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Monitoring *Agromyza* sp. (Diptera: Agromyzidae) Wheat Leafminer with Yellow Sticky Trap

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Abstract. Yellow sticky boards used as traps for early season monitoring of *Agromyza* wheat leafminer (WLM) adults were promising in qualitative assessment of adult populations. The technique is useful in early detection of pest infestations before damage is produced, particularly in the case of *Agromyza* WLM which dominates only in the wheat seedling stage.

Introduction

Previous reports [1, 2] emphasized the importance of *Agromyza* wheat leafminer (WLM) as a new serious pest of wheat in Qassim. These reports also pointed to the fact that this pest might constitute a threat to wheat production in the area due to the noticeably escalating numbers each year since it was first reported in 1986. We attributed this increase to observations on the nature of the leafminer which pupates in the soil, the probability of its over-summering in the soil in the same field and the absence of a crop rotation. Losses inflicted by *Agromyza* WLM to wheat seedlings [1] make early detection on crop germination, and continued monitoring throughout the seedling stage of utmost importance. The habits of this WLM particularly its long pupal resting period in wheat fields from previous season, and the sudden adult colonization of the new crop, calls for quick sampling procedures. Counting live larvae in leaves is laborious and time consuming because usually by the time when mines are visible, 10-20% of the crop leaves were already infested [2]. Sweeping through wheat fields, though an alternative method, does not accurately reflect the actual densities of *Agromyza* eggs and larvae present. Because in addition to being time consuming,

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it poses difficulties and is practically unsuitable with the pivot irrigation system, most common in Saudi Arabia.

Studies made by Musgrave *et al.* [3], Affeldt *et al.* [4], Tyron *et al.* [5], Chandler [6, 7], Parrella & Jones [8], and Zehnder and Trumble [9], confirmed that adult leafminers in the genus *Liriomyza* are more attracted to yellow than to other colours, and the high reflectance throughout the yellow part of the spectrum increases adult catch in yellow sticky traps.

Trapping was found to be an easy and rapid method for detecting adult leafminer population increases [3], and the technique has been suggested for use in attracting *Liriomyza* adults for population monitoring. Chandler [7] found that maximum adult catch was obtained when yellow traps were placed closer to ground level (30 cm) than at higher levels. He also found that *Liriomyza* adult activity was diurnal, and that both sexes are equally responsive to yellow traps.

In preliminary trials in Qassim wheat fields, *Agromyza* WLM was found responsive to yellow traps. Therefore, a study was initiated to investigate the possibility of utilizing this technique for development of reliable sampling methods for population density estimates, early detection of adult emergence before damage is manifested and as adult emergence traps to give more insights about places of pest aestivation, and source of infestation to the new crop.

Materials and Methods

Four wheat fields and two uncultivated baren areas in Qassim region were selected as test sites. These fields were known to be endemic areas for high WLM populations in previous seasons. Yellow sticky boards (35 × 26 cm) were placed at 20 cm above ground level one week before wheat planting and during crop emergence. The yellow colour used was spruce 98 – 34, sun yellow enamel, from Seymour of Sycamore, Inc. Illinois, U.S.A. The sticky matrerial was Temo Bi, non poisonous glue for trapping rats, from Kollant Industries Chemiche S.P.A., Italy.

Six traps were installed along the margins of each wheat field (Fig. 1), to avoid washing the trap by pivot sprinkler system inside fields.

One wheat field was also chosen as a permanent sampling area throughout the season where WLM adults were monitored on weekly basis by placing the yellow sticky traps at the beginning and removing them at the end of each week. Number of *Agromyza* adults trapped was counted and recorded weekly. Attempts to estimate

