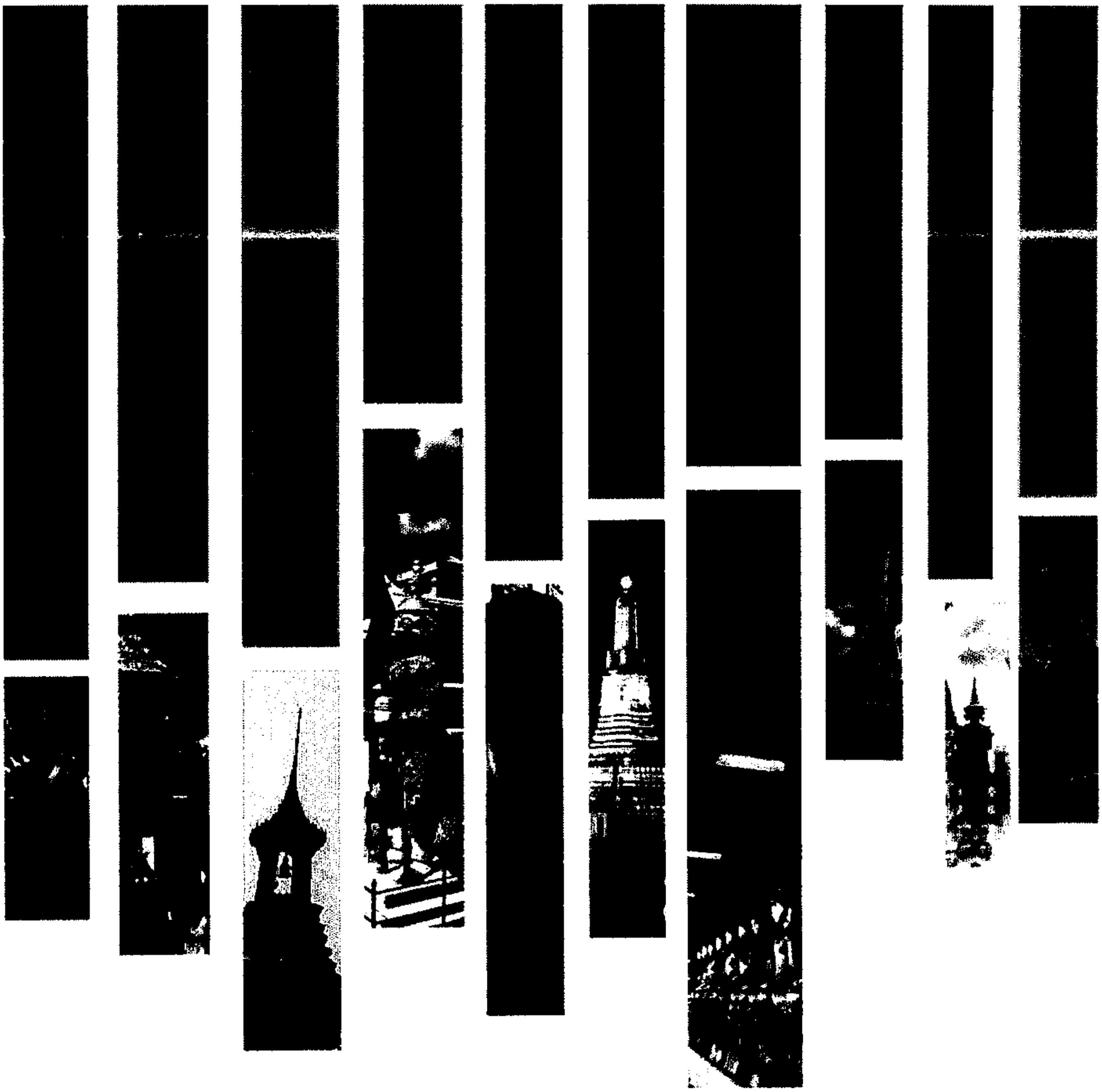

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Laxative Potency of Methanolic Extract of Sea Cucumber (*Holothuria leucospilota*) : Avian Model (pigeons, *Columbia livia*)

J. Rofeta[a], K. Christi[b,*] and S. Sotheeswaran[c]
[a] **Solomons Education Board**, Honiara, Solomon Islands
Email: johnrofeta@gmail.com

