COMPARATIVE STUDY AMONG WATERMELON CRUD EXTRACT, CITRULLINE AND LYCOPENE ON SOME REPRODUCTIVE INDICES IN MALE MICE

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Abstract

Objectives of this project were to study the effect of 70% crude alcoholic extract of watermelon Citrullus lanatus pulp on the reproductive system of male mice as compared with two main component citrulline and lycopene. The mice were divided into four groups, the first group were treated with watermelon 500mg/kg b.w., the second group was treated with citrulline 30mg/kg b.w., third group was treated with lycopene 5mg/kg b.w. and treatment continueous for 38 days while the fourth group used as a control group non treated. The study showed improvement of most parameters related to increase the reproductive efficiency in alcoholic extract of watermelon and citrulline treated group higher than as compared to lycopne and control group.

Key word: watermelon, citrulline, lycopene, sperm parameter.

Introduction:

Nourishment has been linked with animal reproductive success throughout history (1). Dietary habits and essential nutrients to promote successful reproductive out-comes have been identified for the maternal peri-conceptional and peri-natal period, but healthy dietary habits and essential nutrients for paternal reproductive fitness are less clear (2). Watermelon is a fruit of great economic importance, with worldwide production Watermelon is enjoyed by many people across the world as a fresh fruit, partly owing to it being low in calories and highly nutritious and thirst-quenching (3). Watermelon is very rich in phytonutrients such as lycopene a forerunner of β-carotene and a carotenoid of excessive notice because of its antioxidant capacity in scavenging reactive oxygen species, which cause oxidative tissue destruction and loss of proper cell function (4). Epidemiological revisions have established that high consumption of fruits and vegetables that high consumption of fruits and vegetables containing lycopene is associated with reduced incidence of coronary heart disease (5) and some types of prostate and kidney cancer furthermore, provoke sexual and reproductive system. small intestine of rats (6) enables arginine to be a non-essential amino acid by converting most (83%) of citrulline to arginine in the kidney (7). Citrulline-L-arginine is an essential amino acid that has a wide role in reproductive, pulmonary, renal, gastrointestinal, hepatic and immune systems and expedites the healing of wounds (8). Citrulline is used in the nitric oxide system in humans and animals and has potential antioxidant and vasodilatory roles denoted to both of lycopene and citrulline are promote events at cellular levels (9) that’s encouragements of cellular functions through marvel production of nitric oxide and Carotenoids. Now a days, peoples and animals condition are concerned about their health and physical shape and demand more natural ingredient foods of fresh quality such as ready-to-eat, minimally processed or fresh cut fruits and vegetables.

Materials and methods:

Watermelon extraction preparation:

The watermelon Citrullus lanatus was purchased from the local market Baghdad and certified in the Iraqi National Herbarium in Abou–grab,16/9/2013. The fresh watermelon washed with tabe water to remove contaminante on rind (10) and decortications with deseeding manually. The pulp segmented and
blending by electrical blender for 3 minutes then sieving with sieve at mesh 250 μm which were forming slurry shape and saved in clean container (11) after a dried at 40°C mostly for 48±4hr. and the final yield brown dry cake, grinding and sieving then stored at refrigerator 4°C until extraction. Preparation of crude alcoholic extract (70%) and dry of watermelon Citrullus lanatus pulp:

The powdered sample was weighed at 15g and the extraction is employ by used of soxhlet apparatus which contain two part electrical heater and magnetic stirrer with a round glass flask which filled with 300ml of solvent with 50°C, 50 rotation in min. The dry powdered sample put into a thimble part then extraction was clarified with magnetic stirring with vacuum pump machine. The filtrate was concentrated and remove the alcohol from it by using rotary evaporator with 50°C and 200 rotation per minute for 4 hour (12&13). The concentrated extract placed in glass Petri dish with small magnetic bar on magnetic stirrer machine with 40°C and 40 rotation per few minute. All the dried extract collected and kept in frozen at -20°C. The body weight of males were calculated as follow according to (14):

Body weight (gm) = b.w. after treatment – b.w. before. After 38 day of the treatment period finished, the animals were anesthetized and the testis excised and cleaned from the fat tissue. Testes weight and compared to body weight as following: Testicular weight / body weight. ratio = Weight of testis (gm ) / 100 x Weight of animal (gm). Then The volume of testis was calculated by volumetric reedul method according to: Volume of testis = V2 volume of testis after dip - V1 volume of testis before dip.

