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Protective effect of Cicer arietinum on infertility of male rats induced by gibrillic acid

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Abstract

The present study was carried out to elucidate the effect of Cicer arietinum (chick pea) on gibrillic acid (GA_3) induced infertility as well as some serum parameters in male rats.

A total of forty adult male Albino rats (150-180 g) were used in this study. Rats were equally divided into 4 groups. Group one (1^{st}) was kept as a control negative(–ve) and fed on basal diet only, while second (2^{nd}) group fed on basal diet mixed with GA_3 at concentration of 1g/ 100 kg ration as control positive(+ve). On other hand, third (3^{rd}) group was fed on basal diet mixed with chick pea at conc. 5% and forth (4^{th}) group was fed on basal diet mixed with both chick pea (5%) and GA_3 (1 g / 100 kg. ration) for 65 successive days.

At the end of the experimental period, blood samples were collected from each rat for biochemical analysis. Rats were sacrificed for studying the sexual organs weight and epididymal sperm characters.

Rats fed on ration mixed with GA_3 ,showed significant increase in serum levels of AST, ALT,AP, urea, creatinine, total cholesterol and sperm abnormalities; while the levels of serum total protein , albumin , globulin, testosterone and weight of sexual organs decreased significantly as compared with corresponding values of the controls (group one).

Rats fed on ration mixed with combination of chick pea and GA_3 were significantly altered the tested parameters directed toward normal as compared with control positive (+ve) group.

Introduction

Plant foods not only represent major source of nutrients for humans, but also contain "protective factors" against chronic diseases, coronary heart disease, diabetes, infertility and cancer. Plants include some or all of the following characteristics: excellent sources of

omega3 fatty acid, rich sources of anti-oxidant vitamins (vit.E , vit.C, vit .A); rich in fiber and high in protein (**Simopoulos,2001**).

Cicer arietinum is an annual cereal grain which serves as major fed crop. Many nutrients in cereals had been known to contain factor potential for reducing carbohydrate (CHD) as linoleic acid, fiber, vit. E, selenium and phytoestrogen (**Biswas et al.,2001 and Jukantil et al.,2012**). Flavonoids including anti-oxidative and free radical screening activities (**Truswell, 2012 and Geervani, 2009**).

GA3 is a promoting growth and elongation of plants. If used in small amounts but eventually plants grow to tolerance for it (**Kazmierczak , 2003**) .

The present study was carried out to reveal the nutritional and medicinal effects of chick pea to overcome infertility induced by gibrillic acid in rats.

Material and Methods

Material

1- Cicer arietinum (Chick pea): Chick pea was obtained from local market, crushed, milled before mixed with ration and kept in glass bottles.
2- Gibrillic acid (GA3): It was obtained as commercial white tablets from central Lab. For Insecticides at a concentration of 20%

Animals:

Forty mature white rats of an average body weight 150-180 g of spragne dawley strain were used. They were obtained from the laboratory Animal House of Ophthalmic Research Institute, Giza.

Animals were acclimatized to laboratory condition for one week before being used in the current study. Rats were fed on standard ration supplying the essential vitamins and trace elements and water supply was given ad-libitum.

Experimental design:

Forty (40) mature rats were divided into 4 equal groups of 10 rats each for 65 days. The groups were divided as follow:

Group one (G1): served as control negative and fed on basal diet.

Group two (G2): control positive, was fed on basal diet mixed with gibrellic acid (1g/100kg ration).

Group three (G3): fed on basal diet mixed with ground cicer arietinum in a conc.5%.

Group four (G4): fed on basal diet mixed with both gibrillic acid at conc.1 g / 100 kg ration and cicer arietinum at a conc. of 5%.

Serum Samples:

At the end of the experimental period, individual blood sample were obtained from all groups from orbital plexuses and received into clean dry tube. Samples were left to clot at room temperature for 2 hours, stored overnight in a refrigerator at 4°C and centrifuged at 3000 r.p.m for 15 minute to obtain sera which were kept in a deep freeze for biochemical studies (the animals were fasted for 12 hours prior to the sacrificing).

Fertility test:

The effect of chick pea as well as GA3 on fertility of male rats was determined at the end of 65 days of feeding on ration mixed with tested substances, to cover complete spermatogenic cycle (**Hershberger et al., 1969**). Rats were sacrificed for revealing weight of testis and accessary glands as well as epididymal sperm characters according to **Bearden and Fluquery (1980)**.

Biochemical analysis:

The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) (**Reitman and Franked, 1957)** ; alkaline phosphatase (AlP) **(Roy, 1970**) ; total protein (**Sonnen wirth and Jaret,1980**) ; albumin (**Drupt,1974**); urea (**Reises et al., 1965**) ; creatinine (**Faulkner and Ashwood,2001**) and testosterone (**Tietz,1995**) were estimated for all serum samples.

