Journal of
APPLIED
BIOMEDICINE

J. Appl. Biomed.
5: 131–138, 2007
ISSN 1214-0287

ORIGINAL ARTICLE

The role of a mixture of green tea, turmeric and chitosan in the treatment of obesity-related testicular disorders

Mohamed El-Sweedy[1], **Nabil Abdel-Hamid**[2], **Mohamed El-Moselhy**[3]

[1]Biochemistry Departments, College of Pharmacy, Zagazig University, Egypt
[2]College of Pharmacy, El-Minia University, Egypt
[3]Pharmacology Department, College of Pharmacy, El-Minia University, Egypt

Received 25th October 2006.
Revised 16th December 2006.
Published online 16th March 2007.

Summary

In the present investigation, we studied the effect of aqueous green tea extract (GTE), alcoholic turmeric extract (ATE), and water-soluble Chitosan (WSC), individually/or in mixture, on the testicular tissue content of total cholesterol (TC), triglycerides (TG), phospholipids (PL), and thiobarbituric acid reactive substance (TBARS), in addition to nitric oxide (NO) in obese rats.

The testicular weight of the obese rats was increased more significantly than control; TC, TG, PL, TBARS and NO were significantly higher in the obese group. GTE reduced testicular weight and significantly reduced other estimated parameter. ATE significantly increased testicular weight, with apparent peritesticular vascular congestion. It significantly decreased all other studied parameters. WSC significantly increased testicular weight, with significant reduction of all other parameters. The mixture of the three drugs non-significantly decreased testicular weight, and significantly decreased other parameters, except NO, which was significantly more elevated than the obese control. We concluded that obesity induced a significant increase in testicular weight, in addition to TC, TG, PL, TBARS and NO, in comparison to the normal control subjects. An efficient protection against obesity-induced changes was achieved by each individual drug, while the mixture of GTE, ATE and WSC showed less protective potential than each individual drug.We here recommend the use of GTE, ATE in treating obesity-related testicular dysfunction and suggest that attention should be paid to the possible effect of WSC on the bioavailability of other concomitantly-used drugs and suggest a pertinent clinical benefit of both GTE and ATE.

Keywords: obesity – green tea – turmeric – Chitosan – testes – rats

✉ Nabil Mohie Abdel-Hamid, Department of Biochemistry, Faculty of Pharmacy, El-Minia University, El Minia, Egypt
nabilmohie@yahoo.com
☎ +20506913997
+20106426998

INTRODUCTION

Obesity is a common problem in affluent countries. Reduced energy expenditure from exercise or metabolism or both, may be an important contributory factor in developing obesity. Also, failure in reducing food intake sufficiently to maintain energy balance is a another strong cause. Obese persons are at high risk of heart attack, stroke, hypertension, diabetes mellitus, gall bladder

diseases and death (Bray and Gray 1988). More than 90% of body energy is stored as triglyceride in adipose tissue. Protein and glycogen provide only smaller quantities of energy. Triglycerides are stored in fat cells that differ in size from one region in the body to another (Bjorntorp and Ostman 1971).

Rodents showed different genotypes for obesity, some strains have autosomal dominant, others recessive or polygonal mutations (Festing 1979). Likewise, rare forms of human obesity have a pure genetic basis (Bray 1989). In massively obese males, there is a decrease in testosterone, sex hormone binding globulin and free testosterone (Glass et al. 1981) and (Kley et al. 1979) It was reported that spermatogenesis is disrupted by free radical toxicity. Thus, the study of oxidative stress is a determinant in exploring some aspects affecting fertility (Aruldhas et al. 2005).

Changes in the testicular lipid profile, were strongly correlated to testicular degeneration, histochemical and biochemical disturbances (Chowdhury et al. 1990). These changes were also associated with increased lipid, DNA oxidative damage and depletion of lipid-soluble antioxidants (Lucesoli and Fraga 1995).

