

## **TIME-DEPENDENT EFFECT OF ALLYL ISOTHIOCYANATE ON SOME METABOLIC PARAMETERS IN RATS**

By

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### **ABSTRACT**

**Background:** allyl isothiocyanate (AITC) is found in high concentrations in Brassica family vegetables, especially in Brussels sprouts. Recently, they have been used as a nutrition supplement for their preventive and medicinal effect on some types of cancer and other diseases. **Aim of the work:** In this research, nutritional significance of allylisothiocyanate 25  $\mu\text{mol/kg}$  b. w./day and 50  $\mu\text{mol/kg}$  b. w./day was studied by the evaluation of their influence on some parameters of carbohydrate and lipid metabolism in white albino rats after their single (4 h) and 2 weeks oral administration.. **Results:** AITC after 4 h of its ingestion caused glycaemia only at the higher dose. AITC multiple administration strongly disturbed lipid and carbohydrate homeostasis, increasing total cholesterol, free fatty acids and lowering triglycerides in the blood serum. Additionally, AITC at both doses produced insulinaemia **Conclusions:** Whilst consumption of cruciferous vegetables at levels currently considered "normal" seems to be beneficial to human health, this data suggest that any large increase in intake could conceivably lead to undesirable effect. This effect is potentiated with time of action of the examined compounds, whose influence is rather adverse for the majority of metabolic pathways (glycemia at short duration and insulinaemia, cholesterolaemia at long time treatment). Beneficial action of AITC concerned intensified hydrolysis of TG in the blood serum.

**Keywords:** AITC, Metabolism, Rat.

### **INTRODUCTION**

More and more investigations have been performed recently on plants in order to discover their possible anticancer properties. It has been estimated that diets rich in phytochemicals can significantly reduce cancer risk by as much as 20%<sup>(1)</sup>. Based on epidemiological observations, which correlated reduced incidence of cancer (particularly colon and rectal) in populations with intake of Brassicaceae family vegetables, biological studies have dealt with glucosinolates like sinigrin (SIN) and its hydrolysis product allylisothiocyanate (AITC). The conversion of SIN into AITC is catalyzed by myrosinase, a thioglucosidase after plant cellular damage or in the presence of gut microflora<sup>(2),(3)</sup>.

Human exposure to these compounds is undoubtedly widespread and frequent, as many common cruciferous vegetables are a rich source of them (e.g. Brussels sprouts, red, Savoy, white cabbages, cauliflower, broccoli, condiments and salad crops)<sup>(4)</sup>. However,

allylisothiocyanate may be present in following foods per 100 g: syrups (1–8.8 mg), meats (8.7 mg), condiments (5.2 mg), baked goods (0.52 mg), candies and ice cream (0.05 mg)<sup>(5)</sup>, mainly as a component in volatile oil of mustard, which is used in pickling spices (meat) and imitation pineapple flavoring (ice cream). Average human consumption of AITC has been estimated to be less than 1 mg/day (approximately 10  $\mu\text{g/kg}$  body weight)<sup>(6),(7)</sup>.

Biological activity of AITC is wide. Beside its TRPA1 agonistic activities AITC kills bacteria and fungi. Herein, AITC action appears to resemble polymyxin B, which is known to bind to cell membrane and to increase its permeability<sup>(8)</sup>. Also, insecticidal properties of this compound have been reported<sup>(9)</sup>. AITC was also shown to significantly inhibit thioredoxin reductase and acetate kinase at approximately 100  $\mu\text{M}$ <sup>(10)</sup>, enzymes playing an important role in cell growth and proliferation. AITC inhibits proliferation of various types of human cancer cells at the low micromolar range. Inhibition of cell proliferation by AITC was associated with cell cycle arrest and/or

