

The Impact of Concomitant administration of Antiarrhythmic agent (Amiodarone) with Mustard oil on thyroid gland in Experimental Animals



Pharma

KEYWORDS : Amiodarone, Mustard oil; Goitergenic effect.

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ABSTRACT

Natural herb products are used widely in different societies, as they had been used since the Ancient age. Mustard seeds are the small round seeds of various mustard plants, which is a cruciferous vegetable related to broccoli, Brussels sprouts and cabbage. There are three principal types used to make mustard seeds: black mustard (*Brassica nigra*), white mustard (*Brassica alba*) and brown mustard (*Brassica juncea*). Like other Brassicas, mustard seeds contain plentiful amounts of phytonutrients called glucosinolates, so used form many diseases like cancer and inflammation. On the other hand, amiodarone hydrochloride (2-butyl-3-benzofuranyl 4-(2-diethylaminoethoxy)-3,5-di-iodophenyl ketone hydrochloride). It is a Class III antiarrhythmic agent prolonging the action potential duration and hence refractory period of atrial, nodal and ventricular tissues, thereby giving a very broad spectrum of activity. An increase in the refractory period of the atrial cells is a major contributing action to the control of atrial tachyarrhythmias. Aim of the work: This study is aimed to assess the possible goitergenic of amiodarone and mustard oil if they are given together by measuring thyroid function tests including (TSH, T3 and T4), weight of thyroid gland, Heart enzyme CK and the weight of the heart as well as ECG readings, in addition, the liver function enzymes (SGOT and SGPT) all these will be measured. Materials and methods: 30 adult male rabbits (weight range 1250-2000 g and age-9-12 months) were divided randomly to four groups each contain 8 animals except control group contains 6 animals. Second, third and fourth groups were given mustard oil (2g/d), amiodarone (8mg/kg) and combined (mustard oil 2g/d + amiodarone 8mg/kg) intragastric therapy respectively for 2 weeks. First group was kept as control. Results: Combination of mustard oil and amiodarone was shown significant increase in thyroid hormones (T3 and T4). This effect was supported by significant increased in the thyroid gland weight and reduction in body weight. In addition, mustard oil in combination form was reduced the liver function enzyme especially SGOT. Conclusion: from this study we approved that mustard oil may increase the goitergenic effect of amiodarone if they are administered concomitantly for long time. On the other hand this oil has hepato- and cardioprotective effect and can ameliorate the toxic effects of amiodarone on liver and heart.

INTRODUCTION

Natural products are widely distributed in different societies, as they had been used from the Ancient. Mustard seeds are the small round seeds of various mustard plants. The seeds can come from three different plants: black mustard (*Brassica nigra*), brown Indian mustard (*B. juncea*), and white mustard (*B. hirta/Sinapis alba*) (Thimmulappa RK, Mai KH, Srisuma S et al, 2002). Biologically active compounds in various mustards include allyl isothiocyanate, alpha-linolenic acid, sinalbin, sinigrin, gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, and sinapic acid. Components of mustard have been shown to have antimutagenic, antiadipic, antifungal, antimicrobial and antioxidant effects (Sinapis alba L. USDA, NRCS. 2009(2)). On the other hand, amiodarone is 2-butyl-3-benzofuranyl 4-(2-diethylaminoethoxy)-3,5-di-iodophenyl ketone hydrochloride related to Class III antiarrhythmic agent prolonging the action potential duration and hence refractory period of atrial, nodal and ventricular tissues, thereby giving a very broad spectrum of activity.

An increase in the refractory period of the atrial cells is a major contributing action to the control of atrial tachyarrhythmias (Rosenbaum MB, Chiale PA, Halpern MS, et al; 1976 and American Heart Association Guidelines; 2010).

Therefore, the aim of this work is to assess the possible goitergenic effect of amiodarone and mustard oil if they are given together through measuring Thyroid function tests including (TSH, T3 and T4) and the weight of thyroid gland as well as body weight.