The use of herbal medicine increases every day and still finds a wide use worldwide. Traditional herbs have more acceptance than prescription drugs in many cultures with emerging epidemics of obesity. This was mostly attributed to being safer than drugs, from the point of view of patients. Also patients believe that by using this type of medication there is no need for a physician, and it may be a relevant attempt to compensate for drug failure in managing obesity. Some of these alternatives were, green tea and a binding resin (chitosan), which can precipitate lipids, leading to depressed fat absorptivity (Heber 2003). Different studies showed that both green and black tea contain flavonoids such as quercetin, kaempferol and myricetin, which are potent anti-carcinogenics (Herlog et al. 1993). It also reduced the risk of colon, stomach, lung and skin cancers (Challa et al. 1997).

The use of turmeric extract in a group of herbs as a treatment for diabetes, induced fatty liver and showed a significant reduction in triglyceride, total cholesterol and phospholipids-content in diabetic rats (Saravanan and Pari 2005).

The present study was mainly conducted to study possible metabolic disturbances in rat testicular tissue after induction of obesity, and whether some known anti-obesity natural products, as GTE, ATE and a well-defined carbohydrate polymer (WSC) – if used individually or together – may have a corrective potential in relation to testicular dysfunction. This was achieved by an estimation of the testicular content of total cholesterol (TC), triglycerides (TG), phospholipids (PL), thiobarbituric acid reactive substance (TBARS), in addition to nitric oxide (NO) in obese rats.

MATERIALS AND METHODS

Animals and Experimental Design

Sixty male Wistar rats, weighing 100–110 g, of 6–8 weeks old, were obtained from Animal House, Veterinary Medical College, University of Zagazig. The animals were fed a standard pellet diet (purchased from the same animal house) and allowed free access to water for one week to acclimatize. They were housed in a temperature-controlled room (temperature ranged from 19–23 °C), with a 12 hours light and 12 hours dark cycle during the whole period of the experiment. The standard feed used had the following composition: 23.5% protein, 48.9% carbohydrate, 5% lipid, 12% water, 5.7% ash and 4% cellulose. This diet continued only for the control group till the end of the experiment (Llado et al. 1995).

The animals were sub-divided into 6 equal groups, 10 animals/each. The remaining 5 groups were fed high fat (cafeteria) diet for 35 days (Geremias et al. 2006) This diet consisted of 9% protein, 21% carbohydrate, 13.6% lipid, 51.3% water and 5.1% fibers (Serra et al. 1987). The second group was fed only cafeteria diet, served as obese control and was not given drugs. The third group was given green tea (*Camellia sinensis*), 2% aqueous extract as drinking liquid, simultaneously with the cafeteria diet (Challa et al. 1997). The fourth group was given alcoholic turmeric extract (*Curcuma longa*) as (25 mg/kg), daily by intragastric tube. This extract was prepared by extracting 500 g of chopped rhizomes with 1.5 liters of 95% ethanol, by soaking overnight, filtering, re-extracting the sediment and filtering again. Both filtrates were mixed, evaporated in a rotavapor at 40–50 ˚C, under reduced pressure, re-dissolved into alcohol to give a final concentration of 40 mg/dl, and then the dose was calculated to 25 mg/kg, body weight (Saravanan and Pari 2005). The fifth group was given WSC as 4% in the cafeteria diet (Geremias et al. 2006).

The sixth group was given a mixture of the three drugs in the same mentioned doses. All drugs were given by intragastric tube as a single daily dose for 35 days.

After 35 days, on the morning of the next day, the animals were killed, the testicles were removed, kept frozen in liquid nitrogen at –80 ˚C to the day of analysis.

Drugs and chemicals

All drugs and chemicals were of analytical grade and purchased from local suppliers.

