

CHAPTER 3

RESULTS

3.1 Effects of Matoom juice containing Stevia extract as sweetener on aberrant crypt foci formation

3.1.1 Effects of Matoom juice containing Stevia extract as sweetener on the number of aberrant crypt foci at initiation stage

The effect of Stevia extract mixed in Matoom juice on number of ACF in rats colon at the initiation stage was shown in Table 1. No ACF formation was presented in rats not treated with AOM (group 1, 3-7). ACF formation was observed in only rats treated with AOM (group 2). The mean total number of ACF in this group was 142.50 aberrant crypt. This data indicated that treated rats either with Matoom juice containing various concentrations of Stevia extract as sweetener from 0.2-10%w/v or Stevia extract at 10.0% in distilled water was not able to induce ACF formation in colon at the initiation stage.

Table 1. Number of ACF in rats after received Matoom juice that used Stevia extract as sweetener at initiation stage

Treatment	No. of rats	colon ^a		Rectum ^a		Total ^a	
		No.of AC	No.of AC/ACF	No.of AC	No.of AC/ACF	No.of AC	No.ofAC/ACF
H ₂ O+saline (normal control)	8	ND	ND	ND	ND	ND	ND
AOM+H ₂ O (positive control)	8	102.00±11.69	2.58±0.17	40.50±6.21	2.49±0.21	142.50±7.46	2.62±0.315
Matoom juice	8	ND	ND	ND	ND	ND	ND
0.2%(w/v) Stevia in Matoom juice	8	ND	ND	ND	ND	ND	ND
1.0%(w/v) Stevia in Matoom juice	8	ND	ND	ND	ND	ND	ND
10.0%(w/v) Stevia in Matoom juice	8	ND	ND	ND	ND	ND	ND
10%(w/v) Stevia in H ₂ O	8	ND	ND	ND	ND	ND	ND

a) Mean±SD

ND = number of AC (aberrant crypt) is not detectable

3.1.2 Effect of Matoom juice containing Stevia extract as sweetener on the number of aberrant crypt foci at promotion stage

Table 2 shows the effect of Stevia extract mixed in Matoom juice on number of ACF in rats colons at promotion stage. The number of ACF was not observed in vehicle-treated group (group 1) and in rats treated with Matoom juice containing Stevia extract as sweetener (group 3-7). ACF formation was observed in only rats treated with AOM (group 2). The mean total number of ACF in group 2 rats was 407.75 aberrant crypt. Single tubular adenoma at proximal segment was detected in 2 of 8 rats in this group. This result indicated that either Matoom juice containing various concentrations of Stevia extract as sweetener from 0.2-10%w/v or Stevia extract at 10.0% in distilled water was not able to promote ACF formation in rats colon at promotion stage.

Table 2. Number of ACF in rats after received Matoom juice containing Stevia extract as sweetener at promotion stage

Treatment	No. of rats	colon ^a		Rectum ^a		Total ^a	
		No.of AC	No.of AC/ACF	No.of AC	No.of AC/ACF	No.of AC	No.of AC/ACF
H ₂ O+saline (normal control)	8	ND	ND	ND	ND	ND	ND
AOM+H ₂ O (positive control)	8	335.37±95.48*	3.52±0.16	74.71±18.81	3.51±0.28	407.75±95.12	3.52±0.14
Matoom juice	8	ND	ND	ND	ND	ND	ND
0.2%(w/v) Stevia in Matoom juice	8	ND	ND	ND	ND	ND	ND
1.0%(w/v) Stevia in Matoom juice	8	ND	ND	ND	ND	ND	ND
10.0%(w/v) Stevia in Matoom juice	8	ND	ND	ND	ND	ND	ND
10%(w/v) Stevia in H ₂ O	8	ND	ND	ND	ND	ND	ND

b) Mean±SD

ND = number of AC (aberrant crypt) is not detectable

* = single tubular adenoma at proximal segment (2 rats)

3.2 Effects of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on rat body weight

Figure 18 and 19 show the effect of 35 and 100 days administration of Matoom juice containing Stevia extract as sweetener on rat body weight, respectively. The body weights, in either 35 or 100 days administration was not significant differences from the control. These findings were similar of the observations of Xili, *et al* who reported that chronic toxicity study of stevioside showed no significantly difference between normal control and treated groups in body weight (Xili, *et al*, 1992). The body weight in normal control showed no significantly differences to positive control (AOM treated group) which similar to the result of Theresa, *et al* (Theresa, *et al*, 1992).

