herbs *Withaniasomnifera*and *Ocimum sanctum* showed no toxicity effects to dogs. Blood mononuclear cells with herbal fortification found to have more proliferation compared to the control and fulfils the recommendations of NRC on the testing the dietary ingredients in target species.

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