[b] **Biological Sciences, School of Biological and Chemical Sciences, Faculty of Science, Technology and Environment The University of the South Pacific,**
Suva, Fiji Islands
Email: christi_k@usp.ac.fj

[c] **School of Biological and Chemical Sciences Faculty of Science, Technology and Environment The University of the South Pacific**, Suva, Fiji Islands
Email: sotheeswaran@hotmail.com

ABSTRACT
Marine organisms produce many secondary metabolites, which are commonly referred to as natural products. These organisms often exhibit unique chemical responses when they are under stress. Many of these chemicals may have medicinal applications. In this research work methanolic extract from the body wall of holothurian (*Holothuria leucospilota*) was studied *in vivo* to test its effect on passage rate of digesta using avian model. The effect of sea cucumber solvent extract (SCSE) was compared with a known laxative drug (bisacodyl) in 36 feral pigeons fed with various doses (5mg, 20mg, and 40mg) of the extract. Cloaca excretions were collected after administration of the treatments on hourly intervals for initial 6hrs, six hourly intervals for 42hrs and 12hrly interval for 102hrs. Observations on moisture content (%) and chromic oxide recovery (mg) were recorded from these samples. These parameters were used to determine the passage rate (mg/hr) of digesta along the gut for 102 hrs. Mean moisture content for birds fed with 40mg and known drug was relatively higher to other treatments. Results revealed a stimulatory effect of extract on the passage rate of digesta dry matter (mg/hr) and chromic oxide recovery rate (99% and 96%). SCSE of 40mg dose was comparable to the laxative drug. The results indicated that the holothurian extract has a laxative potency.

Keywords: Holothurian extract, laxative, moisture content, chromic oxide recovery, avian model

1. INTRODUCTION

People of the Pacific Islands mainly use the marine resources as a source of food. Their use as source for pharmaceuticals, cosmetics, molecular probes, fine chemicals and agrochemical has not been realized. For the new drug discovery, scientists are exploring marine organism as a huge potential source of drugs. Marine organisms produce many secondary metabolites, which are commonly referred to as natural products. They often exhibit unique chemical responses when they are under stress, disturbed or attacked. Many of these chemicals may have medicinal applications. For example, the chemicals that corals produce to protect them from UV exposure may soon be used in sunscreen protections (Dunlap [1], Rajeev [2], Smirov [3]). The slow moving organisms such as sea cucumber, sea star, sea urchin of Echinodermata have survived successfully in the hostile ocean, amongst many predators. *Holothurians* are sea cucumbers consisting of 1200 species that inhibit a greater number of different habitats than any other group of echinoderms (Allen [4]). *Holothurians* pose wide variety of defense mechanisms in responses to predation. Such defense includes having thick body wall and release of toxic chemicals (Smiley [5], Lawrence [6]). The chemistry of these compounds has been of interest to scientist. This, interest has prompted numerous studies on marine organism to elucidate drugs that may be useful as pharmaceutical. *Holothuria* extracts have been studied (Sotheeswaran [7]) in the Department of Chemistry at USP, Fiji and were shown to exhibit the property of heamolysis in red blood cell. This property is due to the presence of a class of natural product called saponins. This extracts also have molluscidial activity to kill molluscs, which harbour schistosome larvae. Studies of extracts from *holothuria* indicated a range of sterol sulphates (Takada [8], Kita [9). The challenge now is to undertake animal research on the extract of marine organisms to ascertain their usefulness as bioactive drugs that one-day may be found on the shelves of pharmacies. As a natural commercial commodity sea cucumber is sold in Fish market. It has been consumed and used in used in numerous ways by humans in Pacific Island countries including Fiji, however, little studies have been carried out scientifically to investigate its bioactivity on the digestive system. In this study SCSE is fed to pigeons (*Columbia livia*) to determine its comparable laxative effect on the rate of passage of digesta.

2. MAIN BODY

2.1 Materials and methods:

The live *Holothuria* samples were hand-picked in the Suva, Republic of Fiji, reef flat at low tide and placed in bucket filled with seawater. The samples were identified as *Holothuria leucospilota* by Mr. Johnson Seeto, the Curator in the School of Marine studies at the University of the South Pacific. Methanol: water solvent (80:20 v/v) extract was prepared from *H. lecospilota* (n=50) body wall as per (Groweiss [10]). The total SCSE obtained during the extraction process was 15.0g.