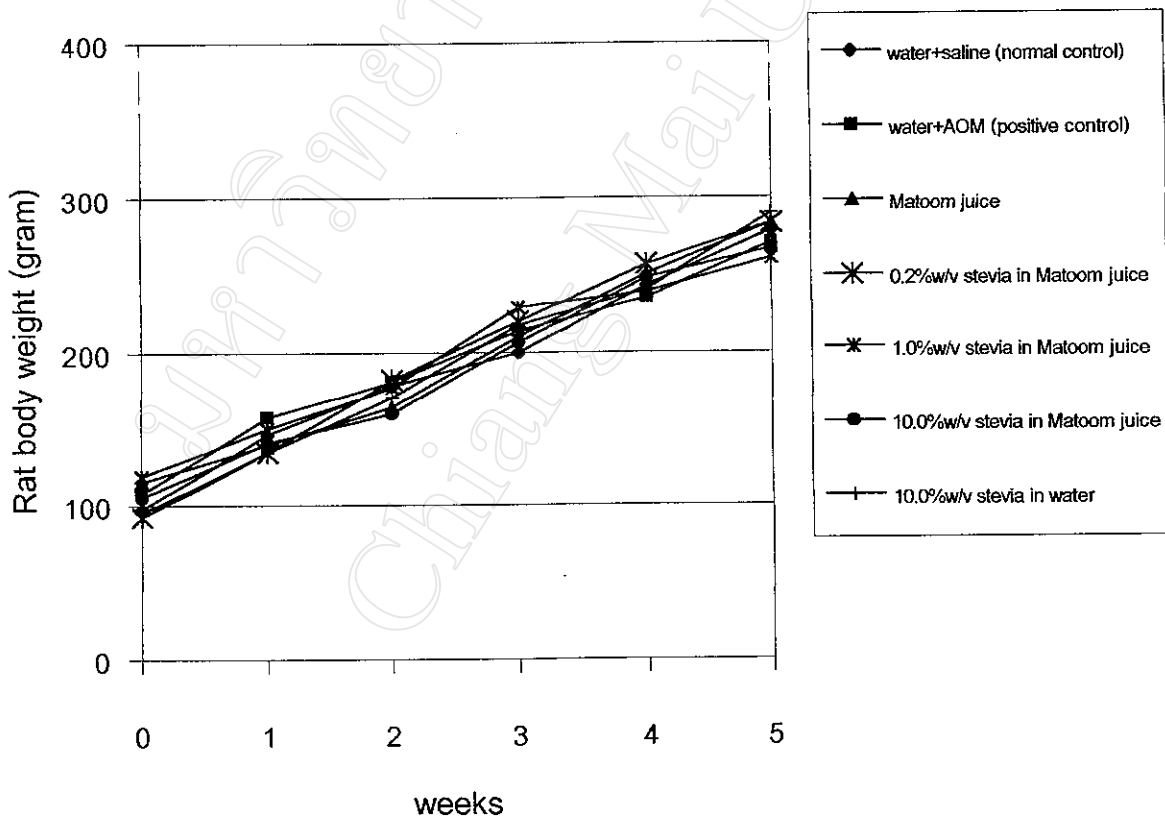


Figure 18. Effects of 35 days administration of Matoom juice containing Stevia extract as sweetener on rat body weight. Values are means from 8 rats/group