### Time-Dependent Effect Of Allvl Isothiocvanate.....

induction of apoptosis<sup>(11)</sup>. What more, **Hwang and Lee**<sup>(12)</sup> reported that AITC plays important roles in cancer metastasis by inhibition of cell adhesion, migration and invasion. AITC was also found to significantly inhibit the production of nitric oxide (NO) and the expression of inducible nitric oxide synthase (iNOS) important signalling molecules in inflammation and cancer<sup>(13)</sup>. Additionally, AITC induces enzymes of xenobiotic metabolism in cell cultures and, when included in the diet, in the livers and peripheral organs of mice and rats<sup>(14)</sup>. AITC also was found to significantly inhibit in a dose-dependent manner the formation of gastric lesions induced by ethanol, hydrochloric acid, ammonia, aspirin and indomethacin in rats<sup>(15)</sup>.

Summing up, many substances that occur naturally in food plant may modify, in significant way, the metabolic processes in their consumers. However, we still know very little about the metabolic effects of glucosinolates where AITC was found in the greatest amounts<sup>(16)</sup>. It is known that AITC given orally for only 3 consecutive days increased liver weight and serum aspartate aminotransferase activity<sup>(17)</sup>. Since epidemiological data indicate that a diet rich in cruciferous vegetables can protect laboratory animals from tumorigenic effects of model carcinogens, overall intake of glucosinolates and their breakdown products in human diet is suggested to be increased (for example by genetic manipulation of plants or by artificial supplementation of the diet)<sup>(11)</sup>.

In order to properly define the balance of benefit and risk of their high consumption, AITC dose levels used in the preclinical studies are far greater than what humans are normally exposed to<sup>(7)</sup>.

Taking into consideration the above-mentioned information, this research project aimed at expanding our knowledge about the influence of high AITC intake on carbohydrate and lipid

parameters in a rat model at different time of their action *in vivo* conditions. Time-related response of the analyzed compounds was included in this study in order to evaluate possible time-interval required for AITC metabolic effect intensification.

#### MATERIALS AND METHODS

**Animals:** 36 healthy, adult, male albino rats weighing 180-200 gm were used that were bred in the animal house. The rats were kept in steel wire cages under hygienic conditions in physiology research laboratory in faculty of medicine Zagazig University. Animals were kept on normal diet that consisted of mixed commercial rat laboratory chow had free access to water, kept at room temperature and were maintained on a 12 h light/dark cycle. The rats were accommodated to new conditions for 5 days before the experiments going on. Rats were administrated AITC at appropriate animal doses, which had no impact on food consumption, rat weight, or general appearance and behavior were used in such doses by many other researchers<sup>(2), (16), (18), (19), (20)</sup>.

**Groups:** After adaptation period, the animals were randomly divided into 6 groups (n=6) for the two investigations (3 groups for each one). The first trial involved single oral administration of AITC at two different doses:

- Group I: Control (CON)
- Group II: rats were administered 25 µmol/kg b. w. AITC,
- Group III: rats were administered 50 µmol/kg b. w. AITC.

The second trial concerned AITC oral administration for 14 days:

- Group IV: Control (CON)
- Group V: rats were administered 25 µmol/kg b. w. AITC,
- Group VI: rats were administered 50 µmol/kg b. w. AITC,

**Experimental protocol:** AITC (Sigma–Aldrich.CH-9471Buchs, Germany) AITC was prepared by solution in distilled water. In the first experiment, the compound was given once and after 4 h the animals were decapitated. In the second research,

### Time-Dependent Effect Of Allvl Isothiocvanate.....

rats received the substance once a day as a single morning dose for 14 days and the animals were decapitated 4 h after the last administration. AITC was orally administered to AITC administered groups rats via stainless stomach tube<sup>(21)</sup>. Animals from groups I and IV received distilled water and served as controls

**Sampling of blood:** were obtained at the time of sacrifice and were allowed to clot for 2 hours at room temperature before centrifuging for 20 minutes at approximately 500 rpm<sup>(22)</sup>. The separated serum was stored at -20° C. Repeated freezing and thawing was avoided.