Epididymus Preparation:

epididymis were gently removed and freed from fat tissue and immediately put in watch glass with 0.1ml of ringer solution on warm hot plate at 37°C then incise by micro scissor to 200 part (15) used by the Spectrophotometric method to evaluate Lag time, Velocity, fraction of rapidly moving sperm as described by (16 and 17). Also Add 0.1ml of fresh semen solutions on slide to evaluated sperm counting after using of hemocytometer with using according to (18): Sperm concentration= Number of sperms / 1000 x 4000/80. Also examining sperm abnormalities by putting a drop of semen with drop of eosin and negrosin stain on edge of slide examining with bright field optics at ×100 according to (19) associated with Acrosomal abnormal- lities according to (20):

Acrosomal abnormalities % = number of counted abnormal acrosome (%) / 200 x 100. Evaluation of live and dead sperms were identified according to (21): Live sperm%= Number of live sperms X100/ Total sperms number.

Animal groups: Total number of adult healthy male mice (Swiss albino) 50 purchased from Laboratory Animal Collage of Medicine University/ Baghdad, weighted 22-34g, and aged 8 weeks, they were housed at a control system temperature 27°C ± 2°C, light schedule 12:12, with ventilated vacumm.

Mice were incubated in plastic cages (13x10x10) cm, and cleaned daily, with substitute wood saw forth time weekly. Mice left for adaptation period for 4 weeks.

Experimental design:

Group 1: Animals were served as control group given orally distilled water for 38 days.

Group 2: Animals were received orally daily watermelon alcoholic extract at dose 500mg /kg B.W for 38 days (11).

Group 3: Animal were received orally daily Citrulline at dose 30 mg/ kg B.W for 38 days (7).

Group 4: Animal were received orally daily Lycopene at dose 5 mg/kg B.W. for 38 days (22).

Results & discussions:

This study showed the watermelon treated group display significant p<0.05 increase in body weight and testicular morphometric parameter than both citrulline and lycopene treated groups in figure (1), table (1). In table 2 there were significant p<0.05 increase in sperm count in watermelon treated group as compared to control group furthermore significant p<0.05 higher than citrulline and lycopene treated group. In table 3 there was significant p<0.05 increase in sperm count and viability & decrease in sperm abnormalities in watermelon treated group as compared to control group furthermore significant p<0.05 higher than citrulline and lycopene treated group. In table 4 there was significant p<0.05 increase in turbidimetric analysis of sperm motility in watermelon treated group as compared to control group furthermore significant p<0.05 higher than
citrulline and lycopene treated group. That result finding in table (1) may be due to ability of watermelon to ameliorate and accelerate the metabolic process by improved food intake (23) and feed behaviors in animals under normal and stress conditions (24) and had the ability to lowered the subcutaneous adipose tissues and increase the brown fat and muscle mass(25,26). In citrulline-L-arginine considered as good appetizer(27)play important role in stimulate the synthesis and releasing of growth hormone (28), essential for body building by reducing triglyceride in subcutaneous adipose tissue and increasing muscle mass (29). That was clinically showed as improvement in body weight (30) &enhanced testicular parameter (31). While Lycopene had little effect on body weight may be due to the slight modulating in lean body mass with reduction in fat tissue due to decrease the production of cytokines, chimikins and adipokines proteins secreted by adipose tissue (32). Further more lycopene considered one of causal factor reduce insulin levels which led to inhibition of Leptin was adipose tissue products; led to reduction in hunger with enhanced feelings of satiety (33). On other hand, Sureda et al., (2010)(34) reported that watermelon-citrulline-L-arginine-NO had the ability to enhanced Insulin levels led to significant increased of growth hormone secretion which considered as the major growth metabolic regulatory hormone interact with the receptor on the surface of cell membrane led to activation of several signaling pathway and increase the body weight or lean body mass(35) . That increase in Growth Hormone was acts synergistically with testosteron synthesis led to magnitude the change in synthesis led to magnitude the change in body mass as well as improvement the testicular parameter that may (36) as in table (2). Watermelon-citrulline-L-arginine had diverse metabolites including ornithine, polyamines, proline, glutamate, glutamite, creatinine, agramine, dimethylarginine (37), spermidine and spermine (38). These metabolite considered as good regulator for integrity and function of cell membrane of sperm cell(39) led to super increase in grow and count of sperm. That mean there was positive correlations between increase in citrulline-L-arginine concentration and sperm concentration (40) as in (table 3). Also watermelon had beneficial activation of the AMP-activated protein kinase (41) led to provoke the rate of glycolysis, resulting in higher rates of ATP generation in spermatozoa (42) and consequently induce formation of stabilizing disulfide cross-links (43) led to increase chromatin compaction with DNA arrangement (44) which considered important determinant factor for sperm head morphology that mean there was correlation between sperm DNA stability and head morphology (45). That mean watermelon and citrulline had the ability to increase viability of sperm (46) and decrease any disorder in sperm morphology, and that result agreed with watermelon contain lycopene which showed that increase in sperm count due to increase the physiological activity of testicular cell associated with reduction in testicular cell associated with reduction in the apoptosis (47). In table (4)There was significant p<0.05 decrease in watermelon and citrulline derived treated group as compared to compared to control group furthermore lesser than lycopene treated group in lag time. Watermelon-citrulline-L-arginine pathway may be increase sperm concentration associated with increase in energy utilization that cause low concentration of calcium medium which may be effected the time lag required for the initiated stimulation needed (48). That’s mean there was negative relationship between lag time and sperm concentration (49). There was significant p<0.05 increase in watermelon derived citrulline treated group as compared to control group furthermore watermelon derived citrulline more than lycopene treated group in fraction of rapid movement, velocity and motility index. In watermelon-citrulline-L-arginine there was increase in sperm concentration and the motile will be the first groups to swim upward into clear medium from a concentrated cell suspension at the bottom mainly dependent on time, increase turbidity of the medium and increase in absorbance. The strongly motile sperms may be the best candidates for fertilization (50) and increase velocity of sperm trough enhance the rate of glycolysis and higher rates of ATP generation (42) and [Ca+2 ] increased the sperm motility and velocity (51) as well as lycopene showed the same increase in sperm motility and velocity due to the antioxidant effect.