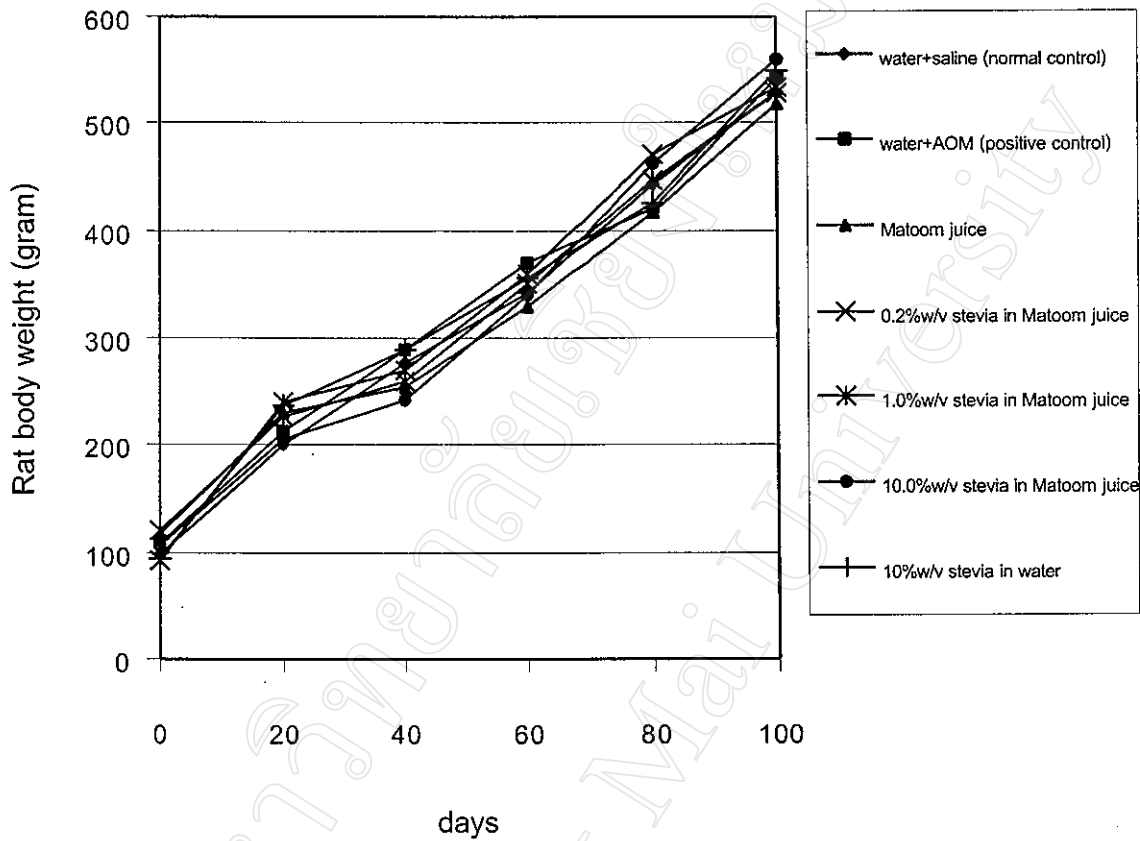


Figure 19. Effects of 100 days administration of Matoom juice containing Stevia extract as sweetener on rat body weight. Values are means from 8 rats/group.

3.3 Effects of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on AST and ALT activities in rats serum

In this results, the normal control of AST and ALT activities in rats serum were consistent with the studied of Hwang, *et al.* The results of AST and ALT activities in this study were 20.56 ± 2.15 and 17.54 ± 1.89 U/L compared to 35 U/L and 19 U/L in the studied of Hwang, *et al.* (Hwang, *et al.*, 2000). No reference value of AST and ALT activities in rat treated with AOM (positive control).

3.3.1 Effects of 35 days administration of Matoom juice containing Stevia extract as sweetener on AST and ALT activities in rats serum

The effect of 35 days administration of Matoom juice containing Stevia extract as sweetener on AST and ALT activities in rat serum were shown in Table 3. The results showed that AST and ALT activities in the rats received Matoom juice used various concentrations of Stevia extract as sweetener and Stevia extract in distilled water were not significantly different from control group. AST and ALT activities were significantly increased in serum of only rat treated with AOM when compared with non-treated group.

3.3.2 Effects of 100 days administration of Matoom juice containing Stevia extract as sweetener on AST and ALT activities in rats serum

The results showed no significant difference in AST and ALT activities in serum of rats treated with Matoom juice containing various concentrations of Stevia extract as sweetener, but only rats received AOM showed significant increase in AST activity. ALT activity in rats treated with AOM was also slightly increased but not statistically significant from non-treated rats (Table 4).

Table 3. Effects of 35 days administration of Matoom juice containing Stevia extract as sweetener on AST and ALT activities in rat serum

Group	Treatment (n=8)	35 days administration	
		AST activity* (U/L)	ALT activity** (U/L)
1	H ₂ O + saline (normal control)	18.25±1.84	16.43±1.68
2	AOM + H ₂ O (positive control)	24.24±2.58 ^a	20.64±3.04 ^a
3	Matoom juice	19.04±2.47	17.35±2.48
4	0.2%(w/v) Stevia in Matoom juice	17.39±2.41	16.74±1.38
5	1.0%(w/v) Stevia in Matoom juice	19.45±3.49	18.79±4.68
6	10.0%(w/v) Stevia in Matoom juice	18.97±2.64	16.86±4.67
7	10.0%(w/v) Stevia in H ₂ O	19.59±2.79	17.38±3.57

Values are mean ± SD

Statistically significant difference from no treatment group (group 1) ^ap<0.05

* α-ketoglutarate and L-aspartate were used as the substrate

** α-ketoglutarate and L-alanine were used as the substrate

Table 4. Effects of 100 days administration of Matoom juice containing Stevia extract as sweetener on AST and ALT activities in rat serum

Group	Treatment (n=8)	100 days administration	
		AST activity* (U/L)	ALT activity** (U/L)
1	H ₂ O + saline (normal control)	20.56±2.15	17.54±1.89
2	AOM + H ₂ O (positive control)	25.79±0.21 ^a	19.35±0.94
3	Matoom juice	21.35±3.24	18.26±1.35
4	0.2%(w/v) Stevia in Matoom juice	21.49±2.68	17.94±1.28
5	1.0%(w/v) Stevia in Matoom juice	19.94±2.89	18.34±2.68
6	10.0%(w/v) Stevia in Matoom juice	20.79±2.33	17.65±4.56
7	10.0%(w/v) Stevia in H ₂ O	19.82±4.01	18.34±0.48

Values are mean ± SD

Statistically significant difference from the normal control (group 1) ^ap<0.05

* α-ketoglutarate and L-aspartate were used as the substrate

** α-ketoglutarate and L-alanine were used as the substrate

3.4 Effects of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on reduced glutathione (GSH) levels in rat liver and intestinal mucosa

3.4.1 Effects of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on reduced glutathione levels in rat liver

The effect of 35 and 100 days treatment with Matoom juice containing various concentrations of Stevia extract as sweetener on reduced glutathione levels in rat liver was shown in Table 5. The result showed that after 35 and 100 days treatment with Matoom juice containing various concentrations of Stevia extract as sweetener, the hepatic GSH content of all treatments was not significant difference compared to the non-treated rats. Hepatic GSH levels in rats treated with AOM were significantly decreased when compared to the non-treated group.