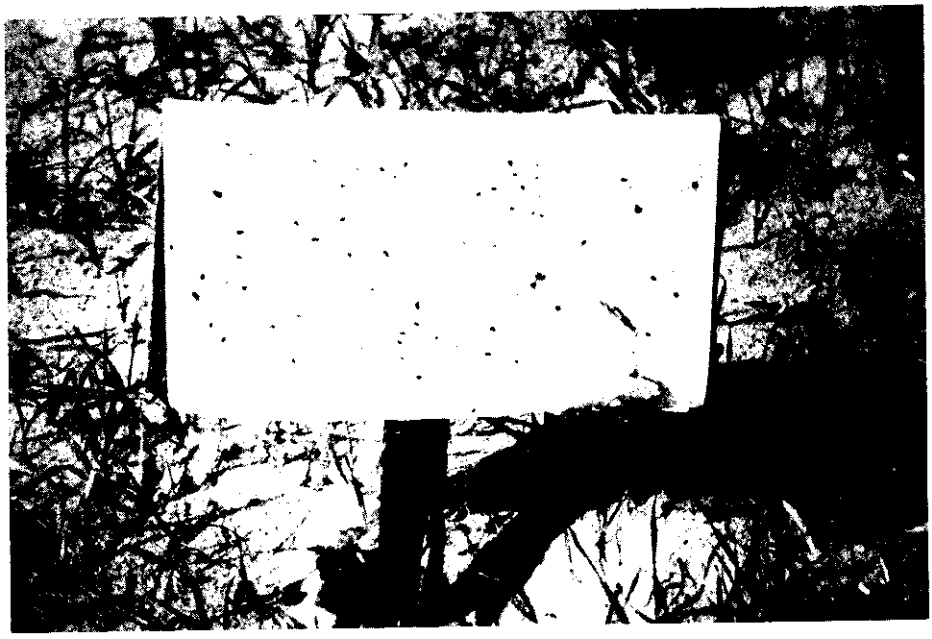

Fig. 1. A close up of yellow sticky trap showing trapped insects including *Agromyza* adults

percentage of infestation in the fields were made by due to the freezing morning temperatures the eggs deposited in wheat leaves did not develop, and thus the infestation level was very low and unrepresentative.

Results and Discussion

Preliminary trials indicated that *Agromyza* WLM adults responded positively to the yellow colour as has been observed by the high ratio of adults trapped on the yellow sticky boards (Fig. 2).

It was clear that the catch on those boards placed inside wheat fields was considerable compared to that obtained in barren areas, even though wheat plants have not emerged yet. This fact strongly indicates that *Agromyza* adults emerge from within wheat field soil and not elsewhere. Field to field migrations are minimum as indicated by the very low catch recovered from comparable traps placed in baren, uncultivated soils. These findings suggest that aestivation of this pest most likely takes place within the boundaries of the same field premises. This indicates that infestation to the new crop is initiated within field premises, then eventually infestation prolifer-

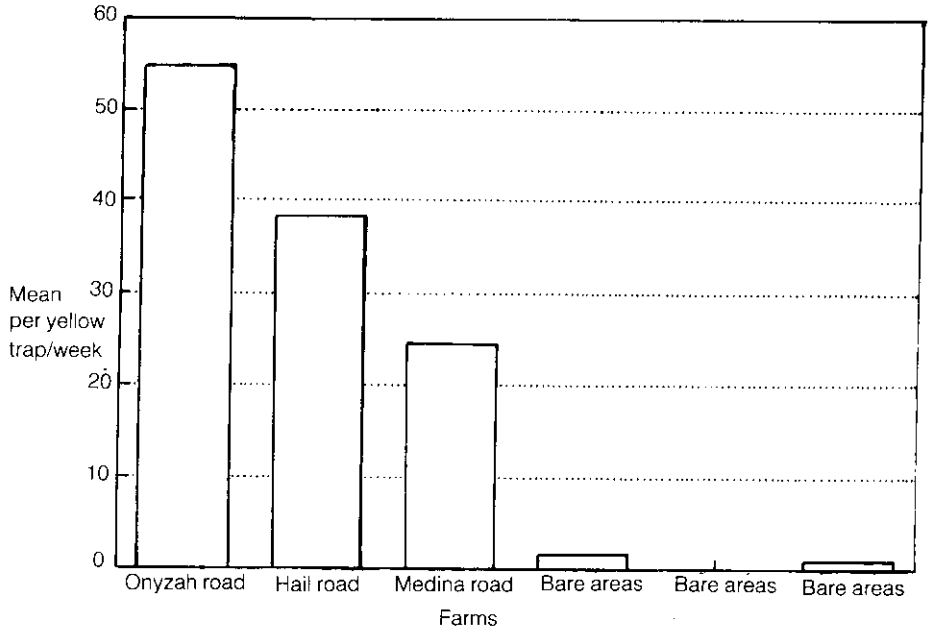

Fig. 2. Number of *Agromyza* wheat leafminer aduts trapped on yellow sticky traps

ates due to shear pest multiplication. This has lead to a high number of aestivating individuals which explains the overall vigorous manifestation of the problem year after year.