**Conclusion**

The major aim of this thesis was to determine the endorse of watermelon nutrient and increase the brown fat and muscle mass (25,26). In citrulline-L-arginine considered as good appetizer(27)play important role in stimulate the synthesis and releasing of growth hormone (28), essential for body building by reducing triglyceride in subcutaneous adipose tissue and increasing muscle mass (29). That was clinically showed as improvement in body weight (30) &enhanced testicular parameter (31). While Lycopene had little effect on body weight may be due to the slight modulating in lean body mass with reduction in fat tissue due to decrease the production of cytokines, chimikins and adipokines proteins secreted by adipose tissue (32). Further more lycopene considered one of causal factor reduce insulin levels which led to inhibition of Leptin was adipose tissue products; led to reduction in hunger with enhanced feelings of satiety (33). On other hand, Sureda et al., (2010)(34) reported that watermelon-citrulline-L-arginine-NO had the ability to enhanced Insulin levels led to significant increased of growth hormone secretion which considered as the major growth metabolic regulatory hormone interact with the receptor on the surface of cell membrane led to activation of several signaling pathway and increase the body weight or lean body mass(35) . That increase in Growth Hormone was acts synergistically with testosteron synthesis led to magnitude the change in synthesis led to magnitude the change in body mass as well as improvement the testicular parameter that may (36) as in table (2). Watermelon-citrulline-L-arginine had diverse metabolites including ornithine, polyamines, proline, glutamate, glutamite, creatinine, agramine, dimethylarginine (37), spermidine and spermine (38). These metabolite considered as good regulator for integrity and function of cell membrane of sperm cell(39) led to super increase in grow and count of sperm. That mean there was positive correlations between increase in citrulline-L-arginine concentration and sperm concentration (40) as in (table 3). Also watermelon had beneficial activation of the AMP-activated protein kinase (41) led to provoke the rate of glycolysis, resulting in higher rates of ATP generation in spermatozoa (42) and consequently induce formation of stabilizing disulfide cross-links (43) led to increase chromatin compaction with DNA arrangement (44) which considered important determinant factor for sperm head morphology that mean there was correlation between sperm DNA stability and head morphology (45). That mean watermelon and citrulline had the ability to increase viability of sperm (46) and decrease any disorder in sperm morphology, and that result agreed with watermelon contain lycopene which showed that increase in sperm count due to increase the physiological activity of testicular cell associated with reduction in testicular cell associated with reduction in the apoptosis (47). In table (4)There was significant p<0.05 decrease in watermelon and citrulline derived treated group as compared to compared to control group furthermore lesser than lycopene treated group in lag time. Watermelon-citrulline-L-arginine pathway may be increase sperm concentration associated with increase in energy utilization that cause low concentration of calcium medium which may be effected the time lag required for the initiated stimulation needed (48). That’s mean there was negative relationship between lag time and sperm concentration (49). There was significant p<0.05 increase in watermelon derived citrulline treated group as compared to control group furthermore watermelon derived citrulline more than lycopene treated group in fraction of rapid movement, velocity and motility index. In watermelon-citrulline-L-arginine there was increase in sperm concentration and the motile will be the first groups to swim upward into clear medium from a concentrated cell suspension at the bottom mainly dependent on time, increase turbidity of the medium and increase in absorbance. The strongly motile sperms may be the best candidates for fertilization (50) and increase velocity of sperm trough enhance the rate of glycolysis and higher rates of ATP generation (42) and [Ca+2 ] increased the sperm motility and velocity (51) as well as lycopene showed the same increase in sperm motility and velocity due to the antioxidant effect.
developed and clarify supported boosting idea. The watermelon administration and citrulline-L-arginine-Nitric oxide pathway on spermatogenesis and spermatogram and motility indecies.

Characterize an upregulation of spermatogenic cells yield in watermelon mice model as a synergestic yield of lycopene and citrulline, experiment joined and determined the sexual hormone levels and their reflection on tissue morphometric and stereological profile of peritubular and seminiferous tubule testes. Finally watermelon and their active ingredient treated group play a DNA keeper and attendant in epididymis extracted spermatozoa. That obviously marked reduction of oxidative stress.

The endpoint increase fertility rate and refereed the approach compatibility the watermelon derived citrulline and lycopene on male productivity of superior testicular fitness and fertilized semen.

Reference :


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Figure 1: The body weight changes parameter of male mice under loading doses of watermelon, citrulline and lycopene for 38 days.