For *in vivo* study adult feral pigeons (*Columbia livia*) were captured locally, housed in well ventilated animal house at the Biological Sciences, School of Biological and Chemical Sciences, Faculty of Science and Technology. The pigeons were housed in individual wooden, and wire netting cages (0.5m x 0.29m x 0.31m) fitted with removable hard plastic sheet beneath the cage floor. This size of the cage allowed the birds to move freely, and extend their wings. Weather [11] suggested that for physiological studies, it is important to have enough space for the bird to move and exercise. Standard animal management practices for bird maintenance and the guidelines of the Faculty Animal Ethics approval were followed throughout the experiment. After two weeks of adaptation, an *in vivo* experiments were conducted using the experimental design consisted of six treatments as described in Table-1.

Table 1: *In vivo* experimental treatment regime (n = 6 birds in each treatment group)

Treatment groups	**Treatment regime**
T_1	5mg of SCSE + 50mg chromic oxide + 20g grower ration
T_2	20mg of SCSE + 50mg chromic oxide + 20g grower ration
T_3	40mg of SCSE + 50mg chromic oxide + 20g grower ration
T_4 (control)	50mg chromic oxide + 20g grower ration
T_5 (known laxative)	5mg bisacodyl + 50mg chromic oxide + 20g grower ration (Bisacodyl from Xierkang Pharmaceutical Co., Ltd, China)
T_6 (blank)	20g grower ration only

Before trials, birds were off fed for overnight.

Chromic oxide used as a marker was an inert compound and it did not absorbed nor interfere with the digestive process in the gut (Shipton [12]). Excreta were quantitatively collected on removable white paper towel placed on hard plastic sheets beneath the cage floor. The samples were collected (after administration of the treatments) at hourly interval for 6 hours, 6 hourly for 36 hours and then 12 hourly intervals up to 102 hours. Each batch of stool was recorded for moisture content (%) and then dry matter (%) were determined by oven drying at 65°C to the constant weight. Color and consistency of the excreta were also recoded. Dried stool sample were ashed at 600°C for five hours and analysed for chromic oxide (mg) recovery by colorimeter method at 540nm based on the procedure by Garcia [13], Noozifar

[14]. In all, 17 samples were collected from each bird. One-way ANOVA (SPSS) was used for statistical analysis of the data.

2.2 Results and Discussion

The birds were ingesting all combination of treatments equally. Grossly there was no adverse effect (morbidity and mortality) recorded in birds after ingestion- overall behavior, food and water consumption, stool abnormality such blood tinged excreta.

The moisture content (%) for all treatments has been compared in Fig 1. In the initial 6hrs period values did not differ significantly (p=0.07). During subsequent period, birds offered with 40mg SCSE (T_3) and bisacodyl (T_5) revealed significantly higher (F=2.801; $p \leq 0.05$) percentage. Observed stimulatory effect remained of shorter duration (12-18hrs) with higher moisture (91-93%) as compared to known laxative (81-85% between 12-30hrs).