Statistical analysis:

Parametric data were statistically analyzed using analysis of variance (ANOVA) test and comparative of means were performed according to Duncan Multiple Range Test for comparative of means according to **Duncan (1955**) using **SPSS 14(2006).**

Results and Discussion:

The present study was carried out in mature male rats to elucidate the effects of chick pea in normal and GA3 induced infertility after its application for 65 days.

1-**Effect on fertility**:

It appears from table (1) that feeding on ration mixed with GA3 (G2) resulted in significant decrease of testis weight, as well as weight of prostate and seminal vesicles. This decrease was concomitant with elevated sperm abnormalities as compared with corresponding values of the control (-ve) group (G1).

On the other side, Rats fed on ration mixed with chick pea for 65 days (G3) showed significantly increased sperm cell count and motility % of the sperm as compared with corresponding values of the control (-ve) group (G1).

On the other hand, Rats fed on ration mixed with GA3 and chick pea, the all tested parameters highly increased and directed toward normal, this results associated with (**Ismail 2013, Samir et al., 2015 and Ataf et a., 2008)** who recorded that administration of chick pea to male rats or rabbit showed significantly increase in sperm motility, sperm count and level of testosterone.

Larionov and Geraimov (2008) and Dana et al., (2011) reported that feeding on chick pea to sheep showed significantly increased sperm concentration, testicular size and sperm production.

All previous data in agreement with our results , this effect of tested plant on sexual organs and sperm character could be attributed to its direct action on lyding cells which secret testosterone hormone which may be due to chick pea high content of phyto estrogen, phosphorus, Zinc and vitamin E . These constituents were considered important factors for fertility and increase the spermatogenic cycle (**Biswas et al 2001, Jukantil et al, 2002, Masliev and Dalyan1969).**

The present results, in group fed on ration mixed with GA3 in agreement with **Ravikumar and Srikumar (2005) and Afaf,2008** who reported that oral administration and dermal injection GA3 for 45 days showed loss of germ cells, reduction in the size of seminiferous tubules and dystrophy of lyding cells; more important decreased sperm cell count and serve as inhibitor of testicular cell function while results of rats fed on ration mixed with GA3 and tested plant correlated with **Kumar et al(2014) and Michael et al (2013**) who observed that abnormal shape of sperm head and weight of testis was noticed in group fed on GA3, directed toward normal when fed on tested plant which may be due to high content of phytoestrogen.

Phytoestrogen act as endocrine disruptors and threaten reproductive health.

2-**Effect on serum biochemical:**

Rats fed on ration mixed with GA3 at concentration 1 g/100 kg ration caused significant increase in the serum levels of ALT, creatinine, urea, cholesterol (table 2, 3). The level of serum total protein, albumin, globulin, testosterone and zinc were significantly decreased (table 4, 5). This may be due to lipid peroxidation of the poly unsaturated fatty acid in cell membranes, break down of membrane –structure and leading to the release of microsomal esterase and other enzyme, such as aminotransferase into extra- cellular compartments including serum.

These findings were coinciding with those reported by **li et al (2004) and Hemmings and Song (2008).**

Feeding on ration mixed with chick pea in combination with GA3 were significantly decreased AST, ALT, cholesterol, Urea and creatinin as compared with G2. These results may be attributed to the presence of anti-oxidants of chick pea which had important beneficial effects on the liver regeneration **Bashandy (2006) and Naghma et al (2008).** More over **Ahuja et al (2006) and Pomery et al (2001)** concluded that tested plant enhances the cholesterol lowering effect in individuals with hypercholestermia.

Fed on GA3 caused significant decrease of total protein, albumin and A: G ratio. Our results in agreement with those reported with **Al Gaby (2008) and Rubio et al (2009).**

Addition of chick pea to diet with GA3 treated rats showed significant increase in total protein, albumin and A: G ratio directed toward normal value.

Form the above mentioned results, it is easy to notice that GA3 clearly affect the liver and its enzymes and this negative effects decrease the liver capacity to synthetize the protein. Also chick pea contain anti-oxidant and flavonoids which improved nutritional status and beneficial effects on liver regeneration **(Junkantil et al 2012**).

Testosterone and zinc level in the serum of rats fed on chick pea and GA3, significantly increase in amount of zinc is a good response for indicate to increase the parameters of fertility. This results agree with **Michael et al(2013) ,Samir (2010) and Kamak (2009)** who reported that chick pea had a direct effect on lyding cells which secret testosterone hormone which may be due to high content of phyto-estrogen, phosphorus and zinc that is constituents considered as an important factors for fertility and increase the spermatogenic cycle **(Maslive and Davlyan 1969).**

Therefore, according to present findings we can conclude that adding of chick pea to the diet of rats is more effective in treatment of infertility induced by GA3 and protect the body from dangerous effect.