Table 2: The testicular morphometrical in male mice under loading doses watermelon, citrulline and lycopene for 38 days.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Watermelon 500mg/Kg.Bw</th>
<th>Citrulline 30mg/Kg.Bw</th>
<th>Lycopene 5mg/Kg.Bw</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Testicular weight g</strong></td>
<td>0.020±0.001</td>
<td>0.058±0.216 A</td>
<td>0.053±0.017AB</td>
<td>0.030±0.0088</td>
</tr>
<tr>
<td><strong>Testicular weight/body weight ratio</strong></td>
<td>0.077±0.020</td>
<td>1.895±0.060A</td>
<td>0.189±0.0458</td>
<td>0.113±0.040C</td>
</tr>
<tr>
<td><strong>Testicular volume cm³</strong></td>
<td>1.75±0.04089</td>
<td>3.16±2.116A</td>
<td>2.60±1.0008</td>
<td>2.50±0.4898</td>
</tr>
<tr>
<td><strong>Testicular dimensions cm</strong></td>
<td>0.018±0.10</td>
<td>0.307±0.3213A</td>
<td>0.272±0.3310 B</td>
<td>0.033±0.2102C</td>
</tr>
<tr>
<td><strong>Testicular density g/cm³</strong></td>
<td>0.011±0.120</td>
<td>0.018±1.264A</td>
<td>0.020±0.216B</td>
<td>0.012±0.155C</td>
</tr>
</tbody>
</table>

Values are presented as mean ±SE
Letter p<0.05 significant different from other groups, n=10

Table 3: The Spermatogram parameter of male mice under loading doses of watermelon, citrulline and lycopene for 38 days.

<table>
<thead>
<tr>
<th></th>
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<th>Watermelon 500mg/Kg.Bw</th>
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<th>Lycopene 5mg/Kg.Bw</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sperm concentration 10⁶/μl</strong></td>
<td>11.99±0.70</td>
<td>18.71±0.42 A</td>
<td>17.31±0.22 B</td>
<td>16.66±0.97 B</td>
</tr>
<tr>
<td><strong>Sperm viability %</strong></td>
<td>85.77±1.36</td>
<td>94.18±1.52 A</td>
<td>91.02±1.46 BA</td>
<td>90.10±0.53 CB</td>
</tr>
<tr>
<td><strong>Total spermbnormalities %</strong></td>
<td>16.53±0.614</td>
<td>3.72±0.131 A</td>
<td>4.81±0.079 B</td>
<td>5.79±0.083 C</td>
</tr>
<tr>
<td><strong>Primary %</strong></td>
<td>7.05±0.58</td>
<td>0.61±0.101 A</td>
<td>0.43±0.30 B</td>
<td>0.27±0.22 C</td>
</tr>
<tr>
<td><strong>Secondary %</strong></td>
<td>9.37±0.872</td>
<td>2.50±0.045 A</td>
<td>3.99±0.072 B</td>
<td>4.08±0.011 B</td>
</tr>
<tr>
<td><strong>Acrosome %</strong></td>
<td>5.86±0.010</td>
<td>0.23±0.0028 A</td>
<td>0.17±0.0031 B</td>
<td>3.39±0.0047 C</td>
</tr>
</tbody>
</table>

Values are presented as mean ±SE
Letter p<0.05 significant different from other groups, n=10

Table 4: The turbidimetric analysis of sperm motility parameter of male mice under loading doses of watermelon, citrulline and lycopene for 38 days.
<table>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Lag time sec.</strong></td>
<td>1.88±0.0062</td>
<td>0.69±0.0095</td>
<td>0.85±0.013</td>
<td>0.92±0.017</td>
</tr>
<tr>
<td><strong>Fraction of rapidly moving sperm</strong></td>
<td>0.0174±0.0010</td>
<td>0.1557±0.0037</td>
<td>0.1408±0.0026</td>
<td>0.0819±0.0058</td>
</tr>
<tr>
<td><strong>Velocity µm/sec</strong></td>
<td>9.07±0.176</td>
<td>15.09±1.08</td>
<td>14.81±1.19</td>
<td>11.57±0.210</td>
</tr>
<tr>
<td><strong>motility index %</strong></td>
<td>1.20±0.048</td>
<td>2.97±0.067</td>
<td>2.44±0.039</td>
<td>2.00±0.761</td>
</tr>
</tbody>
</table>

Values are presented as mean ±SE
Letter p<0.05 significant different from other groups, n=10