

# Effect of Citrullus Colocynthis Seeds Extract on Some Physiological and Biochemical Changes in Male Mice Treated with Anti-Androgenic Drug

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## ABSTRACT

The study aimed to investigate the ameliorative effects of aqueous extract of Citrullus colocyntis some physiological & biochemical parameters in the serum of mice treated with Tamsulosin for 5 weeks. Aqueous extract of Citrullus were prepared and Tamsulosin drug diluted. All preparation administrated orally once in a day by gavages needle. Twenty male albino mice randomly divided to 4 groups at the controlled conditions with pellets and water ad libitum. 1<sup>st</sup> control group received normal saline, 2<sup>nd</sup> group received ACC 20 mg/kg, 3<sup>rd</sup> received Tamsulosin 0.01mg/kg, while 4<sup>th</sup> group received ACC + Tamsulosin. Blood samples were collected, and serum obtained after centrifuged of the blood. Serum kept in eppendorf tubes and stored at deep freeze (-20 °C) till biochemical tests carried out for detection of GOT, GPT, ALP, urea, uric acid and creatinin levels, also lipid fractions TC, TG and HDL-C with glucose concentration were determined, in addition to FSH, LH and testosterone levels. The results of Tamsulosin administration recorded significant increase ( $P < 0.05$ ) in the serum concentration of GOT, ALP, urea, uric acid and glucose in addition to lipid fractions, TC and HDL. While significant decline in the LH and testosterone activities recorded as compared control group. While the animals when co-treated with ACC+ Tamsulosin recorded non-significant differences ( $P > 0.05$ ) in all above parameters as compared control group. So concluded, the animals received long term Tamsulosin when co-treated with ACC improves the deleterious effects of Tamsulosin concerned with hepato-renal function tests parameters in addition to some lipid fractions and hormones.

**Keywords:** Citrullus Colocynthis; Seeds Extract; anti-androgenic; Tamsulosin

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## INTRODUCTION

Citrullus Colocynthis (L.) Schard is one of the natural plants grows in sandy soil belongs to Cucurbitaceae family, have 120 genera and approaching from 825 species (Bendjeddou et al; 2003). The plant composed from phytochemicals as alkaloids, carbohydrates, flavonoids, Tannins, gums and mucilage (Gurudeeban, et al; 2010). Citrullus Colocynthis ( CCT ) used as hypoglycemic and hypolipidemic (Nabil et al; 2007 ), anti-inflammatory, antioxidant, analgesic (Lakshmi B. et al; 2013) and have effects on fertility and functions of the testes (Salla et al; 2015). Testes functioned to produce sperm (spermatogenesis) and androgens primarily testosterone hormone (Hassan KH. and Arrak J.K. 2015; Najdat et al., 2020a). CCT extract have anti-androgenic nature, decreases reversible infertility in male albino rats. So, testes found to have degenerative changes in the seminiferous epithelium and arrest of spermatogenesis at the secondary spermatocyte stage (Mali et al; 2001; Najdat et al., 2020b). Aksha Sharma; 2014, found the significant reduction in weight of testes when the rats treated by the ethanolic extract of 75 mg CCT, also recorded a significant decrement in the level of FSH, LH and testosterone hormones.

Several drugs prescribed for the treatment of infertility and reproductive disorders , some of them listed under anti- androgenic drugs which known as androgen antagonist or testosterone blockers which are class of drugs that impeded androgens like testosterone and DHT from mediating their biological effects by blocking the androgen receptor such as androcure (Fereshteh and Ali;

2012 ) and/or inhibiting androgen production such as Fenesteride and Tamsulosin through inhibition of 5 $\alpha$ -reductase activities (Bei Zhang, et al; 2016; Najdat et al., 2020c). The most application of these drugs, for treatment of prostate cancers, and benign prostatic hyperplasia (BPH), but they incorporated many side effects such as hepatotoxicity, osteoporosis and infertility (Chaturvedi et al; 2003; Najdat et al., 2020d; Abass et al; 2019).