3.4.2 Effects of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on reduced glutathione levels in rat intestinal mucosa.

Table 6 showed the effect of 35 and 100 days treatment with Matoom juice containing various concentrations of Stevia extract as sweetener on reduced glutathione levels in rat intestinal mucosa. The GSH level in intestinal mucosa of all treated groups with Matoom juice containing various concentrations of Stevia extract as sweetener were similar to the non-treated group. GSH levels in rat intestinal mucosa non-treated groups were significant decreased when compared with rats treated with AOM.

Table 5. Effects of 35 or 100 days administration of Matoom juice that containing Stevia extract as sweetener on reduced glutathione levels in rat liver

Group	Treatment (n=8)	GSH levels (nmol/mg protein)	
		35 days	100 days
1	H ₂ O + saline (normal control)	345.25±26.62	339.50±24.17
2	AOM + H ₂ O (positive control)	259.00±28.21 ^a	251.37±36.82 ^a
3	Matoom juice	339.62±25.07	362.12±23.33
4	0.2%(w/v) Stevia in Matoom juice	343.05±30.30	353.62±20.04
5	1.0%(w/v) Stevia in Matoom juice	338.5±28.11	346.37±16.48
6	10.0%(w/v) Stevia in Matoom juice	341.00±29.37	353.37±32.78
7	10.0%(w/v) Stevia in H ₂ O	326.75±33.44	335.50±29.85

Values are mean ± SD

Statistically significant difference from the normal control (group 1) ^ap<0.05

Table 6. Effects of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on reduced glutathione levels in rat intestinal mucosa

Group	Treatment (n=8)	GSH levels (nmol/mg protein)	
		35 days	100 days
1	H ₂ O + saline (normal control)	185.25±27.55	187.09±29.68
2	AOM + H ₂ O (positive control)	118.75±14.83 ^a	132.50±29.62 ^a
3	Matoom juice	173.50±15.36	180.37±24.07
4	0.2%(w/v) Stevia in Matoom juice	172.75±20.14	158.12±24.12
5	1.0%(w/v) Stevia in Matoom juice	184.25±17.16	164.37±26.30
6	10.0%(w/v) Stevia in Matoom juice	176.75±27.95	167.12±17.86
7	10.0%(w/v) Stevia in H ₂ O	181.62±33.36	180.50±34.18

Values are mean ± SD

Statistically significant difference from the normal control (group 1) ^ap<0.05

3.5 Effects of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on glutathione-S-transferase (GST) activity in rat liver and intestinal

In this results, the normal and positive control of GST activities in rats liver and intestinal mucosa were consistent with the studied of Bandaru, *et al.* They reported that in normal and positive groups (treated with AOM); GST activities in rats liver were 688 ± 128 and 720 ± 114 , in rat intestinal mucosa were 336 ± 64 and 372 ± 50 nmol/mg/min, respectively (Bandaru, *et al.*, 2000). The results of the normal and positive control of GST activities in rats liver and intestinal mucosa in this study were 578.87 ± 23.11 , 720.87 ± 34.92 , 330.37 ± 31.12 and 456.12 ± 33.22 nmol/mg/min, respectively.

3.5.1 Effects of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on glutathione-S-transferase activity in rat liver

Using glutathione and CDNB as substrates, GST activity was measured in the cytosolic fraction. The Effect of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on GST activity in rat liver was shown in Table 7. The results showed that after 35 or 100 days administration of Stevia extract in Matoom juice containing various concentrations of Stevia extract as sweetener from 0.2-10%w/v or Stevia extract at 10.0% in distilled water, the hepatic GST activities in all treated groups were not statistically significantly different from the non-treated group. GST activity in rat liver was significantly increased in AOM treated group (group 2) when compared to the non-treated rats (group1).

3.5.2 Effects of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on glutathione-S-transferase activity in rat intestinal mucosa

The results in Table 8 showed that after receiving of Matoom juice containing Stevia extract as sweetener for 35 or 100 days, the GST activity in intestinal mucosa in

all rats treated with Matoom juice containing various concentrations of Stevia extract as sweetener were slightly increased but not significantly difference when compared to non-treated rats. The GST activities in rat treated with AOM was significantly increased over the non-treated group.