Table 1 shows a follow up of *Agromyza* adult population throughout the season in farm No. 45 on Onaizah Rd. A high turn out of adults was evident during the first month right after crop emergence; with the peak occurring during the third week after crop emergence. (*i.e.* between 4-16.1.89). Despite the high adult leafminer population in this farm, the actual leaf infestation was rated as low during late December, 88 and early January 89, but was rather negligible during January and Febraury. This was attributed to the occasionally freezing temperatures that prevailed during that period reaching -1 on Dec. 31, 88, -4 on Jan. 7, 89 and -5 on Feb. 6, 89. Females have apparently oviposited in wheat leaves, but the eggs and/or hatching larvae eventually died before further development. This was verified by dissecting and examining wheat leaf samples showing damaged tips. Most *Agromyza* larvae encountered during this period were found dead in their early developmental stages.

From Table 1 and Fig. 3 we can see that the heavy adult turn out continued until mid-February (end of seedling stage). One peak occurred in mid-January being perhaps a mixture of emerging adults (from aestivation) and first generation indi-

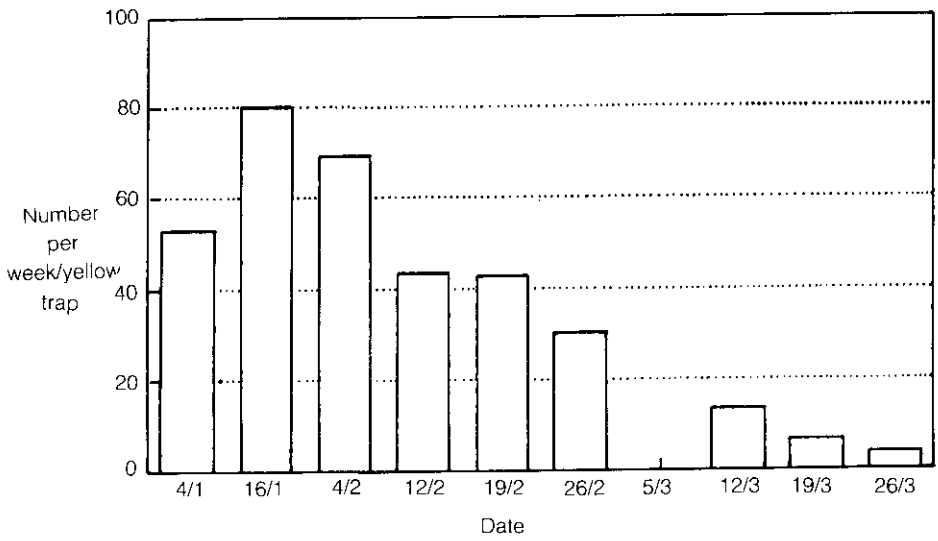

Fig. 3. Number of *Agromyza* adults trapped on yellow sticky traps during 1988/89 in Gassim (wheat field No. 45)

Table 1. Number of *Agromyza* adults trapped in yellow sticky traps during season 88/1989 in Qassim wheat field No. 45.

Trap	No. of *Agromza* adults trapped/week						$\bar{X} \pm$ S.D.
Date	1	2	3	4	5	6	
4.1.89	25	63	79	82	29	38	52.7+25.3
16.1.89	105	79	107	74	74	68	80.0+22.9
4.2.89	73	113	96	49	79	67	68.3+22.5
12.2.89	37	224	32	57	63	48	43.5+15.1
19.2.89	41	44	35	60	62	52	43.0+12.1
26.2.89	31	40	26	21	16	47	30.2+11.7
5.3.89	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	
12.3.89	12	18	11	14	10	15	13.3+02.9
19.3.89	7	13	4	8	5	2	6.5+ 3.8
26.3.89	3	2	2	5	4	6	3.7+ 1.6

N.S. = No sampled due to dust storm

viduals. Since the generation time of this pest is about one month, [1], it should be mentioned that the situation of *Agromyza* leafminer this season is rather confused and complicated by the abnormally cold weather that dominated during the seedling stage. This has resulted in very low infestation levels and delayed development, which is expected to result in lower populations next season.

Although, the yellow sticky trap technique seems to be efficient and promising for qualitative detection of adult populations, its usefulness in monitoring adult populations of *Agromyza* WLM populations before and at the onset of infestation (before damage is produced) is of particular interest because *Agromyza* wheat leafminer populaitons predominate only for a short period during the early part of the growing season. Of course, more detailed information and better understanding about behavior and aspects of ecology and biology of this pest are urgently needed and is achieved through increased basic research in this direciton for improved monitoring and management systems.