The etiology of some disorders and disease of male reproductive organs which hamper with fertility such as cancer and BPH were the most problems of males in the world for the age related hormonal imbalance (Hanieh et al; 2012 ). Inhibitors of 5 $\alpha$ -reductase inhibited huge production of DHT in the prostate tissue that results inhibition proliferation of cells (Tagaya et al; 2009, Abbas et al; 2019). Furthermore, currently phytotherapeutic agents included almost 50% of all medicines prescribed for BPH in Italy, also 90% in Germany and Austria (Zegarra et al; 2007 ).

For overcome side effects of industrial drugs to be achieved through supplementation of complementary and alternative medicine such as medicinal plants, so the current study carried out to investigate the ameliorating and improvement effects of aqueous extract of CCT when used with or without anti-androgenic drugs through evaluation some physiological, biochemical and histopathological parameters in male albino mice treated with Tamsulosin.

## MATERIALS AND METHODS

## *Effect of Citrullus Colocynthis Seeds Extract on Some Physiological and Biochemical Changes in Male Mice Treated with Anti-Androgenic Drug*

### **Preparation of aqueous extract of Citrullus Colocynthis (ACC).**

The fruit Citrullus Colocynthis were purchased from local herb market in Kirkuk city. The seeds of the fruit were pulverized in electrical grinder for grinding of all seeds and obtained powder which was kept in cool, dry place. 200 gm of powder weighed by Denver analytic balance. The powder dissolved in 1000 ml of distilled water, and stored for 24 hr at R.T. In the second day the mixture was shaken continuously for 12hr with shaker water bath at 60°C (Memmert GmbH. Germany). The mixture was filtered using sterile gauze and incubated overnight in incubator (GFL. GmbH. Germany) (Nmila et.al; 2000).

Concentrated filtrate 200 ml (1gm/ml) were diluted by 0.9% normal saline to be used for the treatment of animals with dose 20 mg/kg B.W.

Tablets of Tamsulosin Hydrochloride 0.4 mg were freshly prepared daily by dissolving in 0.9% normal saline and diluted to be used 0.01 mg/kg B.W.

All preparations intubated orally by gavages needle once daily for 5 weeks.

### **Animal acclimatization and grouping**

Twenty-five male albino mice weighing  $25 \pm 1$  gm were obtained from college of veterinary medicine/ university of Baghdad, kept in standard cages for 2 weeks for acclimatization in animal house 12 hr light/dark cycles at temperature  $23 \pm 2$  °C. Standard pellet diet and water ad libitum. Animals were divided randomly to four groups (n= 5/ group).

Control group: received 0.1ml normal saline only.

ACC group: received aqueous extract of Citrullus Colocynthis 20mg/kg B.W.

Tamsulosin group: received 0.01 mg/kg B.W Tamsulosin HCL

ACC + Tamsulosin group: received ACC 20 mg/kg B.W + Tamsulosin 0.01 mg/kg B.W.

### **Collection and preparation of samples**

At the end of five weeks, the blood samples were gathered from animals by facial vein puncture for detection of blood parameters by fully automated system ( 3-part differentiation hematology system) ( Mythic 18),

while the second part of the blood were obtained by heart puncture and kept in jell tubes for separation of serum by centrifuge (Centerion , UK ). The serum was stored in eppendorf tubes in deep freeze (-20 °C) for biochemical tests.

The Liver enzymes (GOT, GPT, ALP) and other biochemical parameters in the serum (uric acid, urea, creatinin and glucose) were assayed using commercial kits (AGAPPE) from agape diagnostics SWITZERLAND GmbH, by Semi- Auto Chemistry Analyzer. For estimation of Lipid fractions used commercial kits from Randoux, UK, with instrument spectrophotometer (Schimadzo, Japan). Serum concentration of FSH, LH, and testosterone were evaluated by commercial kits, used AFIAS 6 (Automatic Fluorescence Immuno- Assay).

### **Statistical Analysis:**

Statistical Analysis: Conventional statistical methods were used to calculate means and standard errors. Analysis of variance (ANOVA) applied to test for any significant differences ( $P < 0.05$ ).

### **RESULTS**

The alternations of biochemical parameters in the serum of mice treated with different preparations as show in table 1, that the administration of Tamsulosin 0.01 mg/kg B.W. recorded significant increase ( $P < 0.05$ ) in the concentration of GOT ( $209.6 \pm 9.8$ ), ALP ( $378 \pm 4.1$ ) urea ( $51.4 \pm 3.1$ ) and uric acid ( $357 \pm 10$ ) as compared control and other treated groups.