**Measurement of Carbohydrate and Lipid Parameters:** The serum was used for the determination of blood insulin, glucose, free fatty acids (FFA), triglycerides (TG), total cholesterol (CHL) and high-density lipoproteins (HDL). Insulin was assayed via a solid phase Enzyme Amplified Sensitivity Immunoassay for insulin was performed on microtitreplates by using the kit specific for insulin INS-EASIA, KAP1251 (BioSource Europe S.A.-Rue de L'Industrie, 8-B- 1400 Nivelles-Belgium). Glucose was determined by enzymatic colorimetric method, ENDPOINT (Joaquim Costa, 18, 2<sup>a</sup> planta. 08390 Montgat- Barcelona-Spain) after enzymatic oxidation in the presence of glucose oxidase. Serum FFA was assayed by quantitative colorimetric method using enzymatic<sup>TM</sup> FFA Assay Kits supplied by (Bioassay systems, USA), and Triglycerides were assayed by the method of **Fossati**,<sup>(23)</sup> (Cat. No. BK 8148 CGPO - PAP Method). Total cholesterol levels were measured by the enzymatic method of **Tietz**<sup>(24)</sup>. High-density lipoproteins were obtained from blood serum after precipitation of lipoproteins other than HDL using phosphotungstic acid and magnesium chloride according to **Demacker et al.**,<sup>(25)</sup>

**Statistical analysis:** The data obtained in the present study were

expressed as mean  $\pm$  SD for quantitative variables and statistically analyzed according to the methods described by **Kirkwood (1989)**<sup>(26)</sup>. The statistical analysis is done by using SPSS program (version 17) (SPSS Inc. Chicago, IL, USA). The metabolic effects of AITC in rats were evaluated by ANOVA test analysis of variance that used to compare means of more than two groups. Subsequent post hoc analysis to determine significant differences between two groups were performed by least significant difference (LSD) test. Test was considered significant at P values < 0.05.

### RESULTS

#### Effects of single oral administration of AITC on Carbohydrate and Lipid metabolism:

In the short term trial (4 h), the only metabolic changes were observed after high dose AITC administration. The single treatment of AITC 50  $\mu\text{mol/kg b. w}$  caused increment of glycaemia ( $P < 0.01$ ) when compared with the control group. On the other hand, both doses of AITC failed to affect the blood serum insulin, free fatty acids, triglycerides, total cholesterol and high-density lipoproteins ( $P > 0.05$ ) (Table 1).

#### Effects of 2 weeks oral administration of AITC on Carbohydrate and Lipid metabolism:

Surprisingly, the 2 weeks experiment brought many significant metabolic changes caused by the both doses of AITC. These changes concerned the alteration of blood serum parameters: FFA, total CHL and TG. The concentration of FFA, and total CHL was significantly elevated after 14 days administration of 25  $\mu\text{mol/kg b. w}$ . AITC, ( $P < 0.001$ ) and ( $P < 0.01$ ) respectively and after administration of 50  $\mu\text{mol/kg b. w}$  AITC ( $P < 0.001$ ) and ( $P < 0.01$ ) respectively when compared with control group. Serum TG concentration was reduced after administration of both doses ( $P < 0.01$ ) compared with the control group. Additionally, AITC at both doses caused considerable increase in the serum insulin concentration (AITC<sub>25</sub> ( $P < 0.01$ ); AITC<sub>50</sub> ( $P < 0.05$ ) when compared with the control group. On the other hand, both doses of AITC administration did not alter serum HDL and glucose concentrations ( $P > 0.05$ ) (Table 2) (Figure 1).