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استخدام المصائد الصفراء اللاصقة لمراقبة ظهور صانعة الأنفاق في أوراق القمح في الحقول (*Agromyza* sp., Diptera, Agromyzidae)

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ملخص البحث. تم استخدام اللوحات الصفراء اللاصقة كمصائد لبالغات حشرة صانعة الأنفاق في أوراق القمح (*Agromyza* sp.) التي تصيب القمح في القصيم، بعد أن ثبت أنها تنجذب نحو اللون الأصفر. اتضح أن هذا التكنيك وافٍ في المراقبة والتقويم الكيفي لكثافة البالغات في الحقل، كما أنه مفيد للإنذار المبكر عن وجود الآفة قبل ظهور أعراض الإصابة على النبات، خاصة في حالة صانعة الأنفاق والتي تسود فقط في طور البادرة.

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Clinicopathological and Toxicological Studies of Oral Administration of *Rhazya stricta* Extract "Harmal" in Rats

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Abstract. *Rhazya stricta* (Apocyanaceae) is abundantly found in Saudi Arabia and is used in traditional medicine for the treatment of rheumatism.

In this study, the lyophilized aqueous extract of the aerial parts of the plant was given orally to mature rats at a dose of 400 mg/kg body weight daily for a period of 8 weeks. This resulted in insignificant changes in the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, the serum levels of urea nitrogen, total proteins, albumin and inorganic phosphorus. There was, however, significant elevations ($P < 0.01 - P < 0.001$) in serum globulins, and serum calcium together with significant decreases ($P < 0.01$) in serum triglycerides and A/G ratio.

Treatment with the extract also resulted in a significant decrease ($P < 0.05 - P < 0.001$) in the values of RBCs and WBCs counts, Hb%, MCV and MCHC, together with a slight decrease in MCH. The extract was not lethal when given in doses up to 4.0 gm/kg and did not produce any gross or microscopic changes.

Introduction

Rhazya stricta (Apocyanaceae) is a small shrub widely disributed in Western Asia and abundantly found in Saudi Arabia. The leaves and roots of the plant produced more than fifty indole alkaloids, three of which had cytotoxic activity. These were vallesiachotamide [1], sewarine [2, P. 540] and tetrahydrosecamine [3]. Two more alkaloids stricticitine and strictine from the leaves of the plant were also isolated [4]. Moreover, robinin and two glycosides from the leaf extract of the plant were recently isolated by Andersen *et al.* [5].

Rhazya stricta is a bitter tonic and curative for choronic rheumatism [3, 6, P. 222, 7, P. 3911, 8]. The alcoholic extract of *R. stricta* showed a marked leucopenic effect in rats when given orally (20 mg/kg) and that a single i.p. injection of 15 mg/kg significantly reduced the white blood cell count in 7-10 days [9]. The anticancer activity of some of its alkaloids was also reported [10].

The present study was conducted to investigate the clinical and pathological effects of oral administration of the aqueous extract of the plant in rats.

Materials and Methods

The plant material was collected in April 1986 from Qassim area, Saudi Arabia. Its identity was verified by Prof. Hassan Moustafa Hassan, professor of Plant Taxonomy, College of Science, King Saud University, Riyadh, Saudi Arabia. The air dried aerial parts were extracted with hot water. The water filtrate was lyophilized using a labconco freeze dryer – 18 model 75018. The lyophilized aqueous extract was freshly prepared and used as 10% (W/V) solution in normal saline.

Adult male wistar rats weighing between 180-200 gm body weight (b. wt) were used. These were divided into two groups of twenty rats each and were fed on a standard pelleted diet with free access to water. One group was left as control, given normal saline only, whereas, the other group was orally dosed with the aqueous solution of the extract at a rate of 400 mg/kg. b.wt. daily for a period of 8 weeks. After the end of the experiment, all rats were sacrificed and ten whole blood and serum samples were obtained. Whole blood samples were collected in lithium heparinized plastic bottles for haemogram determination, while serum samples were collected in 10 dry and clean bottles and stored at –20°C until analysed.

Animals were dissected for post-mortem examination. Specimens from the liver, kidneys, spleen, intestine, heart and mesentric lymph nodes were fixed in 10% buffered formol saline solution and processed and stained with haematoxyline and eosin for microscopic examination [11, p. 209].