Table (1): Effect of Cicer arietnum in reference to GA3 on sexual organs weight and epididymal sperm characters.(Mean± SE)

Group	Conc.	Weight of sexual organ/ 100 g b.wt				Sperm characters		Abnormality %
		Testis	Epidid.	Prost.	S.V.	Count 10^6/ mm^3	Motility %	
G1 (C-ve)	--	1.7 ±0.06	0.71 ±0.03	0.69 ±0.03	0.8 ±0.04	579 ± 5.8	82 ±5.0	2.1 ± 0.069
G2 (GA3)	1g/100 kg ration	1.04 ±0.03*	0.77 ±0.039	0.17 ±0.01***	0.378 ±0.25***	354 ± 6.1***	30 ±7.16	37.7 ±1.8
G3 (Chick pea)	5%	1.904 ±0.013**	0.81 ± 0.02*	0.66 ±0.003	0.831 ± 0.005	610 ±7.8*	85 ±2.3	1.66 ±0.06
G4 (GA3+ Chick pea)	5% + 1g/100kg	1.098 ± 0.04*	0.751 ±0.02	0.25 ±0.018***	o.42 ±0.03***	406 ± 7.1***	45 ±3.39	19.6 ±0.9

*P< 0.01 **P<0.05 ***P<0.001

Table (2): Effect of cicer arietinum in reference to GA3 on activities of transaminases and alkaline phosphatase

Group	Conc.	ALT µ / L	AST µ/ L	AP µ / L
G1 (C-ve)	-	11.90 ± 0.4^{a}	25.3 ± 0.31^{a}	40.58 ± 0.76^{bc}
G2 (GA3)	1g/100kg ration	20.33 ± 0.29^{e}	51.6 ± 1.087^{e}	27.97 ± 0.88^{a}
G3(Chickpea)	5%	11.84 ± 0.27^{a}	26.06 ± 0.33^{a}	37.3 ± 0.63^{b}
G4 (chickpea + GA3)	5%+ 1g / 100 kg ration	14.79 ± 0.49^{c}	33.4 ± 1.06^{b}	50.19 ± 0.23^{d}

Significant at P< 0.05 using ANOVA test
A,b,c,d…..significantly different between comparison group within the same letter and column using Duncan multiple range test at P< 0.05

Table (3): Effect of cicer arietinum in reference to GA3 on urea, creatinin and cholesterol

Group	conc	Urea mg / dl	Creatinin mg/ dl	Cholesterol mg/ dl
G1(C-ve)	-	16.5± 0.23^{b}	0.78± 0.08^{b}	136.0± 5.04^{ab}
G2 (GA3)	1g/100kg ration	23.5± 2.12^{de}	1.5± 0.04^{e}	155.7± 9.86^{c}
G3(chickpea)	5%	17.88± 1.7^{e}	0.81± 0.03^{a}	138.9± 10.21^{ab}
G4(chickpea+GA3)	5%+ 1g/100 kg ration	17.93± 1.8^{bc}	0.95± 0.04^{d}	140.7± 5.6^{ab}

Values with different letters in the same column are significantly different at p< 0.05

Table (4): mean value of total serum protein, albumin, globulin and A:G ratio in normal and GA3 treated rats fed diets containing chick pea

Group	Conc.	Total protein g / dl	Albumin G /dl	Globulin g/dl	A:G ratio
G1(C –ve)	-	10.47± 0.4^{c}	4.6± 0.17^{c}	5.85±0.38^{c}	0.8± 0.06^{b}
G2(GA 3)	1g/100kg ration	7.09± 0.37^{a}	2.63 ± 0.13^{a}	4.45± 0.36^{b}	0.59± 0.05^{a}
G3(chickpea)	5%	8.87± 0.43^{b}	4.39± 6.25^{c}	4.5± 0.29^{b}	0.97± 0.02^{d}
G4(chickpea+GA3)	5%+1g/100 kg ration	8.03± 0.25^{b}	3.8± 0.15^{bc}	4.25± 0.25^{b}	0.9± 0.08^{c}

Values with different letters in the same column are significantly different at p< 0.05

Table (5): Effect of Cicer arietinum in reference to GA3 on zinc and testosterone

group	Conc.	Zinc mg / dl	Testosterone ng /ml
G1 (C –ve)	-	5.89 ± 0.06^{e}	2.44± 0.002^{c}
G2 (GA3)	1gm/100kg ration	5.14 ± 0.14^{d}	0.54 ± 0.043^{a}
G3(chickpea)	5%	5.78 ± 0.27^{cd}	3.82 ± 0.063^{d}
G4(chickpea+ GA3)	5%+1gm/100 kg ration	4.66 ± 0.17^{abc}	0.88 ± 0.016^{b}

Values with different letters in the same column are significantly different at p< 0.05

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