Methods

The testes were thawed, weighed (as wet weight), the gross features were observed, recorded, decapsulated and cut into small pieces (Wang et al. 1997). Lipids were extracted from tissues by the method of Folch et al. (1957). TC was estimated by the method of Zlatkis et al. (1953). To 0.1 ml of the lipid extract, 9.9 ml of ferric chloride – acetic acid reagent were added, mixed, allowed to stand for 15 minutes and centrifuged. 5 ml of the supernatant were mixed with 3 ml conc. sulfuric acid. The developed color was read after 20 minutes at 560 nm against the reagent blank.

TG were determined by the method of Bucolo and David (1973) in an aqueous liquid prepared from the lipid extract (chloroform/methanol, 2:1). A certain volume of the lipid extract was evaporated in a boiling water bath, the sediment was dissolved in 0.2% Triton X100 to give an aqueous medium (Ide et al. 2004).

The phospholipid content was colorimetrically determined in the total lipid extract, depending on the phosphorus content, without acid digestion, to exclude interference of inorganic phosphorus (Raheja et al. 1973). Egg yolk was extracted by chloroform/methanol, and serially diluted for preparation of the standard curve, from which the PL content was calculated.

Nitric oxide was extracted from the tissue in 100 mM phosphate buffer, pH 7.4, containing 17 mM sulfanilamide and 0.4 mM N–(1–naphthyl) ethylenediamine dihydrochloride as described elsewhere (Nims et al. 1995). NO was then spectrophotometrically-determined utilizing copper – cadmium alloy as a reducing agent. This method basically relied on the reduction of nitrate into stable nitrite (Gries reaction) (Sastry et al. 2002). A standard curve was obtained from different concentrations of sodium nitrite (Bauche et al. 1998).

Lipid peroxidation product (malondialdehyde) was colorimetrically estimated as TBARS according to the method of Niehius and Samuelsson (1968). Briefly, 0.1 ml of tissue homogenate in Tris-HCl buffer (pH 7.5) was treated with 2 ml of thiobarbituric acid 0.37–15% trichloroacetic acid and 0.25 N HCl, 1:1:1 (TBA – TCA – HCl) reagent. The mixture was then placed in water bath for 15 minutes, cooled and the absorbance of the clear supernatant was measured at 535 nm.

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). Values of $p<0.05$ were considered statistically significant (Duncan 1957).

RESULTS

Table 1 shows that feeding the animals with cafeteria diet increased testicular weight significantly more than the control. It also significantly elevated the testicular content of TC, TG, PL, TBARS and NO. Treatment with GTE significantly decreased these values, in comparison to obese rats, except TC, which was left to increase significantly more than than the obese control. ATE significantly induced peri-testicular congestion in the veins of all treated animals. It significantly increased testicular weight compared to the obese control; and it significantly decreased other parameters in comparison to obese control. Chitosan treatment significantly increased testicular weight compared to obese control), but also significantly decreased other values compared to obese control subjects. The mixture of the three drugs non-significantly elevated testicular weight compared to obese control, but significantly decreased TC, TG, PL, TBARS, although it significantly elevated NO content compared to obese control.

DISCUSSION

The base of information about obesity has dramatically expanded in the last three decades. The importance of fat distribution as a health risk has added a new dimension to this problem (Bray and Gray 1988). It was proved that hormone-sensitive lipase (HSL) is essential for spermatogenesis. This enzyme is necessary for hydrolysis of triacylglycerol and cholesteryl esters in many tissues, including ovaries and testes. This role of the enzyme shed light on the importance of lipids in studying testicular derangements (Osuga et al. 2000). Nevertheless, the absence of HSL from Leydig cells (testicular cells, responsible for testosterone production) did not affect the plasma testosterone level. Thus, oligospermia did not result from hypogonadism in some isolated testicular cells *in vitro* (Sassone–Corsi 1979).

This controversial testosterone level depending /or non-dependent on HSL was a reason why serum testosterone level was not estimated in the present work. In the present study, the induction of obesity lead to a significant increase in testicular weight, TC, TG, PL, TBARS and NO contents. This variation was also recorded before in some strains of mice, compared to their homozygous lean controls, in the form of lipid accumulation, associated with decreased lipolysis in isolated Leydig cells. These changes in the testicular endocrine function of obese mice were interpreted as a possible consequence of pituitary dysfunction (Khun-Velten et al. 1986).

