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Role of Nitroglycerine Injection in Japanese Quail (*Coturnix japonica)* Testes Tissues Parameters

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Abstract: This study aimed to evaluate injection Japanese quail males *Coturnix japonica* with nitroglycerine NTG subcutaneous in testes tissues parameters. 45 males of Japanese quails used in this study divided into 3 treatments, 3 replicate. Males injected subcutaneous one time weekly for six weeks by 0.5 ml of 2.5 and 5 mg of NTG dissolved with sesame oil while control group left without injection. Males slaughtered and testes weight, testes relative weight, seminiferous tubules, and interstitial cells volume density and relative weights (mg/g) and contents diameters used in this study. Results show that significant increasing (P≤0.003) in volume density percentage of spermatogonia and significant decreasing (p≤0.05) in relative weight of Sertoli cells for 2.5 mggroup and significant increasing (P≤0.03) in Sertoli cells volume density percentage for 5 mg group compared with control group. Significant decreasing (p≤0.05) in relative weight of basement membrane, significant increasing (P≤0.0001) in seminiferous tubules diameter and significant decreasing (p≤0.009) in testes relative weight (g kg^{-1}) and total seminiferous tubules contents for all treatments compared with control group.

Keywords: Nitroglycerune, Quail, Testes, seminiferous tubules, nitric oxide

Nitroglycerine (NTG) is a colorless oil soluble in alcohol, molecular formation is C_3H_5 $(ONO_2)_3$, content of nitrogen is (18.5%) (Jiang Fang 2001). In human body, NTG rapidly absorbed and has very short half time, about 2 minutes (Serven tet al 1989). NTG used in cardiovascular ischaemia through liberation of Nitric Oxide (NO), because the local concentration of NO in the tissues responsible for any biological effects (Agvald 2001). Moncada et al (2006) expressed that NO is an important effective of cellular function as a vasodilator, neurotransmitter and conceder as a physiological and pathological process. Moreover, it plays important physiological effects by activation of guanylate cyclase (platelet aggregation, smooth muscle tonus) (Severina et al 2003, Vanholt 2018). All effects of NO in smooth muscles tone are relised with the enzyme guanylate cyclase (GC) producing cyclic guanosine monophosphate (cGMP) and NO activates the enzyme GC via reversible interaction with it (Moncada 1991). Davidoff (1995) show that NO synthase is reported to function in leydic and sertoli cells of men testes. So, NO make a blood following in testicles in rats (Sharma et al 1998). Using of nitrate esterase (including NTG) make a vasodilation effects due to releasing of NO that make an activation of guanylate cyclase which catalyzed the conversion of guanonsine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) (Orai et al 1999). No generated in human, animal and birds tissues from L. Arginine (Titrov 2012, 2018, AL-Bayar 2010). NO produced from L. Arginine make vasodilation of Turkey and hens leading an improvement in testes weight, sperms motility and concentration while, it make high significant decreasing in dead sperms, abnormal sperms, and acrosomal abnormality and high significant increase in semniferous tubules diameter and germinal cells layer (AL-Bayar 2010). And it can improve sperms energy production and sperms motility (Lefievre 2007). While NO inducted in roosters mild stressed can improve sperms motility after cryopreservation (Feyzi 2018). This study conducted to evaluate the effect of injection NTG in Japanese quails males in testes, seminiferous tubules and interstitial cells contents traits.

MATERIAL AND METHODS

This study carried out in the poultry farm, Dept. of animal production, Agriculture College (Iraq) according to the protocol approved by the University of Anbar, Ethics-Committee to study the effect of injection NTG in Japanese quails males in testes tissue parameters. 45 males of Japanese quail used in this study (45 days age) divided into 3 treatments, 3 replicate for each one (5 males in each replicate). Males injected subcutaneous one time weekly for 6 weeks by 0.5 ml with nitroglycerine (NTG) dissolved with 500 and 1000 mg per100 ml of sesame oil for each treatments (T1 and T2) respectively while, (T0) left without injection as a control group. At the end of this study, males weighted and slaughtered (two males from each replicate), tested extracted and packed in plastic containers content 10% of formalin, then processed as Uni et al (1998) and Tako

et al (2004). Testes weighted in sensitive scale and relative weight calculate as below:

$$\text{Testes relative weight} = \frac{\text{Testes weight}}{\text{Life body weight}} \times 100$$

Morphometric analysis method for estimated seminiferous tubules and interstitial cells volume density (%), relative weight (gm kg^{-1}) and contents diameters used in this study as Weible (1979).