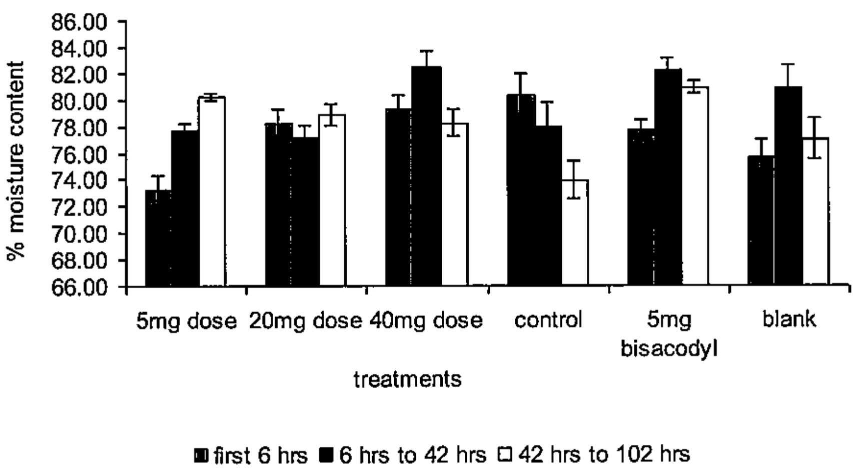

Fig 1: Effect of SCSE on stool moisture (%) in birds during the experiment

Results suggested that the 40mg SCSE and bisacodyl could have mobilized water excretion in the gut at this stage. Laxative drugs such as bisacodyl have been shown to interfere with enzyme systems involved in ion transport, which increases the concentration of the intestinal fluid, and lead to an osmotic effect. Some may directly prevent water re-absorption in the colon and may even promote water excretion directly from the intestinal cells to the lumen (Galbraith [15]). Therefore, high concentration of SCSE in the gut of the birds might be responsible for the low water absorption and subsequently increase excreta moisture (%) comparable to bisacodyl. Other studies have shown that stimulant laxative such as the bisacodyl can directly affect the walls of either the small or large intestine and cause an increase in the peristaltic movement of the digesta. The mode of action is within 6 to 24hrs after the administration of the drugs. Other laxative drugs such as senna may simply irritate the smooth muscle cells of the gut leading to defecation reflex arc (Mascolo [16]). Chen [17] reported sea cucumbers use in the treatment of constipation in China. Constipation is slow passage of bowel movement along the gut. Hard and dry stools of constipation occur, when the colon absorbs too much water or there is low water intake. This happens because the colon's muscle contractions are slow or sluggish, causing the stool to move through the colon too slowly.

Passage rate of dry matter (mg/hr) was high for birds fed with 5mg of SCSE (T_1) and the least was observed for blank for the first 6 hours of ingestion (457.21 ± 0.12(T_1), 401.12 ± 0.43(T_2), 423.16 ± 0.56 (T_3), 340.78 ± 0.87(T_4), 432.76 ± 0.78(T_5) and 245.23 ± 0.34 (T_6)). However, the difference with other treatments was non-significant (p=0.08). Between 6-42hrs the rate declined for T_1 and T_2, while it increased significantly ($_p$≤0.05) for T_3 and T_5 (Fig2). This suggests that the 40mg SCSE (T3) and 5mg bisacodyl (T5) may have affected the movement of faeces transit along the bird's gut during this time period. However, the rate declined over the last phase (42-102hrs), where the rate of all the treatments recorded to be similar.

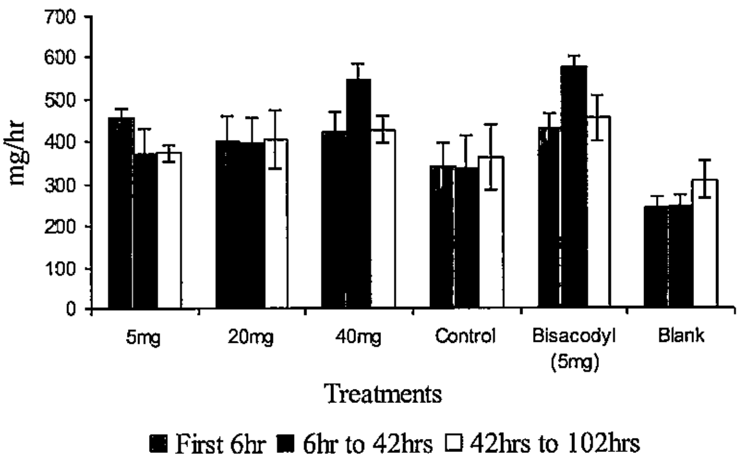

Fig 2: Effect of SCSE on chromium oxide passage rate in birds during the experiment

The recovery of chromic oxide (mg) recovered for all treatments varied in excreta collected during the first 6hrs (Table 2). There was significantly (p≤0.05) higher recovery rate at 6, 12, 30 and 36hrs from birds treated with 40mg SCES (T_3) and at 6 and 12hrs for bisacodyl (T_5). The excretion rate of chromic oxide from all treatments reduced below 50% after 42hrs. Overall chromic oxide recovery were 99% and 96% for T_3 (40mg SCSE) and T_5 (laxative drug). This may suggest that the flow of digesta along the gut in the treatments (40mg and bisacodyl) were relatively faster than 5mg and 20mg dose. Studies in dogs (Hills [18]) have shown that chromium oxide recovery is almost complete. Therefore, original amount of chromic oxide taken up with the feed was expected to be recovered in the excreta. Study carried out by Williams [19] in rabbits showed that 99.9% of chromic oxide was recovered in stool, when it was used as a marker. The rate of digesta during this period 0-42hr after the administration of the dose correlates with the literature (Galbraith [15] where the onset of action for bisacodyl was 6 to 24hrs after its administration. However, after 42hrs, the rate subsides. This suggests that, the optimum action of bisacodyl is between 6-42hrs. Similar results were observed for 40mg (T_3) of SCSE, where the chromium passage rate along the gut was also high between 12-42hrs (Fig 2).