MATERIALS AND METHODS

Thirty rabbits were used in this study taken from faculty's animal house with weight of 1250-1500g and age of (9- 12) months. They were isolated in a relatively controlled environment at a temperature of about 37°C with free access for tap water and food ad libitum. They were acclimatized to laboratory conditions for five days before commencement of treatment. Duration of experiment was continued for 2 weeks (Burger A; 1976). Then they were split into four groups randomly of six animals in each group. Second, third and fourth groups were allowed to take 2g/kg of mustard oil (freshly prepared by squeezing the black mustard seeds by cold method, 8mg/kg of amiodarone and combination (2g/kg of mustard oil +8mg/kg of amiodarone oil) respectively, while first group was kept as

control only taken 15 mg / kg physiological normal saline. The preparations were given to all groups by Orogastric tube for 2 weeks.

At the end of the experiment the blood samples were collected by the heart puncture and prepared the sera to measure thyroid gland hormones T4 (Ekins RP, Ellis SM), T3 (Klee GG; 1996) and thyroid stimulating hormone (TSH) (Wheeler MH, Lazarus JH; 1994) by the (ELISA method). In addition, weights of thyroid gland was taken and compared with a control group.

Results of representative values were expressed as mean \pm standard error of the mean (S.E.M). Comparisons between intra groups were made using independent Student's t-test (Version 18). P-Values below 0.05 were considered statistically significant.

RESULTS

Combined agent (mustard + amiodarone) produced significant increase in the level of T3 from 1.78 \pm 0.334 pg/ml in control group to 3.81 \pm 0.397 pg/ml in combined group. In addition, the weight of thyroid gland was significantly increased (P<0.05) from 0.673 \pm 0.063 g in control group to 1.20 \pm 0.080 g in combined group. This effect was supported by significant reduction (P<0.05) in total body weight of animals from 1850 \pm 42.3g in control group (before intake of combined) to 1650 \pm 56.6g after combined administration. On the other hand, administration of mustard oil concurrently with amiodarone produced significant hepatoprotective effect (P<0.05). Mustard reduced cardiac enzyme creatine kinase (CK) but insignificantly (P>0.05). This may be related to the duration of this experiment.

Figure (1a): The effect of oral administration of mustard oil (2g/d) and amiodarone 8mg/kg on average (mean \pm SE) thyroid function tests in adult male rabbits for 2weeks

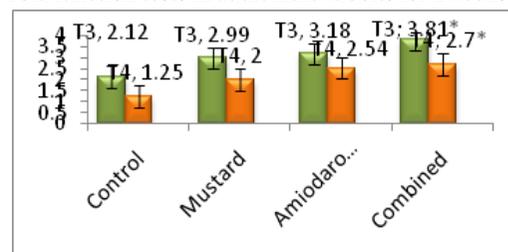


Figure (1b): The effect of oral administration of mustard oil (2g/d) and amiodarone 8mg/kg on average (mean±SE) thyroid function tests in adult male rabbits for 2 weeks

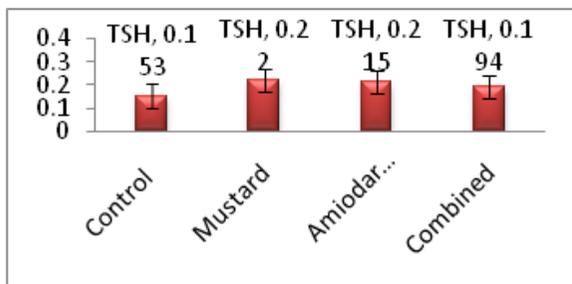


Table (1): The effect of oral administration of mustard oil (2g/d) and amiodarone 8mg/kg on average (mean±SE) thyroid function tests in adult male rabbits for 2 weeks

Parameters	MeanT3 (pg/ml) M±SE	MeanT4 (ng/dl) M±SE	Mean TSH (uU/L) M±SE
Control	1.78±0.334	1.25±0.312	0.153±0.065
Mustard p-value	2.99±0.52 0.20	2.00±0.392 0.26	0.22±0.07 0.62
Amiodarone p-value	3.18±0.522 0.37	2.54±0.481 0.17	0.215±0.09 0.88
Combined p-value	3.81±0.397 0.045*	2.70±0.204 0.046*	0.194±0.08 0.96

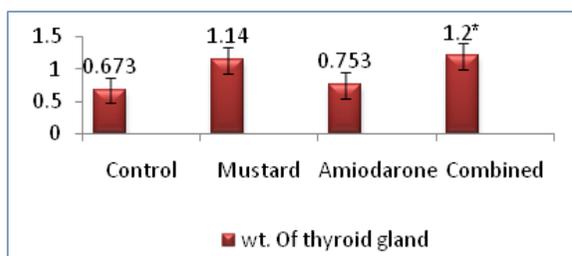
*Significant as compared with control at P<0.05