Volume density = Relative weight × testes relative weight

Parameters of seminiferous tubules contents studied is spermatogonia, spermatocytes, speamatides, sperms, spermatogenic cells, Sertoli cells, Lumen, Vacuoles, Basement membrane and total seminiferous tubules contents. Parameters of interstitial cells contents studied is myoid cells,Leydic cells, Blood vessels, Interstitial spaces, Total intestinal contents and Ratio of total seminiferous tubules contents to total intestinal contents. Parameters calculated as a mean of 3 sections for each slide.

Statistical analysis: Complete random design (CRD) within three treatments, 3 replicates used in this experiment. Data analyzed by using GLM model procedure of SAS (Statistical analysis system) (SAS 2001). Including concentrations in NTG. Means for treatments compared by using Duncan's polynomial using different significance levels to determine significant differences between the averages (Duncan 1955).

RESULTS AND DISCUSSION

Results in Table 1 show a significant increasing (P≤0.003) in volume density percentage of spermatogonia for 2.5 mg group and significant increasing (P≤0.03) in Sertoli cells volume density percentage for 5 mg group compared with control group (0 mg), and there is no significant differences in other seminiferous tubules contents volume density percentage. In Table 2, there is significant decreasing in relative weight of Sertoli cells for 2.5 mg treatment and significant decreasing in relative weight of basement membrane and total seminiferous tubules contents for all treatments compared with control group. Results in Table 3 and 4 refers to non-significant differences in volume densities and relative weights for all contents of testes interstitial cells for all treatments compared with control groups.

Data in Figure 1 show that significant increasing (p≤0.0001) in seminiferous tubules diameter for all treatments compared with control group, and there is no difference in lumen diameter and epithelial height for treatments compared with control group. Data in Figure 2 show significant decreasing (P≤0.009) in testes relative weight (g kg^{-1}) for 2.5 and 5 mg Nitroglycerine injection groups compared with control group (0 gm Nitroglycerine injection).

The biological effects of NG indirectly supported the impact of NO on NG-mediated action then make a

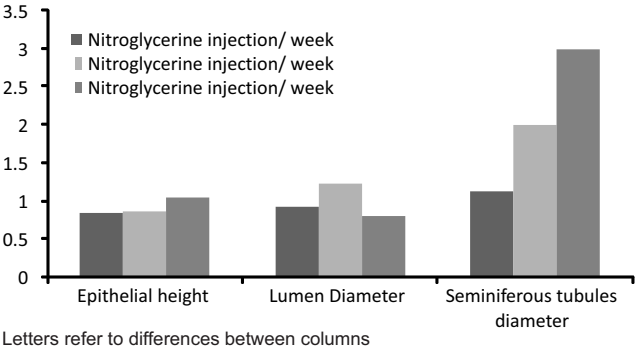

Fig. 1. Effect Nitroglycerine injection to Japanese quail males in seminiferous tubules contents diameters

Table 1. Effect Nitroglycerine injection to Japanese quail males in seminiferous tubules contents volume density percentage

Traits	Nitroglycerine injection/ week			Mean	SEM*	Significant level
	0 mg	2.5 mg	5 mg			
Spermatogonia	5.77b	9.72a	4.68b	6.72	0.71	0.003
Spermatocytes	7.38	8.33	6.94	7.55	0.42	NS**
Speamatides	8.11	6.65	8.77	7.84	0.82	NS
Sperms	6.58	6.79	7.31	6.89	0.49	NS
Spermatogenic cells	27.85	31.5	27.7	29.02	1.15	NS
Sertoli cells	1.02ab	0.43B	1.26a	0.9	0.13	0.03
Vacuoles	3.5	2.63	3.36	3.16	0.39	NS
Lumens	2.12	3.07	2.19	2.46	0.52	NS
Basement membrane	5.04	3.87	3.36	4.09	0.37	NS

Letters refer to differences between columns
*SEM: standard error of means
**NS: No significant differences between columns

mitochondrial dysfunction (Sydow et al 2004, Esplugues et al 2006) This reduction may be due to role of nitric oxide in cells oxidation by mitochondrial respiration inhibition (Dungel et al 2011). This inhibition due to the action of NG induced in vivo and in vitro by decreasing mitochondrial oxygen consumption (Brown 1999, Beretta 2008). Then production of mitochondrial reactive oxygen species ROS (Fink et al 2000, Beretta et al 2008). Furthermore, exposure of mitochondria to NG decreased electron transfer preferentially (Dungel et al 2011). Then ROS make DNA damage and structurally modify biological macromolecules such as protein, thereby resulting in metabolic diseases (Veron and Tang 2013). For all this causes, we can explain the significantly decreasing in volume density, relative weights of sertoli cells, relative weights of total seminiferous tubules contents, testes weights and relative testes weights in this study for NTG injection treatments.