Table 1: **Variations in testicular total cholesterol, triglycerides, phospholipids, thiobarbituric acid reactive substance and nitric oxide in obese rats, treated with green tea, turmeric extracts and chitosan for 35 day** (values are given as mean±SEM, n=10)

Group Parameter	Control	Obese control	Green tea	Turmeric	Chitosan	Green tea + Turmeric + Chitosan
Weight of single testis (g)	0.87 ±0.03	0.99* ±0.05	0.93 ±0.02	1.30# ±0.02	1.10# ±0.03	0.96 ±0.01
Total Cholesterol (mg/g wet tissue)	11.3 ±1.1	14.7* ±0.1	17.1# ±0.1	7.2# ±0.1	9.8# ±0.1	12.2# ±0.1
Triglycerides (mg/g wet tissue)	31.10 ±0.73	40.00* ±0.80	20.00# ±2.40	11.10# ±0.60	9.30 ±0.32#	4.00# ±0.32
Phospholipids (mg/wet tissue)	43.20 ±0.53	100.00* ±1.10	25.40# ±0.37	35.10# ±0.48	17.60# ±0.33	56.80# ±0.70
TBARS (nmol/g wet tissue)	403.0 ±2.1	546.6* ±3.4	358.1# ±3.4	366.0# ±4.1	433.0# ±4.2	388.1# ±4.1
Nitric Oxide (nmol/g wet tissue)	490.0 ±3.6	625.1* ±3.8	180.0# ±2.5	536.1# ±3.1	586.0# ±3.8	802.0# ± 2.8

* Significantly different from control
# Significantly different from obese control

The increased cholesterol levels were reported to be an important risk of testicular cancer (Dobson 2005)

Spermatozoa are rich in polyunsaturated fatty acids and more liable for lipid peroxidation by reactive oxygen species (ROS).

The oxidation product (TBARS) increases in most spermatogenic disturbances (Sharma and Agarwal 1996). Increased testicular lipids, in conjunction with obesity may lead – in addition to varices – to obstructive azospermia, which was reported to be associated with increased tissue nitrite and TBARS (Basar et al. 2006).

Treatment with GTE significantly decreased testicular TG, PL, TBARS, NO and non-significantly testicular weight, but couldn't retain TC content. It was shown that testicular cholesterol exists in three different forms: free, ester and sulfate. The free form is about 91% at all ages, which increases by maturation (Connor et al. 1997). The antioxidant activity of GTE was previously reported in mice. It also protected DNA from oxidative damage (Shi et al. 1994).

It also inhibited both lung (Wang et al. 1992) and liver (Wang et al. 1988) carcinomas. Increased NO generation in testes showed an inhibitory effect on steroidogenesis by Leydig cells, both *in vivo* (Adams et al. 1994) and *in vitro* (Del Punta et al. 1996). Thus, decreasing NO level may activate spermatogenesis, that may be inhibited by obesity-related NO accumulation.

Our results revealed that turmeric treatment significantly decreased TC, TG, PL, TBARS and NO contents, although it significantly increased testicular weight, in comparison to obese rats. The decreased content of lipids concomitantly with elevated weight, is most probably due to the prominent congestion of peri-testicular veins. This congestion wasn't clear in other groups. Turmeric has many active components, but curcumin is the most potent ingredient. It is a powerful ani-inflammatory and anti-oxidant and has greater effects in preventing free radical damage, compared with vitamins C, E and superoxide dismutase (Sharma 1976).