Table (2): The effect of oral administration of mustard oil(2g/d) and amiodarone 8mg/kg on average (mean±SE) weight of thyroid gland(g) and body weight(g) in adult male rabbits for 2 weeks

Parameters	Mean wt. of thyroid gland(g) M±SE	Mean body wt. (g) at zero time M±SE	Mean body wt. (g) end of treatment M±SE
Control	0.673±0.063	1266±21.0	1250±60.1
Mustard p-value	1.14±0.138 0.05	1500±26.7	1412±44.1 0.006*
Amiodarone p-value	0.753±0.046 0.055	1433±21.1	1350±95.0 0.34
Combined p-value	1.20±0.080 0.034*	1850±42.3	1650±56.6 0.001*

*Significant compared with control (baseline) group at (P<0.05)

Figure (2a): The effect of oral administration of mustard (2g/d) and/or amiodarone 8mg/kg on average (mean±SE) weight of thyroid gland in adult male rabbits for 2 weeks.



*Significant compared with control group at (P<0.05)

Figure (2b): The effect of oral administration of mustard (2g/d) and/or amiodarone 8mg/kg on average (mean±SE) body weight of adult male rabbits for 2 weeks.

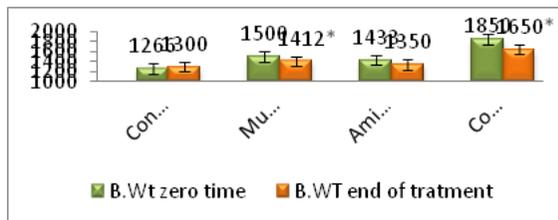


Table (3): The effect of oral administration of mustard (2g/d) and/or amiodarone 8mg/kg on average (mean±SE) liver function enzymes in adult male rabbits for 2 weeks.

Parameters	Mean SGOT(U/L) M±SE	Mean SGPT (U/L) M±SE
Control	52.02±5.57	69.4±7.97
Mustard p-value	60.15±12.3 0.061	73.2±7.9 0.435
Amiodarone p-value	106.3±17.2 0.009*	90.79±13.7 0.274
Combined p-value	66.5±5.57 0.01*	74.5±12.3 0.257

*Significant compared with control group at (P<0.05)

Figure (3): The effect of oral administration of mustard (2g/d) and/or amiodarone 8mg/kg on average (mean±SE) liver function enzymes in adult male rabbits for 2 weeks.

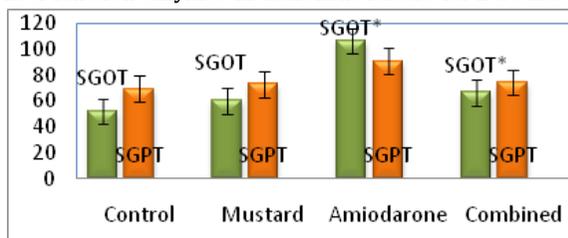
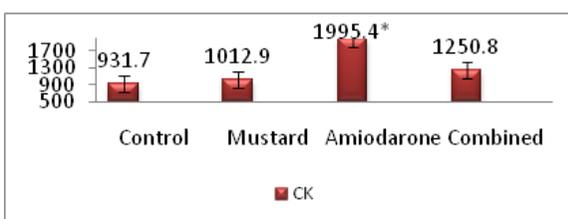


Table (5): The effect of oral administration of mustard (2g/d) and/or amiodarone 8mg/kg on average (mean±SE) heart parameters in adult male rabbits (n=6) for 2 weeks

Parameters	Mean CK(U/L) M±SE	Mean Wt. of the heart (g) M±SE
Control	931.7±638.4	3.93±0.604
Mustard p-value	1012.9±284.3 0.631	4.46±0.495 0.07
Amiodarone p-value	1995.4±995.7 0.044*	5.28±0.33 0.56
Combined p-value	1250.8±375.4 0.07	4.88±1.54 0.10