Table 7. Effect of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on glutathione-S-transferase activity in rat liver

Group	Treatment (n=8)	GST activity* (nmol/min/mg protein)	
		35 days	100 days
1	H ₂ O + saline (normal control)	578.87±23.11	798.62±45.96
2	AOM + H ₂ O (positive control)	720.87±34.92 ^a	977.75±67.60 ^a
3	Matoom juice	566.62±31.33	801.62±52.73
4	0.2%(w/v) Stevia in Matoom juice	567.50±24.95	810.02±54.19
5	1.0%(w/v) Stevia in Matoom juice	577.62±31.19	802.87±34.01
6	10.0%(w/v) Stevia in Matoom juice	589.01±25.89	821.62±32.34
7	10.0%(w/v) Stevia in H ₂ O	590.87±35.75	838.62±58.24

Values are mean ± SD

Statistically significant difference from the normal control (group 1) ^ap<0.05

* CDNB was used as the substrate

Table 8. Effects of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on glutathione-S-transferase activity in rat intestinal mucosa

Group	Treatment (n=8)	GST activity* (nmol/min/mg protein)	
		35 days	100 days
1	H ₂ O + saline (normal control)	330.37±31.12	358.50±32.75
2	AOM + H ₂ O (positive control)	456.12±33.22 ^a	443.37±26.58 ^a
3	Matoom juice	356.37±31.81	330.75±25.73
4	0.2%(w/v) Stevia in Matoom juice	364.87±23.46	349.87±34.52
5	1.0%(w/v) Stevia in Matoom juice	349.25±31.54	345.12±30.06
6	10.0%(w/v) Stevia in Matoom juice	362.25±35.28	349.87±27.10
7	10.0%(w/v) Stevia in H ₂ O	358.12±29.84	368.12±32.27

Values are mean ± SD

Statistically significant difference from the normal control (group 1) ^ap<0.05

* CDNB was used as the substrate

3.6 Effects of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on glutathione-S-transferase subunit level in rat liver and intestinal mucosa

In this study, the normal control of GST isoenzyme levels in rats were consistent with the studied of Geoffrey, *et al*. The normal control of GST isoenzyme levels were reported as follow; GST-alpha> GST-mu 25 times and GST-mu>GST-theta 10 times (Geoffrey, *et al*, 1993). The results of GST isoenzyme levels of GST alpha, mu and theta in this study were 16.13, 1.71, 0.23 AU, respectively. No reference value of GST isoenzyme levels in rat treated with AOM (positive control).

3.6.1 Effects of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on glutathione-S-transferase subunit level in rat liver

After administration of Matoom juice containing various concentrations of Stevia extract as sweetener for 35 days, it was shown in Table 9 that the hepatic GST Ya and Yc subunits levels were not different from non-treated rats, but the levels were increased in rats treated with AOM. The Ya and Yc subunits levels in rats treated with AOM were induced 1.3 and 1.25 fold to non-treated group, respectively. GST YT subunit levels were decreased in rats treated with Matoom juice while GST Yb subunit were not different in all groups treated with Matoom juice containing various concentrations of Stevia extract as sweetener. GST Yb and YT subunits levels were decreased in rats treated with AOM. The levels of GST alpha classes (both Ya and Yc subunits) and GST mu (Yb subunit) levels in rats treated with Matoom juice containing various concentrations of Stevia extract as sweetener were not different from non-treated rats but in AOM-treated rats the level of GST alpha class were increased but GST mu levels were decreased. GST theta (YT subunit) levels in rats treated with Matoom juice containing various concentrations of Stevia extract as sweetener were not different when compared with non-treated group. The GST subunits in the liver of rats after 100 days administration of Matoom juice containing various concentrations of Stevia extract as sweetener was shown in Table 10. The GST alpha (both Ya and Yc

subunits) and GST mu levels in rats treated with Matoom juice containing various concentrations of Stevia extract as sweetener were not different from the non-treated group, except rats treated with AOM GST alpha level was increased and GST mu level was decreased. The Yc and Ya subunits in rats received AOM were induced 1.26 and 1.17 fold, respectively. GST theta class levels in rats treated with Matoom juice, 10.0%w/v Stevia extract in distilled water and AOM were decreased.