This anti-oxidant activity is clear on the testicular level, which is manifested in decreased TBARS and NO contents. This will correct the possible inhibitory effect of elevated NO on Leydig cell steroidogenesis, that may be inhibited by obesity (Adams et al. 1994). The elevated testicular TC content induced by obesity may be due to

impaired utilization in steroidogenesis, which may have been corrected by turmeric administration (Lin et al. 1995). The changes in lipid content seem to be more apparent in PL. PL are always more prevalent than TC and TG in testicular tissue (Oshima and Carpenter, 1968).

The role of turmeric in testicular protection may also be referred to its anti-apoptotic property (Mohanty et al. 2006). In the present work, chitosan treatment significantly decreased testicular TC, TG, PL, TBARS and NO contents, but elevated testicular weight significantly more than obese subjects. From the results shown in the Table, it seems that increased testicular weight is not correlated to fat content which was significantly depressed.

It was reported that oral administration of WSC to rats whether alone or mixed with aloe vera extract could prevent the atherogenic process associated with hyperlipidaemia by depressing blood levels of TC, TG, low density lipoprotein and very low density lipoprotein cholesterol (Geremias et al. 2006). It is important to point out that WSC is not an efficient drug for treating obesity, but it is a preventive medication, that can inhibit fat absorption (Choi et al. 2002) and without dietary surveillance, it will not be efficient (Ho et al. 2001).

Chitosan is a natural non-toxic polysaccharide, havinga chemical composition as poly-N-acetyl glucosaminoglycan, which is a bioabsorbable polymer known to accelerate wound healing (Ozmeric et al. 2000). In our study, chitosan increased testicular weight, whether taken alone or in mixture. It decreased lipid content, so the increased weight may be attributed to increased protein content at the expense of lipid value. This is most probably because WSC has been reported to interact with cell membranes, enhancing peptide and protein uptake, but interfering with lipid uptake (Poropratto et al. 2005). On the other hand, WSC showed an activation of intestinal immune functions and prevented tumor growth, probably through activation of natural killers and chemotaxis (Maeda and Kimura 2004). This effect, in addition to antioxidant properties – seen in our work – can be considered as a synergistic benefit of its networking pathways.

In another study, WSC showed potent antioxidant properties in tissues by decreasing TBARS and increasing antioxidant enzymes, catalase and superoxide dismutase (Jeon et al. 2003). This protective action against some hepatotoxic chemicals was also noticed by inhibiting malondialdehyde formation triggered by carbon tetrachloride (Yan et al. 2006). In the present study, we noticed that the use of GTE, ATE with WSC induced a non-significant decrease in testicular weight, a significant decrease in TC, TG, PL, TBARS and a significant increase in NO content, if compared to obese rat values. As previously stated, the effect on testicular weight, in spite of decreasing lipid figures is attributed to another pathway for chitosan, by which tissue protein may be increased and consequently also tissue weight (Poropratto et al. 2005).

The idea of polyherbal formulation in medical practice was extensively applied a long time ago, including GTE for management of obesity (Saper et al. 2004). Mostly, the presence of WSC in the mixture used in this study didn't elicit effects as satisfactory as if each individual component had been used solely. Although WSC alone was reported to adjust the metabolic functions controlling fertility (Choi et al. 2002), it seems that addition of other herbs may decrease this potential. On the other hand, WSC alone increased NO production, but it was more synergistically increased when an additional drug was simultaneously used as interferon – gamma (IFN – gamma) in an *in vitro* study (Seo et al. 2000). The previous results on chitosan-containing mixtures concerning NO production are in agreement with our results

The presence of ATE in the mixture, mostly augmented the effect of WSC, due to the antioxidant, adaptogenic, ani-inflammatory and anti-infectious activities of its curcumin content (Srinivas 1992). These effects, taken together, improve fertility and testicular performance, through controlling both lipoperoxidation and NO production, which simultaneously affect sperm motility (Romeo et al. 2003).