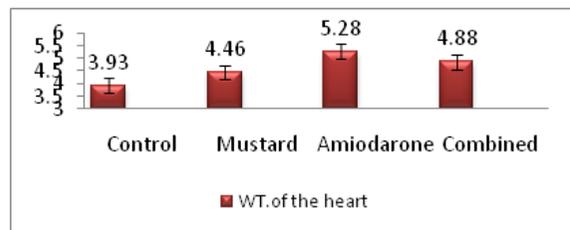
*Significant compared with control group at (P<0.05) –CK: creatinin kinase

Figure (7a): The effect of oral administration of mustard (2g/d) and/or amiodarone 8mg/kg on average (mean±SE) heart parameters in adult male rabbits (n=6) for 2 weeks



*Significant compared with control group at (P<0.05)-

Figure (7b): The effect of oral administration of mustard (2g/d) and/or amiodarone 8mg/kg on average (mean±SE) heart parameters in adult male rabbits (n=6) for 2weeks



DISCUSSION

The normal thyroid gland secretes sufficient amounts of the thyroid hormones- triiodothyronine (T3) and tetraiodothyronine(T4, thyroxine) to normalize growth and development, body temperature, and energy levels. These hormones contain 59% and 65% (respectively) of iodine as an essential part of the molecule (Dong BJ and Greenspan FS, 2012).

This is the first study concentrated on the possible interaction between mustard oil and amiodarone in an animal model.

There are commonly foods known as Goitrogenic foods (vegetables of the Brassica family), which mean that they contain substances prevent thyroid gland from getting its necessary amount of iodine. If eaten in excess, they interfere with the healthy function of thyroid gland, tilting in the direction of hypothyroidism, hyperthyroidism or even development of goiter (Mary JS; 2005).

In the present study, concomitant administration of mustard oil and amiodarone was resulted in significant increased in thyroid hormones T4 and T3. This effect was accompanied by increasing the weight of thyroid gland as well as reduction in the body weight of tested animals. All these findings are indicated the possible goiterogenic and hyperthyroidism of both agents. Amiodarone is a benzofuranic-derivative iodine-rich drug widely used for the treatment of tachyarrhythmias. It often causes changes in thyroid function tests (Martino E, 2001).

In 14-18% of amiodarone-treated patients, there is overt thyroid dysfunction, either amiodarone-induced thyrotoxicosis (AIT) or amiodarone-induced hypothyroidism (AIH) (Martino E, 2001). Amiodarone contains about 37.3% by weight of iodine, so abnormalities in thyroid function are common. Amiodarone is structurally similar to thyroxine (a thyroid hormone), which contributes to the effects of amiodarone on thyroid function (British National Formulary; 2012). It often causes changes in thyroid function tests (typically an increase in serum T(4) and rT(3). (Martino E, 2001).

On the other hand, extracts of Brassica plants including mustard showed anti-thyroidal activity in vitro. Excess iodide reversed the anti-TPO activity to same extent but could not neutralise it (Chandra AK,2004). In hyperthyroids, goitrogens may help suppress thyroidal function until normal thyroidal functioning can be restored. However, this may not be a good strategy.

Goitrogens work by interfering with the thyroidal uptake of iodine. While many hyperts try to limit thyroid output by iodine restriction, this strategy can backfire. Iodine restriction will cause the thyroid to increase in size (goiter) in an effort to filter more blood to get more iodine.

When iodine is then re-introduced to the diet, medications or accidentally ingested, the larger thyroid gland has the capacity for greater thyroid hormone production (Babademez, M. A.; et al. 2010).

With regarding to our combination, both of them contain iodine. Presence of iodine may allow TSHR-Ab to be effective in stimulating production of thyroid hormones as well as damaging thyroid cells directly and release antigen to the immune system resulted in goiter development (Benbassat et al., 2000, and Noel et al., 2002)

However, some studies found that hepatotoxicity and induction of atypical ventricular tachycardia occurs quite commonly in patients undergoing treatment with amiodarone. Rosenbaum MB, et al. (1976) and Passman RS, et al. (2012)

In this study, mustard oil showed significant hepatic and cardioprotective effect as it reduced the biochemical markers including liver and cardiac function enzymes, especially SGOT and CK. This effect may be related to the presence of

Polyphenols and flavones that possess antioxidant activity. This activity is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators and reductants of ferryl hemoglobin (Gebicka, L. and E. Banasiak, 2009). All these effects may reduce free radical damaging the vital organs. (Nair S and R. Gupta. 1998)

CONCLUSION

From this study we approved that mustard oil may increase the goiterogenic effect of amiodarone if they are administered concomitantly for long time. On the other hand this oil has hepato- and cardioprotective effect and can ameliorate the toxic effects of amiodarone on liver and heart. The free radicals scavenging effects of this agent could be attributed to its higher polyphenols and flavones contents.