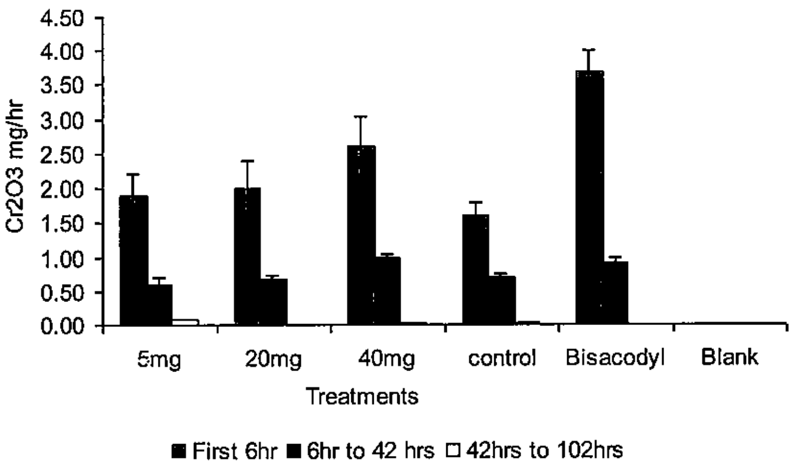

Fig.2: Effect of SCSE on chromium oxide passage rate in birds during the experiment

Table 2: Recovered chromic oxide (mg, mean ±SE) during the experiment

Time interval	Hrs after feeding	SCSE doses 5mg (T_1)	20mg (T_2)	40mg (T_3)	Control (T_4)	Bisacodyl (5mg) (T_5)
Hourly	1	1.5±0.23	1.3±0.15	1.4±0.38	0.9±0.09	1.8±0.01
	2	2.2±0.11	1.7±0.49	1.7±0.58	1.0±0.15	2.3±0.02
	3	2.4±0.38	2.2±0.27	1.0±0.58	1.0±0.02	2.7±0.00
	4	2.9±0 .22	2.8±0.21	2.3±0.58	1.4±0.11	3.0±3.30
	5	3.1±0.11	3.9±0.91	2.8±0.27	2.2±0.08	3.8±0.27
	6	2.6±0.31	3.4±0.91	*4.6±0.23	3.0±0.17	*8.5±0.23
6 Hourly	12	3.5±0.33	3.8±0.85	*6.9±0.24	4.0±0.29	*7.8±0.58
	18	4.4±0.44	5.3±0.45	4.5±1.31	5.1±0.10	4.0±0.11
	24	6.6±0.63	6.7±1.85	4.5±2.55	6.7±0.44	5.6±0.05
	30	3.6±0.56	3.8±0.24	*6.2±1.09	5.6±1.10	3.5±0.70
	36	5.2±0.20	3.3±0.91	*7.1±1.50	3.9±0.59	4.1±0.10
	42	0.9±0.12	1.4±0.32	4.5±1.90	2.2±0.35	0.9±0.02
12 Horly	54	0.3±0.11	0.1±0.09	1.8±1.57	1.0±0.27	0.2±0.00
	66	0±0	0±0	0±0	0.2±0.02	0±0
	78	0±0	0±0	0±0	0.1±0.06	0±0
	90	0±0	0±0	0±0	0±0	0±0
	102	0±0	0±0	0±0	0±0	0±0
Overall recovery		78%	79%	99%	77%	96%

Note: * significant at 5%; blank (T6) is not included

This study showed laxative effect of sea cucumber (*H. leucospilota*) solvent extract in the birds. This product could be developed for its application in veterinary medicine. Further studies would also be carried out to other holothurian species as well as using mammalian as model.

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