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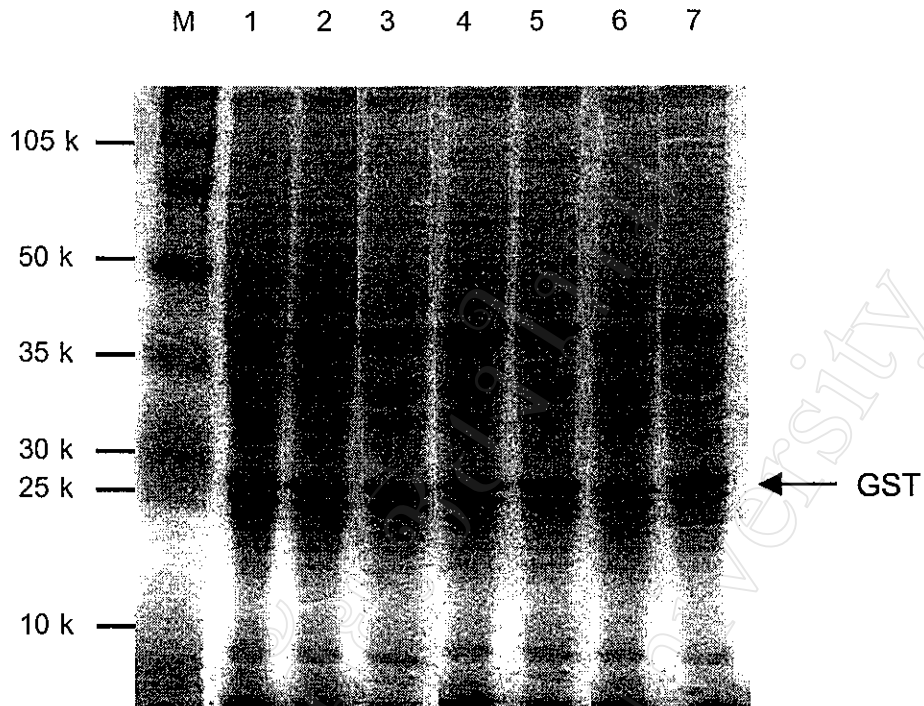


Figure 20. Coomessie blue stain of rats cytosolic GSTs on running SDS-PAGE.

The fractions are as follows:

(M) molecular weight marker

Lane 1: Hepatic cytosol from non-treated rats

Lane 2: Hepatic cytosol from rats treated with distilled water and AOM

Lane 3: Hepatic cytosol from rats treated with Matoom Juice

Lane 4: Hepatic cytosol from rats treated with 0.2%w/v Stevia extract in Matoom Juice

Lane 5: Hepatic cytosol from rats treated with 1.0%w/v Stevia extract in Matoom Juice

Lane 6: Hepatic cytosol from rats treated with 10.0%w/v Stevia extract in Matoom Juice

Lane 7: Hepatic cytosol from rats treated with 10.0%w/v Stevia extract in distilled water

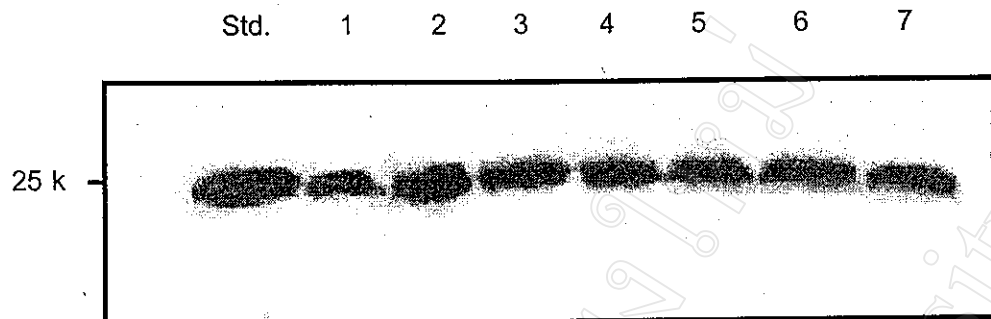


Figure 21. Western blot analysis using antibodies raised against GST Yc subunit of rats hepatic cytosol

The fractions are as follows: (Std.) Standard liver GST (Sigma)

- (1) Hepatic cytosol from non-treated rats
- (2) Hepatic cytosol from rats treated with distilled water and AOM
- (3) Hepatic cytosol from rats treated with Matoom Juice
- (4) Hepatic cytosol from rats treated with 0.2%w/v Stevia extract in Matoom Juice
- (5) Hepatic cytosol from rats treated with 1.0%w/v Stevia extract in Matoom Juice
- (6) Hepatic cytosol from rats treated with 10.0%w/v Stevia extract in Matoom Juice
- (7) Hepatic cytosol from rats treated with 10.0%w/v Stevia extract in distilled water