**Time-Dependent Effect Of Allvl Isothiocvanate.....**

**Table (1):** Effects of single oral administration of AITC 25 µmol/kg b. w and 50 µmol/kg b. w on Carbohydrate and Lipid metabolism:

Parameters ( $\bar{x} \pm SD$ )	CON (n=6)	AITC <sub>25</sub> (n=6)	AITC <sub>50</sub> (n=6)	F test	P value
Serum glucose (mg/dl)	100.1±7.8 (89-112)	98.1±10.5 (82-114)	122±13.4** (98-135)	9.032#	<0.05(S)
Insulin level (µIU/ml)	25.2±3.98 (20.8-31.5)	25.37±4.44 (20.5-31)	25.35±4.96 (23-28)	0.004	>0.05(NS)
Serum FFA (µMol)	197.5±7.3 (189-210)	196±8.8 (187-211)	198.2±9.3 (188-215)	0.102	<0.001(S)
Serum triglyceride (mg/dl)	164±9.8 (146-172)	163.5±10.5 (144-173)	164.7±12 (143-175)	0.018	<0.001(S)
Serum total cholesterol (mg/dl)	172.5±5.3 (167-182)	172.7±7.7 (166-188)	173±17 (166-186)	0.009	>0.05(NS)
Serum high density lipoprotein HDL (mg/dl)	60.5±4.8 (52-66)	60.2±5.8 (53-68)	60.3±6.5 (51-70)	0.005	>0.05(NS)

(#) significant ANOVA test (P<0.05).

(\*\*) significant when compared with control group (P<0.01).

**Table (2):** Effects of 2 weeks oral administration of AITC 25 µmol/kg b. w and 50 µmol/kg b. w on Carbohydrate and Lipid metabolism

Parameters ( $\bar{x} \pm SD$ )	CON (n=6)	AITC <sub>25</sub> (n=6)	AITC <sub>50</sub> (n=6)	F test	P value
Serum glucose (mg/dl)	103±8.7 (95-115)	102.1±10.7 (92-117)	107.2±6.9 (98-135)	0.575	>0.05(NS)
Insulin level (µIU/ml)	25.4±404 (20.7-33.2)	31.8±3.7** (28.1-38.3)	30.7±1.8* (28.9-33.5)	5.6#	<0.05(S)
Serum FFA (µMol)	198.5±8.9 (190-215)	238±16.6*** (210-254)	234±15.6*** (205-251)	14.3#	<0.05(S)
Serum triglyceride (mg/dl)	164.8±9.6 (148-175)	149.8±7.5** (140-159)	150.3±6.5** (141-60)	6.8#	<0.05(S)
Serum total cholesterol (mg/dl)	172.8±6.6 (166-185)	191.2±8.9** (179-206)	192.3±10.5** (176-205)	9.2#	<0.05(S)
Serum high density lipoprotein HDL (mg/dl)	59.8±6.2 (52-68)	62±5.6 (55-72)	61.2±7.6 (53-74)	0.169	>0.05(NS)

(#) significant ANOVA test (P<0.05).

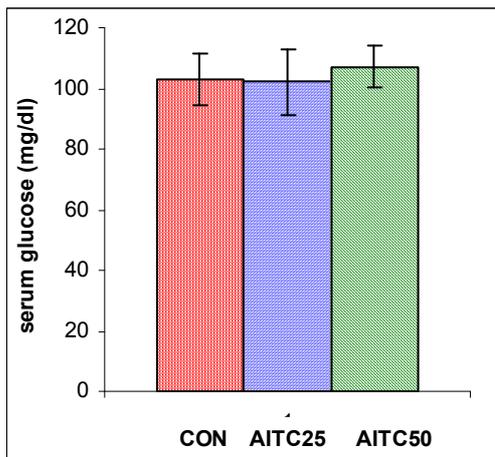
(\*) significant when compared with control group (P<0.05).

(\*\*) significant when compared with control group (P<0.01).

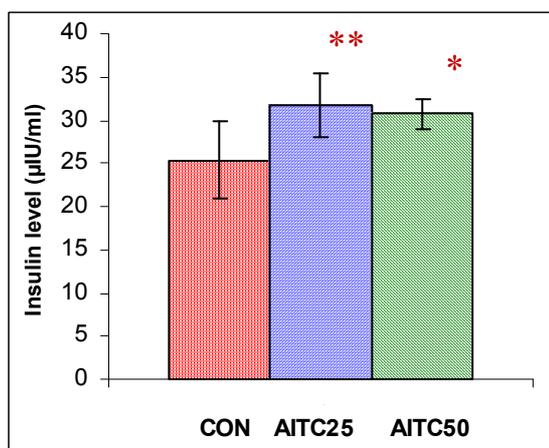
(\*\*\*) significant when compared with control group (P<0.001).