Erythrocytes (RBC) and leucocytes (WBC) counts were estimated using the double improved neubauer chamber [12]. Haemoglobin (Hb%) was estimated using the acid haematin method [13]. Packed cell volume (PCV) was determined in double capillary tube preparations using a microhaematocrit centrifuge, [14]. The formulae of Baker *et al.*, [15, pp. 558-560] were employed for calculations of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

A group of serum chemical variables were determined colorimetrically using commercial kits supplied by BioMerieux (France). These were:

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [16], total proteins [17], serum urea nitrogen [18], triglycerides and inorganic phos-

phate [19, pp. 675, 887], while serum Ca^{2+} was determined using the Boehringer Manheim Calcium kit following the method of Connerty and Briggs [20].

Serum protein fractionation was made by using 5 l samples from treated and control rats. These were applied to agarose gel slabs (5401-001 Hydragel protein, LKB-Sebia). Electrophoresis was run for 20 min. using LKB equipment and methods (Sebia 91130 Issy Les Moulineaux, France).

Following staining with amidoblack and destaining in 5% acetic acid, the electrophoretograms were scanned for the percentages of albumin, α, β and γ-globulins fractions and the A/G ratio in LKB-5300 "preference" densitometer programmed for protein analysis.

Toxic effects

The LD_{50} for the extract was determined in rats [21, p. 60]. Rats were given oral doses ranging between 400-4000 mg/kg.b.wt., and were kept under observation for 24 hr. during which symptoms of toxicity and mortality were recorded.

Statistical analysis

Data were statistically analysed using paired student "t" test [22].

Results

Table 1 shows that the daily oral administration of *Rhazya stricta* aqueous extract to mature rats in a dose of 400 mg/kg.b.wt. for 8 weeks produced an insignificant increase in the activity of serum ALT, AST and serum urea concentration. There was however, a significant increase ($P < 0.01$-$P < 0.001$) in serum calcium α, β and γ-globulins together with a significant decrease in serum triglycerides, and A/G ratio. A slight decrease in serum total proteins, albumin and inorganic phosphorus were also observed. Table 2 shows that total RBCs, WBCs counts, Hb%, PCV, MCV and MCHC were significantly decreased ($P < 0.05$-$p < 0.001$) as a result of treatment with the plant extract, however, MCH is slightly decreased.

Toxic effects

The LD_{50} experiment revealed that *Rhazya stricta* aqueous extract in doses ranging from 400-4000 mg/kg.b.wt. of rats produced no deaths or even toxic symptoms.

Gross and histopathological findings

No post-mortem or histopathological changes or lesions were found.

Table 1. Effect of oral administration (Means ± SE) of an aqueous extract from, *Rhazya stricta* to rats at a dose rate of 400.0 mg/kg.b.wt. daily for a period of 8 weeks.

Variables	Control n = 10	Treated n = 10	Variables	Control n = 10	Treated n = 10
ALT (unit/ml)	158.5 ± 9.67	164.8 ±16.3	Total proteins (gm/l)	71.7 ±4.88	70.26 ±2.5
AST (unit/ml.)	283 ± 6.82	293.4 ± 7.85	Albumin (gm/l)	46.6 ±1.06	42.13 ±3.0
Urea Nitrogen (mg/l)	397.6 ±10.48	410.5 ±19.74	α-globulins (gm/l)	15.87 ±0.15	16.8 ±0.23 **
Triglycerides (gm/l)	63.0 ±5.95	33.0 ± 2.93 ***	β-globulins (gm/l)	5.86 ±0.05	7.17 ±0.14 ***
Calcium (mg/100 ml)	14.62 10.6	18.6 ± 0.83 **	γ-globulins (gm/l)	2.71 ±0.015	3.85 ±0.066 **
Inorganic phosph (mg/100 ml.)	20.22 ± 1.67	19.12 ± 0.83	A/G ratio	1.9 ±0.035	1.5 ±0.12 **

** $P < 0.01$

*** $P < 0.001$

Table 2. Effect of oral administration (Means ± S.E.) of an aqueous extract from *Rhazya stricta* to rats given daily in doses of 400 mg/kg. b.wt. for 8 weeks on blood characteristics.