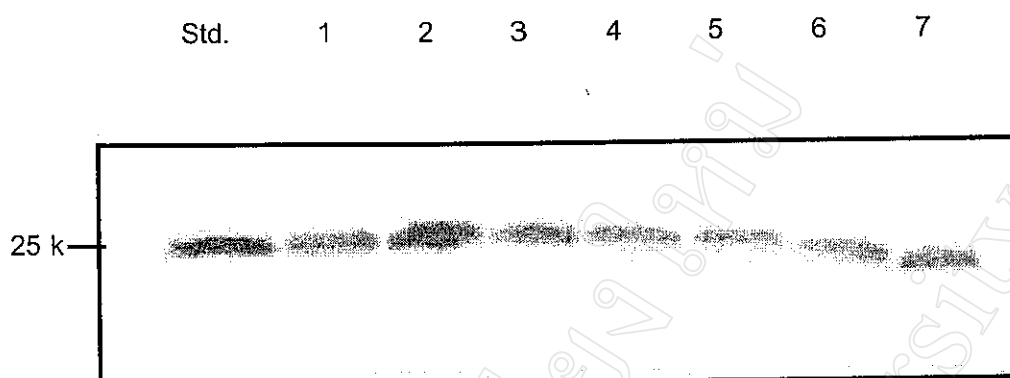


Figure 22. Western blot analysis using antibodies raised against GST Ya subunit of rats hepatic cytosol

The fractions are as follows: (Std.) Standard liver GST (Sigma)

(1) Hepatic cytosol from non-treated rats

(2) Hepatic cytosol from rats treated with distilled water and AOM

(3) Hepatic cytosol from rats treated with Matoom Juice

(4) Hepatic cytosol from rats treated with 0.2%w/v Stevia extract in Matoom Juice

(5) Hepatic cytosol from rats treated with 1.0%w/v Stevia extract in Matoom Juice

(6) Hepatic cytosol from rats treated with 10.0%w/v Stevia extract in Matoom Juice

(7) Hepatic cytosol from rats treated with 10.0%w/v Stevia extract in distilled water



Figure 23. Western blot analysis using antibodies raised against GST Yb subunit of rats hepatic cytosol

The fractions are as follows: (Std.) Standard liver GST (Sigma)

- (1) Hepatic cytosol from non-treated rats
- (2) Hepatic cytosol from rats treated with distilled water and AOM
- (3) Hepatic cytosol from rats treated with Matoom Juice
- (4) Hepatic cytosol from rats treated with 0.2%w/v Stevia extract in Matoom Juice
- (5) Hepatic cytosol from rats treated with 1.0%w/v Stevia extract in Matoom Juice
- (6) Hepatic cytosol from rats treated with 10.0%w/v Stevia extract in Matoom Juice
- (7) Hepatic cytosol from rats treated with 10.0%w/v Stevia extract in distilled water

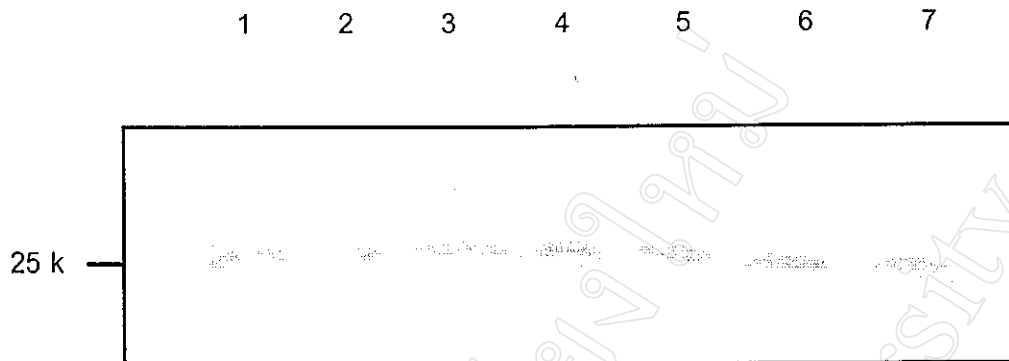


Figure 24. Western blot analysis using antibodies raised against GST YT subunit of rats hepatic cytosol

The fractions are as follows:

- (1) Hepatic cytosol from non-treated rats
- (2) Hepatic cytosol from rats treated with distilled water and AOM
- (3) Hepatic cytosol from rats treated with Matoom Juice
- (4) Hepatic cytosol from rats treated with 0.2%w/v Stevia extract in Matoom Juice
- (5) Hepatic cytosol from rats treated with 1.0%w/v Stevia extract in Matoom Juice
- (6) Hepatic cytosol from rats treated with 10.0%w/v Stevia extract in Matoom Juice
- (7) Hepatic cytosol from rats treated with 10.0%w/v Stevia extract in distilled water

Table 9. Effects of 35 days administration of Matoom juice containing Stevia extract as sweetener on glutathione-S-transferase subunits levels in rat liver

Group*	Treatment	GST subunit (arbitrary unit)			
		Yc	Ya	Yb	YT
1	H ₂ O + saline (normal control)	7.15	0.82	0.64	0.18
2	AOM + H ₂ O (positive control)	9.34	1.03	0.33	0.10
3	Matoom juice	7.51	0.61	0.55	0.13
4	0.2%(w/v) Stevia in Matoom juice	6.84	0.74	0.72	0.19
5	1.0%(w/v) Stevia in Matoom juice	7.30	0.89	0.61	0.21
6	10.0%(w/v) Stevia in Matoom juice	7.73	0.93	0.77	0.17
7	10.0%(w/v) Stevia in H ₂ O	6.79	0.68	0.58	0.20