CONCLUSION

We thought that obesity can be considered as an important contributory factor underlying testicular dysfunction. This is clearly shown by increased testicular weigh, concomitantly with increased TC, TG, PL, elevated oxidative stress indicated by increased lipoperoxidation and NO production. Each drug when used individually, corrected these testicular parameters in the direction of improving testicular function. The most protective drug that alleviated oxidative stress, was GTE, if compared with ATE and WSC. All three drugs individually decreased testicular lipid profile in obese animals. The use of a mixture containing chitosan mostly did not introduce any more observable benefit than individual drugs, mostly due to the incompatibility of both extracts to chitosan polymers. Although our results were designed on laboratory animals, human studies may introduce more satisfactory and reliable results. We can recommend the use of GTE and ATE for treating most cases of expected testicular dysfunction, whether in obese, or non-obese individuals. However, care should be

exercised in prescribing chitosan with drugs that may be affected by WSC polymer form, that might lead to poor bioavailability and usefulness.

REFERENCES

Adams ML, Meyer ER, Sewing BN, Ciuro TJ: Effects of nitric oxide-related agents on rat testicular dysfunction. J. Pharmacol. Exp.Ther. 269:230–237, 1994.

Aruldhas M, Subramanian S, Sekar P, Vengatesh G, Chandrahasan G, Govindarajulu P, Akbarsha MA: Chronic chromium exposure-induced changes in testicular histoarchitecture are associated with oxidative stress: study in a non-human primate (*Macaca radiata* Geoffroy). Hum. Reprod. 20:2801–2813, 2005.

Basar MM, Kisa Ü, Tuglu D, Yilmaz E, Basar H, Caglayan O, Batislam E: Testicular nitric oxide and thiobarbituric acid reactive oxygen substance levels in obstructive azoospermia: A possible role in pathophysiology of infertility. Mediators of inflamm. article ID: 27458, pp. 1–5, 2006.

Bauche F, Stephan JP, Touzalin AM, Jegou B: In vitro regulation of an inducible type NO synthase in the rat seminiferous tubule cells. Biol. Reprod. 58:431–438, 1998.

Björntorp P, Ostman J: Human adipose tissue dynamics and regulation. Adv. Metab. Disord. 5:277–327, 1971.

Bucolo G, David H: Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem. 19:476–482, 1973.

Bray GA: Classification andevaluation of obesities. Med. Clin. North Am. 73:161–184, 1989.

Bray GA, Gray DS: Obesity: Part I: Pathogenesis. West. J. Med. 149:429–441, 1988.

Challa A, Rao DR, Reddy BS: Interactive suppression of abberant crypt foci induced by azoxymethane in rat colon by phytic acid and green tea. Carcinogenesis 18:2023–2026, 1997.

Choi HG, Kim JK, Kwak HD, Cho JR, Kim TY, Kim BJ, Jung KY, Choi BK, Shin MK, Choo YK: Effects of high molecular weight water-soluble chitosan on *in vitro* fertilization and ovulation in mice fed a high-fat diet. Arch. Pharmacol. Res. 25:178–183, 2002.

Chowdhury AR, Gautam AK, Bhatnagar VK: Lindane-induced changes in morphology and lipid profile of testes in rats. Biomed. Biochim. Acta 49:1059–1065, 1990.

Connor WE, Lin DS, Neuringer M: Biochemical markers for puberty in the monkey testes: Desmosterol and docosahexanoic acid. J. Clin. Endocrinol. Metab. 82:1911–1916, 1997.

Del Punta K, Charreau EH, Pignataro OP: Nitric oxide inhibits Leydig cell steroidogenesis. Endocrinology 137:5337–5343, 1996.

Dobson R: High cholesterol may increase the risk of testicular cancer. BMJ 330:1042, 2005.

Duncan BD: Multiple range test for correlated heteroscedastic means. Biometrics 13:359–364, 1957.

Festing MFW (ed): Animal Models of Obesity. Oxford Univ Press, NY 1979.

Folch J, Lees M, Sloane-Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509, 1957.