REFERENCE

- American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care (November 2010). "Part 8: Adult Advanced Cardiovascular Life Support". *Circulation* 122 (18 Suppl): S729-S767. | -Babademez M. A.; Tuncay, K. S.; Zaim, M.; Acar, B.; Karasen, R. M. (2010). "Hashimoto Thyroiditis and Thyroid Gland Anomalies". *Journal of Craniofacial Surgery* 21 (6): 1807-1809. | -Benbassat C. C., Mechlis-Frith S., Cohen M., and Blum I. (2000). Amiodarone-induced thyrotoxicosis type 2: A case report and review of the literature. *Am J Med Sci.* 320 (4): 288-291. | -British National Formulary (BNF) (2012). *Drugs of arrhythmia*. Royal Pharmaceutical Society of Great Britain, pp: 71-72 | -Burger A, Dinichert D, Nicod P, Jenny M, Lemarchand-Beraud T, Vallotton MB 1976 Effect of amiodarone on serum triiodothyronine, reverse triiodothyronine, thyroxine, and thyrotropin. *J Clin Invest* 58:255-259 | -Chandra AK, Mukhopadhyay S, Lahari D, Tripathy S (2004 May). Goitrogenic content of India cyanogenic plant foods & their in vitro anti-thyroidal activity. *Indian J Med Res;* 119 (5): 180-5 | -Dong BJ and Greenspan FS (2012). *Thyroid and antithyroid drugs in: Basic & Clinical Pharmacology*. Katzung BG, Masters SB and Trevor AJ (ed.). Mac Graw Hill (12th edition), pp:681-696. | -Ekins RP, Ellis SM (1975). The radio-immunoassay of free thyroid hormones in serum. In Robbins J, Braverman LE (eds). *Thyroid research, proceedings of the Seventh International Thyroid Conference*, Boston. Amsterdam, Ecerpta Media: 597 | -Gebicka, L. and E. Banasiak (2009). Flavonoids as reductants of ferryl hemoglobin. *Acta Biochim. Pol.*, 56: 509-513. | -Klee GG (1996). Clinical usage recommendations and analytic performance goals for total and free triiodothyronine measurements. *Clin Chem;* 42:155-159. | -Martino E, Bartalena L, Bogazzi F, Braverman LE (2001 Apr). The effects of amiodarone on the thyroid. *Endocr.*; 22(2):240-54. | -Mary JS (2005). *The Thyroid Diet Revolution: Manage Your Metabolism for Lasting Weight Loss*, Book (2nd ed.) | -Nair, S., R. Nagar and R. Gupta (1998). Antioxidant phenolics and flavonoids in common Indian foods. *J. Assoc. Physicians India.*, 46: 708-10. | -Noel R.R., Raphael B., and Burek C.L. (2002). Iodine: an environmental trigger of thyroiditis. *Autoimmun. Rev.* 1 (1-2): 97-103 | -Passman RS, Bennett CL, Purpura JM, Kapur R, et al. (2012) "Amiodarone-associated Optic Neuropathy: A Critical Review" *Am J of Med*, 125:5, (447-53) | -Rosenbaum MB, Chiale PA, Halpern MS, et al. (1976). "Clinical efficacy of amiodarone as an antiarrhythmic agent". *Am. J. Cardiol.* 38 (7): 934-44. | -Sinapis alba L. USDA, NRCS. 2009. *The PLANTS Database* (<http://plants.usda.gov>, November 2009). National Plant Data Center, Baton Rouge, LA 70874-4490 USA. | -Thimmulappa RK, Mai KH, Srisuma S et al (2002). Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. *Cancer Res* 2002 Sep 15;62(18):5196-5203. | -Wheeler MH, Lazarus JH (1994). *Diseases of the thyroid*. London, glasgow, weinheim, New York, Melbourne, madras: chapman and hall medical ,