* One group pooled sample (8 rats in each group)

Values are the mean from two, independent experiments, each done in duplicate

ND was not detectable

Arbitrary unit (adjusted volume) was OD x mm²

Table 10. Effects of 100 days administration of Matoom juice containing Stevia extract as sweetener on glutathione-S-transferase subunits levels in rat liver

Group*	Treatment	GST subunit (arbitrary unit)			
		Yc	Ya	Yb	YT
1	H ₂ O + saline (normal control)	16.13	1.94	1.71	0.23
2	AOM + H ₂ O (positive control)	20.42	2.28	0.98	0.14
3	Matoom juice	16.10	2.06	1.64	0.19
4	0.2%(w/v) Stevia in Matoom juice	18.91	1.82	1.75	0.26
5	1.0%(w/v) Stevia in Matoom juice	17.88	1.98	1.81	0.30
6	10.0%(w/v) Stevia in Matoom juice	16.38	1.88	1.68	0.21
7	10.0%(w/v) Stevia in H ₂ O	19.76	1.61	1.57	0.17

* One group pooled sample (8 rats in each group)

Values are the mean from two, independent experiments, each done in duplicate

ND was not detectable

Arbitrary unit (adjusted volume) was OD x mm²

3.6.2 Effects of 35 or 100 days administration of Matoom juice containing Stevia extract as sweetener on glutathione-S-transferase subunits levels in rat intestinal mucosa

After 35 days administration, as showed in Table 11, the Yc subunit in all treated groups were increased when compared to the non-treated rats. The Yc subunit levels in rats treated with AOM, Matoom juice, Matoom juice containing Stevia extract as sweetener at dose 0.2%w/v, 1.0w/v, 10.0%w/v and 10.0%w/v Stevia extract in distilled water were also induced about 1.81, 1.18, 1.46, 1.13, 1.23 and 1.32 fold to the non treated group, respectively. The Ya subunit levels in all treated rats were not detectable, excepted in rats treated with AOM. The GST mu level in all groups treated with Matoom juice containing Stevia extract as sweetener were not different but decreased in only rats treated with AOM. GST theta subunit levels in all treated rats were not able to detect.

After 100 days administration, as showed in Table 12, the amount of Yc subunit and GST mu (Yb subunit) levels in rats treated with Matoom juice containing various concentrations of Stevia extract as sweetener were not different, except rats treated with AOM were induced 1.66 and 1.79 fold, respectively when compared to the non-treated rats. The GST Ya subunits levels in rats treated with Matoom juice containing various concentrations of Stevia extract as sweetener and Stevia extract in distilled water were slightly lower than the non-treated group, excepted in rats received AOM were induced 2.28 fold. GST theta class levels were not detectable in all treatments.

Table 11. Effects of 35 days administration of Matoom juice containing Stevia extract as sweetener on glutathione-S-transferase subunits levels in rat intestinal mucosa

Group*	Treatment	GST subunit (arbitrary unit)			
		Yc	Ya	Yb	YT
1	H ₂ O + saline (normal control)	0.43	ND	0.35	ND
2	AOM + H ₂ O (positive control)	0.78	0.34	0.13	ND
3	Matoom juice	0.51	ND	0.41	ND
4	0.2%(w/v) Stevia in Matoom juice	0.63	ND	0.37	ND
5	1.0%(w/v) Stevia in Matoom juice	0.49	ND	0.29	ND
6	10.0%(w/v) Stevia in Matoom juice	0.53	ND	0.33	ND
7	10.0%(w/v) Stevia in H ₂ O	0.57	ND	0.47	ND

* One group pooled sample (8 rats in each group)

Values are the mean from two, independent experiments, each done in duplicate

ND was not detectable

Arbitrary unit (adjusted volume) was OD x mm²

Table 12. Effects of 100 days administration of Matoom juice containing Stevia extract as sweetener on glutathione-S-transferase subunits levels in rat intestinal mucosa

Group*	Treatment	GST subunit (arbitrary unit)			
		Yc	Ya	Yb	YT
1	H ₂ O + saline	0.81	0.21	0.54	ND
2	AOM + H ₂ O	1.35	0.48	0.11	ND
3	Matoom juice	0.76	0.14	0.58	ND
4	0.2%(w/v) Stevia in Matoom juice	0.89	0.23	0.63	ND
5	1.0%(w/v) Stevia in Matoom juice	0.68	0.19	0.50	ND
6	10.0%(w/v) Stevia in Matoom juice	0.93	0.12	0.48	ND
7	10.0%(w/v) Stevia in H ₂ O	0.88	0.14	0.61	ND

* One group pooled sample (8 rats in each group)

Values are the mean from two, independent experiments, each done in duplicate

ND was not detectable

Arbitrary unit (adjusted volume) was OD x mm²