*Time-Dependent Effect Of Allvl Isothiocvanate.....*

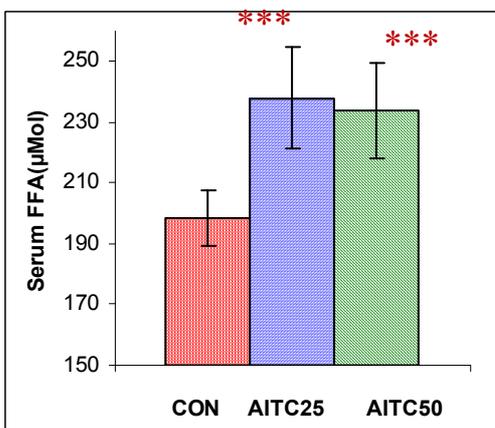
**Figure (1): Effects of 2 weeks oral administration of AITC on Carbohydrate and Lipid metabolism parameters**



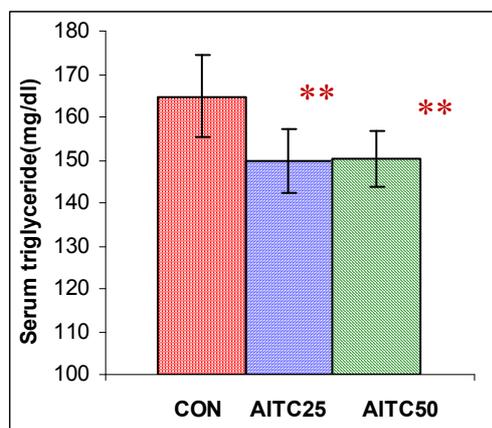
**Fig (1a):** Comparison between **Serum glucose concentration (mg/dl)** in the studied groups at the end of experimental period



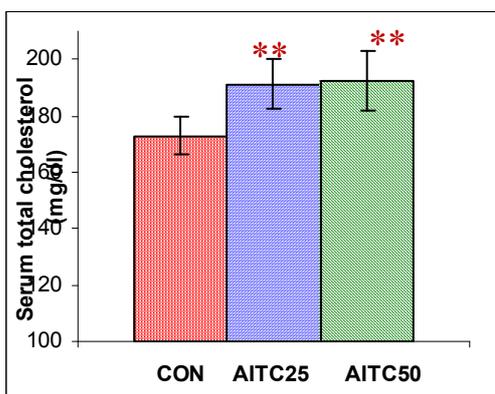
**Fig (1b):** Comparison between **serum Insulin level (µIU/ml)** in the studied groups at the end of experimental period



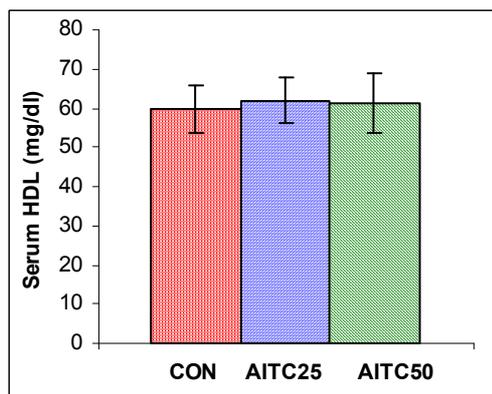
**Fig (1c):** Comparison between **Serum FFA concentration (µMol)** in the studied groups at the end of experimental period



**Fig (1d):** Comparison between **Serum triglyceride concentration (mg/dl)** in the studied groups at the end of experimental period



**Fig (1e):** Comparison between **Serum total cholesterol concentration (mg/dl)** in the studied groups at the end of experimental period



**Fig (1f):** Comparison between **Serum high density lipoprotein HDL concentration (mg/dl)** in the studied groups at the end of experimental period

(\*) significant when compared with control group (P<0.05).