Geremias R, Pedrosa RC, Locatelli C, de Favere VT, Coury-pedrosa R, Laranjeira MC: Lipid lowering activity of hydrosoluble chitosan and association with *Aloe vera* L and *Brassica olearacea* L. Phytother. Res. 20:288–293, 2006.

Glass AR, Burman KD, Dahms WT, Boehm TM.: Progress in endocrinology and metabolism: Endocrine function in human obesity. Metabolism 30:89–104, 1981.

Heber D: Herbal preparations for obesity: are they useful? Prim. Care 30:441–463, 2003.

Herlog MGL, Hollman PCH, Putte BV: Content of potentially carcinogenic flavonoids of tea infusion, wines and fruit juices. J. Agric. Food Chem. 41:1242–1246, 1993.

Ho HC, Tai ES, Eng PH, Tan CE, Fork AC: In the absence of dietary surveillance, chitosan does not reduce plasma lipids or obesity in hypercholesterolaemic obese Asian subjects. Singapore Med. J. 42:6–10, 2001.

Ide T, Tsunoda M, Mochizuki T, Murakami K: Enhancement of insulin signaling through inhibition of tissue lipid accumulation by activation of peroxisome proliferator-activated receptor (PPAR) in obese mice. Med. Sci. Monit. 10:BR388–BR395, 2004.

Jeon TI, Hwang SG, Park NG, Jung YR, Shin SI, Choi SD, Park DK: Antioxidative effect of chitosan on chronic carbon tetrachloride induced hepatic injury in rats. Toxicology 187:67–73, 2003.

Kley HK, Solbach HG, McKinnan JC, Kruskemper H: Testosterone decrease and estrogen increase in male patients with obesity. Acta Endocrinol. 91:553–563, 1979.

Kuhn-Velten N, Codjambopoulo P, Haider SG, Passia D, Kley HK, Herberg L, Staib W, Goslar HG: Biochemical and histochemical studies on the pituitary-testicular axis in obese (C57Bl/6J.ob/ob) mice. Int. J. Androl. 9:123–131, 1986.

Lin DS, Connor WE, Wolf DP, Alexander M: Uneven distribution of monkey sperm. FASEB J. 9:A 81, 1995.

Llado I, Pico C, Palou A, Pons A: Protein and amino acid intake in cafeteria fed obese rats. Physiol. Behav. 58:513–519, 1995.

Lucesoli F, Fraga CG: Oxidative damage to lipids and DNA concurrent with decrease of antioxidants in rat testes after acute iron intoxication. Arch. Biochem. Biophys. 316:567–571, 1995.

Maeda Y, Kimura Y: Antitumor effects of various low-molecular weight chitosans are due to increased natural killer activity of intestinal intra-epithelial lymphocytes in sarcina-180-bearing mice. Nutrition 134:945–950 , 2004.

Mohanty I, Arya S, Gupta SK: Effect of *Curcuma longa* and *Ocimum sanctum* on myocardial apoptosis in experimentally-induced myocardial ischemic – reperfusion injury. BMC Complement. Altern. Med. 6:3–14, 2006.

Niehius WG, Samuelsson D: Formation of malondialdehyde from phospholipids arachidonate duringmicrosomal lipid peroxidation. Eur. J. Biochem. 6:126–130, 1968.

Nims RW, Darbyshire JF, Saavedra JE, Christodoulou D, Hanbauer I, Cox GW, Grisham MB, Laval F, Cook, JA, Krishna, MC, Wink D.A: Colorimetric methods for the determination of nitric oxide concentration in neutral aqueous solutions. Methods: A Companion Methods in Enzymology 7:48–54, 1995.

Oshima M, Carpenter MP: The lipid composition of the prepubertal and adult rat testis . Biochim. Biophys. Acta 152:479–497, 1968.

Osuga J, Ishibashi S, Oka T, Yagyu H, Tozawa R et al.: Targeted disruption of hormone-sensitive lipase results in male sterility and lipocyte hypertrophy, but not in obesity. Proc. Natl. Acad. Sci. U.S.A. 97:787–792, 2000.