The significant increasing in seminiferous tubules diameter for all treatments due to the role of NTG in vasodilatation of smooth muscles through releasing NO. Some researchers explain the mechanism of generating NO

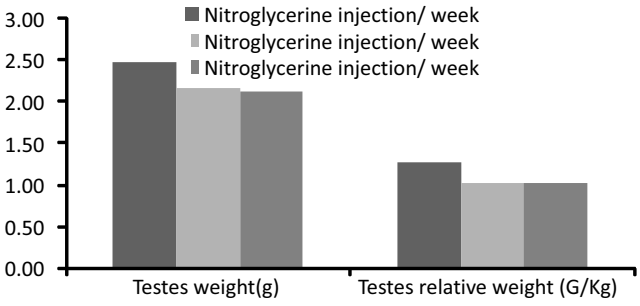

Fig. 2. Effect Nitroglycerine injection to Japanese quail males in testes weights and relative weights

Table 2. Effect Nitroglycerine injection to Japanese quail males in seminiferous tubules contents relative weight

Traits	Nitroglycerine injection week^{-1}			Mean*	SEM	Significant level
	0 mg	2.5 mg	5 mg			
Spermatogonia	7.4ab	9.94a	4.86b	7.4	0.73	0.009
Spermatocytes	9.5	8.5	7.21	8.4	0,52	NS**
Speamatides	10.33	6.76	9.13	8.74	0.96	NS
Sperms	8.39	6.96	7.63	7.66	0.59	NS
Spermatogenic cells	35.36	32.16	28.84	32.21	1.42	NS
Sertoli cells	1.3a	0.44b	1.29a	1.01	0.15	0.05
Vacuoles	4.47	2.68	3.38	3.51	0.44	NS
Lumens	2.7	3.33	2.24	2.75	0.58	NS
Basement membrane	6.51a	3.96b	3.47b	4.22	0.41	0.05
Total seminiferous tubules contents	15a	10.42b	10.35	11.92	0.93	0.05

Letters refer to differences between columns
*SEM: standard error of means
**NS: No significant differences between columns

Table 3. Effect Nitroglycerine injection to Japanese quail males in interstitial testes cells contents volume density (%)

Traits studied	Nitroglycerine injection week^{-1}			Mean	SEM*	Significant level
	0 mg	2.5 mg	5 mg			
Myoid cells	2.99	3.72	4.89	3.87	0.42	NS**
Leydic cells	0.8	0.88	0.87	0.85	0.13	NS
Blood vessels	0.44	0.44	0.73	0.53	0.07	NS
Interstitial spaces	1.9	1.31	2.11	1.77	0.32	NS
Total intestinal contents	6.14	6.36	8.62	7.04	0.76	NS
Ratio of total seminiferous tubules contents to total intestinal contents	2.41	1.6	1.41	1.81	0.26	NS

*SEM: standard error of means
**N. S.: No significant differences between columns

Table 4. Effect Nitroglycerine injection to Japanese quail males in interstitial testes cells contents relative weight

Traits studied	Nitroglycerine injection week^{-1}			Mean	SEM*	Significant level
	0 mg	2.5 mg	5 mg			
Myoid cells	3.86	3.82	4.99	4.22	0.41	NS.**
Leydic cells	1.03	0.89	0.87	0.93	0.13	NS
Blood vessels	0.56	0.44	0.73	0.58	0.07	NS
Interstitial spaces	2.47	1.31	2.13	1.97	0.35	NS
Total intestinal contents	7.94	6.49	8.74	7.72	0.77	NS
Ratio of total seminiferous tubules contents to total intestinal contents	2.41	1.6	1.41	1.81	0.26	NS

*SEM: standard error of means
**N. S.: No significant differences between columns

from NTG *in vivo*. FeridMurad demonstrated that vasodilating effect of nitrate esters (including NTG) was due to the fact that they release nitric oxide (NO), leads to activation of guanylate cyclase, which catalyzes the conversion of guanosine triphosphate (GTP) to Cyclic guanosine monophosphate cGMP (Oraii et al 1999). The increasing of cGMP level leads to smooth muscle relaxation (Oraii et al 1999). NTG resulting in activation of soluble GC and cGMP mediated vasodilation (Oplet et al 2016).

CONCLUSION

The injection of NTG subcutaneous for Japanese quail males led to decreasing testes weight, relative weight and relative weights of total seminiferous tubules contents, then increasing diameters seminiferous tubules.

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