(\*\*) significant when compared with control group (P<0.01).

(\*\*\*) significant when compared with control group (P<0.001).

### Time-Dependent Effect Of Allyl Isothiocyanate.....

#### DISCUSSION

The result of the present trials, following different duration of AITC ingestion shows that allyl isothiocyanate absorbed from gastrointestinal track quickly and revealed high biological activity on biochemical parameters. This result is in good agreement with other reports showing that the bioavailability of AITC is extremely high, as nearly 90% of orally administered AITC is absorbed<sup>(7)</sup>. Pharmacokinetic studies using orally administered isothiocyanates proved that ITCs undergo rapid absorption from the upper gastrointestinal tract. The rate of appearance of <sup>14</sup>C in the blood after dosing rats with [<sup>14</sup>C]allyl isothiocyanate (25 to 250 µmol/kg) is rapid, with a peak concentration of 10 to 100 µM occurring at 3 h<sup>(16)</sup>. The largest amount, accounting for 46% of the ingested dose, was present in the caecum and colon<sup>(2)</sup>. These data could explain why, in the present 4-hour trial, metabolic changes were observed after AITC administration. Higher dose of AITC caused glycaemia which could be explained by AITC caused a significant increase in adrenal sympathetic efferent nerve activity and AITC-induced adrenal catecholamine secretions are elicited through activation of adrenal sympathetic nerves via TRPA1 stimulation<sup>(6), (27)</sup>. Generally, adrenaline is known to increase hepatic glycogenolysis without simultaneous insulin elevation in the present conditions.

The obtained results from long term AITC administration (the alteration of FFA, TG and total CHL in the serum) could be explained by that AITC acts on liver and fat tissues. Previous studies showed that isothiocyanates are conjugated with glutathione and then sequentially metabolized to mercapturic acids (dithiocarbamates), which undergo the renal excretion<sup>(19)</sup>. The obtained metabolic changes in the present trial showed that besides the mercapturic acid pathway, isothiocyanates may follow enterohepatic circulation. In rats dosed

with (<sup>14</sup>C) allyl isothiocyanate, the higher value for biliary excretion confirms that some material undergoes such routes of metabolism. In tissues, the greatest amounts of radioactivity are retained in the liver, kidneys and intestinal mucosa<sup>(16)</sup>. This enterohepatic circulation of allyl isothiocyanate may exert in this way an impact on some intestinal and/or liver enzymes. It is highly probable that the noted elevation of cholesterol was caused by intensified cholesterol esterification by putative AITC influence on acyl-CoA:cholesterol acyltransferase. ACAT activation in the intestinal cells and/or the liver enhances the uptake of intestinal cholesterol and/or the incorporation of hepatic cholesterol into lipoproteins resulting in elevation of total serum cholesterol<sup>(28)</sup>. Enhancement of total serum cholesterol observed also in the performed trial could additionally prove this presumption.

A TG drop was also observed with concomitant exceptionally distinct FFA enhancement in the serum. Because of the above-mentioned enterohepatic circulation of allyl isothiocyanate, it is quite possible that its presence limited chylomicron synthesis in the intestine and led to the TG drop<sup>(2)</sup>. However, it may be also amplified by enhancement of lipoprotein lipase activity, which is a critical step in clearance of plasma TG, highly regulated by nutritional and hormonal status<sup>(16)</sup>. In mammals, the main modulator of LPL is insulin<sup>(29)</sup>. Enhancement of this hormone in the present research could additionally confirm this assumption.