Ozmeric N, Ozcan G, Haytac CM, Alaadinoglu EE, Sargon MF, Senel S: Chitosan film enriched with an antioxidant agent, taurine, in fenestration defects. J. Biomed. Mater. Res. 51:500–503, 2000.

Poropratto C, Bianco ID, Correa SG: Local and systemic activity of the polysaccharide chitosan at lymphoid tissues after oral administration. J. Leukoc. Biol. 78:62–69, 2005.

Raheja RK, Charanjik K, Singh A, Bhatia JS: New colorimetric method for estimation of phospholipids without acid digestion. J. Lipid Res. 14:695–697, 1973.

Romeo C, Ientile R, Impellizzeri P et al.: Preliminary report on nitric oxide-mediated oxidative damage in adolescent variocele. Hum. Reprod. 18:26–29, 2003.

Saper RB, Eisenberg DM, Phillip RS: Common dietary supplements for weight loss. Am. Fam. Physician 70:1731–1738, 2004.

Saravanan R, Pari L: Antihyperlipidemic and antiperoxidative effect of Diasulin, a polyherbal formulation in alloxan induced hyperglycemic rats. BMC Complement. Altern. Med. 5:14–21, 2005.

Sassone-Corsi P: Transcriptional checkpoints determining the fate of male germ cells. Cell 88:163–166, 1997.

Sastry KVH, Moudgal RP, Mohan J, Tyagi JS, Rao GS: Spectroph-otometric determination of serum nitrite and nitrate by copper-cadmium alloy. Anal Biochem. 306:79–82, 2002.

Seo WG, Pae HO, Kim NY, OH GS, Pak IS, Kim YH, Kim YM, Lee YH, Jun CD, Chung HT: Synergistic cooperation between water-soluble chitosan oligomers and interferon-gamma for induction of nitric oxide synthesis and tumoricidal activity in murine peritoneal macrophages. Cancer Lett., 159:189–195, 2000.

Serra F, Bonet T, Palou A: Amino acid enzyme activities in brown and white adipose tissues and the liver of cafeteria rats: effects of 24 hours starving. Arch. Int. Physiol. Biochim. 95:263–268, 1987.

Sharma OP: Antioxidant properties of curcumin and related compounds. Biochem. Pharmacol., 25:1811–1825, 1976.

Sharma RK, Agarwal A: Role of reactive oxygen species in male infertility. Urology 48:835–850, 1996.

Shi TS, Wang ZY, Smith TJ, Hong JY, Ho CT, Yang CS: Effects of green tea and black tea on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone bioactivation, DNA methylation and lung tumorigenesis in A/J mice. Cancer Res. 54:4641–4647, 1994.

Srinivas L, Shalini VK, Shylaja M: Turmerin: A water-soluble antioxidant peptide from turmeric (*Curcuma longa*). Arch. Biochem. Biophys. 292:617–623, 1992.

Wang ZY, Bickers DR, Mukhtar H: Interction of epicatechin derived from green tea with rat hepatic cytochrome P-450. Drug Metabol. Disposal 16:93–103, 1988.

Wang ZY, Hong MT, Reuhl KR, Conney AH, Yang CS: Inhibition of N-nitrosodi-methylamine and 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone-induced tumorigenesis in A/J mice by green tea and black tea. Cancer Res. 52:1943–1947, 1992.

Wang SW, Chiao YC, Tsai SC et al.: Inhibition of bufalin on pituitary and testicular function in rats. J. Pharmacol. Exp. Ther. 283:528–532, 1997.

Yan Y, Wanshun L, Baoqin H, Bing L, Chenwei F: Protective effects of chitosan ligopolysaccharide and its derivatives against carbon tetrachloride-induced hepatic damage in rats. Hepatol. Res. 35:178–184, 2006.

Zlatkis A, Zak B, Boyle G: A method for the determination of serum cholesterol. J. Clin. Med. 41:486–492, 1953.