Group	RBCs $\times 10^{-6}$	WBCs $\times 10^{-3}$	Hb %	PCV %	MCV Cu mm	MCHC %	MCH u ug
Control n = 10	5.702 ± 0.158	7.3 ± 0.084	9.44 ± 1.13	40.0 ± 2.9	23.5 ± 1.93	77.9 ± 7.43	20.14 ± 1.55
Treated	4.758 ± 0.316 *	5.63 ± 0.482 **	6.24 ± 0.411 *	29.2 ± 2.03 *	8.24 ± 0.77 ***	23.56 ± 1.98 ***	16.25 ± 2.73

* $P < 0.05$

*** $P < 0.001$

Discussion

The experiment showed that the oral administration of *R. stricta* aqueous extract to mature rats resulted in insignificant changes in serum enzyme activities of ALT, AST, serum urea nitrogen, total proteins, albumin, inorganic phosphorous and MCH. There were however, significant increases in serum globulins, and calcium levels together with significant decreases in serum triglycerides, A/G ratio, RBCs and WBCs counts, Hb%, PCV% MCV and MCHC when compared with the control values.

Increased serum globulins and decreased A/G ratio obtained in this study might be due to stimulation of the liver to synthesize α and β-globulins on one hand and to stimulation of the lymphocytes to produce γ globulins on the other, especially when the total WBCs count was low in the treated group, indicating the presence of immunopotentiating factor in the plant extract.

The significant increase in serum calcium level in the presence of slightly lowered phosphate concentration may be attributed to increased intestinal absorption of calcium. This could be due to increased formation of calcium binding proteins, as a result of some factors(s) present in *R. stricta* having an action similar to vitamin D_3.

The significant ($P < 0.001$) decrease in serum triglycerides level could be attributed to decreased availability of conjugated bile salts. Farah *et al.* [23], noted a decrease in the level of serum total bilirubin in sheep treated with *R. stricta* extract. This could be due to a selective increase in the activity of the liver to conjugate bile components including bile acids and their subsequent loss through the intestines.

Moreover, it could be speculated that some components in *R. stricta* reduced the rate of esterification of fatty acids to triglycerides. The significant decrease in RBCs and WBCs counts with the subsequent decrease in Hb%, PCV%, MCV and MCHC might be due to suppression of the bone marrow. This finding is in accordance with Siddiqui and Bukhari [9] who reported that *R. stricta* extracts showed a marked leucopenic effect in rats when given orally in a dose of 20 mg/kg.b.wt. They also added that a single intraperitoneal injection of 15 mg/kg. b. wt. significantly reduced the WBCs count for a period of 7-10 days. This marked leucopenic effect might be most probably due to the cytotoxic effect of the extract on bone marrow as was observed by Mukhopadhyay *et al.* [10] who reported anticancer activity of some alkaloids of *R. stricta*.

From the obtained results, we may conclude that the aqueous extract of *R. stricta* is nontoxic to rats at 4.0 gm/ kg.b.wt. with hypolipidaemic and immunopotentiative effects.

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دراسات إكلينيكية مرضية وسمية لتناول خلاصة نبات الحرمل عن طريق الفم في الفئران

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ملخص البحث. يوجد نبات الحرمل بوفرة في المملكة العربية السعودية وهو يستخدم شعبيًا كعلاج لبعض الأمراض مثل الروماتيزم. ولقد تم في هذه الدراسة إعطاء الخلاصة المائية عن طريق الفم للفئران البالغة في جرعة مقدارها ٤٠٠ مجم/ كجم من وزن الفئران يوميًا ولمدة ثمانية أسابيع متصلة. ولقد أدى ذلك إلى حدوث تغيرات غير معنوية في نشاط إنزيمي ALT and Ast وكذا في بولينا المصل والبروتينات الكلية ـ والزلال والفسفور غير العضوي. كما كان هناك زيادة معنوية في جلوبيولينات المصل والكالسيوم. كما صاحب ذلك نقص معنوي في الجليسيريدات الثلاثية ونسبة الزلال للجلوبيولينات. كذلك أدى العلاج بخلاصة الحرمل إلى حدوث نقص معنوي في عدد خلايا الدم الحمراء والبيضاء وكذا في نسبة الهيموجلوبين وحجم الخلايا المضغوطة ومتوسط حجم الكريات، وكذا متوسط محتوى الكريات من الهيموجلوبين.

لم يؤد تعاطي خلاصة نبات الحرمل إلى حدوث وفيات في حيوانات التجارب حتى عندما أعطى في جرعات مقدارها ٤ جم/ كجم إضافة إلى عدم حدوث أي تغيرات مرضية.