Nevertheless, it is commonly known that the serum FFA is derived mainly from adipose tissue. The elevated supply of fatty acids to circulation by putative action of AITC on adipocytes is proved by the previous in vitro experiments where AITC potentiated basal lipolysis process at 10 µM<sup>(30)</sup>. Such AITC concentration in the blood is achieved after dosing rats with 25–50 µmol/kg allyl isothiocyanate<sup>(16)</sup>, the doses also used in the present research

### Time-Dependent Effect Of Allyl Isothiocyanate.....

Because both AITC doses stimulated significant insulin secretion without any rise in blood glucose concentration, a direct AITC influence not only on liver and intestine, but also on pancreas can be suggested. This organ previously was shown to be affected by glucosinolate breakdown derivatives<sup>(31)</sup>. It is not also possible to exclude indirect AITC action on this organ by the above-mentioned excessive release of FFA from adipose tissue. According to Boden<sup>(32)</sup>, acute increases in plasma levels of long-chain fatty acids can raise plasma insulin levels by stimulating insulin secretion or by decreasing insulin clearance.

Despite stimulated hepatic glycogenolysis<sup>(27)</sup>, surprisingly, the blood serum glucose level was unchanged. This phenomenon could be explained, once again, by the observed exceptionally high FFA levels in this research. As indicated by other researchers<sup>(33)</sup>, elevated serum FFA increase hepatic gluconeogenesis as well as reduce the ability of insulin to suppress this process in the liver, balancing the serum glucose concentration.

To conclude, the results of this study demonstrate that AITC biological activity depends on the time of administration. The metabolic effect of AITC is multidirectional, indicating its impact on many organs like liver as well as pancreas and intestine in rats. Unfortunately, its influence is adverse for the majority of metabolic pathways of carbohydrates and lipids in rat model and potentiated with the time of its action. Short AITC action enhanced glycaemia (at higher dose). Long AITC treatment leads to insulinaemia (action on pancreas), and cholesterolaemia (action on intestine and liver) and serum FFA increase (action on fat cells). Beneficial sides of their activity concerned triglycerides drop in the blood serum. AITC would be urged that any further increase (abuse?) in dietary AITC should be also preceded by

comprehensive investigations of their metabolic action.

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**Time-Dependent Effect Of Allyl Isothiocyanate.....**

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## Time-Dependent Effect Of Allvl Isothiocvanate.....

### تأثيرات أليل ايزوثيوسيانات المتوقفة علي الوقت علي بعض مؤشرات الايض في الفئران.

توجد مادة أليل ايزوثيوسيانات بتركيزات عالية في عائلة الكرنب وخاصة كرنب بروكسل وقد استخدمت خضروات هذه العائلة حديثا كغذاء للوقاية من حدوث السرطان.

#### الهدف من البحث

دراسة تأثير أليل ايزوثيوسيانات بجرعات مختلفة ولفترات زمنية مختلفة علي بعض مؤشرات ايض الكربوهيدرات والدهون في فئران التجارب.

#### نتائج البحث.

وجد أنه بعد اربعة ساعات من تناول الجرعة الكبيرة من أليل ايزوثيوسيانات حدث زيادة ذات دلالة احصائية في مستوي الجلوكوز في دم الفئران في حين ان تناول أليل ايزوثيوسيانات لمدة طويلة ادي الي تغيرات ملحوظة في ايض الكربوهيدرات والدهون في صورة ارتفاع في تركيز الانسولين والكوليستيرول والاحماض الدهنية الحرة وانخفاض في مستوي الدهون الثلاثية غي دم الفئران .

#### المستخلص من البحث:

في حين ان تناول الخضروات الصليبية بمستويات تعتبر طبيعية يمكن ان يكون مفيد لصحة الانسان الا انه اي زيادة في تناول هذه الخضروات قد يؤدي الي تأثيرات غير محمودة. هذه التأثيرات تعتمد علي طول فترة تناول المادة قيد البحث في صورة زيادة مستوي الجلوكوز في الدم مع الاستعمال لفترة قصيرة وزيادة مستوي الانسولين والكوليستيرول مع الاستعمال لفترة طويلة . في حين انه هناك تأثيرات محمودة لاستعمال أليل ايزوثيوسيانات في صورة تقليل مستوي الدهون الثلاثية في الدم.