While group of animals treated with aqueous extract of Citrullus Colocynthis 20mg/kg B.W and co- treated group with aqueous extract of Citrullus Colocynthis + Tamsulosin recorded significant increase ( $p < 0.05$ ) in the concentration of GOT ( $173 \pm 4.5$ ), ( $167.2 \pm 3.1$ ), ALP ( $338.8 \pm 4.4$ ), ( $331 \pm 3.5$ ), urea ( $40 \pm 2.7$ ), ( $40.4 \pm 2.7$ ), and uric acid ( $288 \pm 3.7$ ), ( $226 \pm 6.3$ ) respectively as compared to the control group. But there were no significant differences ( $P > 0.05$ ) in the level of GPT ( $21 \pm 0.7$ ) ( $21.8 \pm 0.8$ ) and creatinin ( $0.3 \pm 0.03$ ), ( $0.32 \pm 0.03$ ) respectively as compared control group (Table 1).

Table 1: Effect of aqueous extract of Citrullus Colocynthis seeds, Tamsulosin and ACC+ Tamsulosin on the level of some biochemical parameters in the serum of male mice.

Groups	GOT	GPT	ALP	Urea	Uric acid	Creatinin
	u/l	u/l	u/l	mg/l	mmol/l	mg/l
Control	$147.4 \pm 3.3$ a	$19.0 \pm 0.7$ a	$311.8 \pm 2.7$ a	$32.6 \pm 2.0$ a	$267 \pm 6.6$ a	$0.34 \pm 0.02$ a
ACC extract	$173 \pm 4.5$ b	$21.0 \pm 0.7$ ab	$338.8 \pm 4.4$ b	$40.0 \pm 2.7$ b	$288 \pm 3.7$ b	$0.30 \pm 0.03$ a
Tamsulosin	$209.6 \pm 9.8$ c	$24.6 \pm 1.5$ b	$378 \pm 4.1$ c	$51.4 \pm 3.1$ c	$357 \pm 10$ c	$0.42 \pm 0.04$ a
ACC + Tamsulosin	$167.2 \pm 3.1$ bd	$21.8 \pm 0.8$ ab	$331 \pm 3.5$ b	$40.4 \pm 2.9$ ab	$266 \pm 6.3$ a	$0.32 \pm 0.03$ a

a, b, c, d: Small letters refer to the significant differences ( $p < 0.05$ ) between groups at the horizontal arrows.

On other hand the results of lipid profile and glucose concentration revealed in table 2, were TC, TG and glucose concentration in Tamsulosin treated group recorded significant ( $P < 0.05$ ) increase ( $112.6 \pm 2.7$ ),

( $124.6 \pm 3.3$ ), and ( $19.9 \pm 0.5$ ) respectively as compared control & other treated groups. But HDL-C level ( $27.6 \pm 1.2$ ) recorded significant decrease ( $P < 0.05$ ) as compared control and other treated groups.

## Effect of Citrullus Colocynthis Seeds Extract on Some Physiological and Biochemical Changes in Male Mice Treated with Anti-Androgenic Drug

While treatment of animals with ACC recorded a significant decrease ( $P < 0.05$ ) in the level of TC ( $64.4 \pm 3.0$ ) and glucose ( $8.5 \pm 0.5$ ) as compared control group. On other hand, co-treatment of animals with ACC +

Tamsulosin recorded significant increase ( $P < 0.05$ ) in the concentration of TC ( $87.4 \pm 2.9$ ) and TG ( $116.4 \pm 2.9$ ) but significant decrease ( $P < 0.05$ ) in the level of glucose ( $10.8 \pm 0.4$ ) as compared control group.

Table 2: Effect of aqueous extract of Citrullus Colocynthis seeds, Tamsulosin and ACC+ Tamsulosin on the level of lipid fractions in the serum of male mice.

Groups	Cholesterol mg/dl	Triglyceride mg/dl	HDL-C mg/dl	Glucose mmol/l
Control	$71.0 \pm 1.4$ a	$85.2 \pm 1.4$ a	$48.6 \pm 2.3$ ab	$16.8 \pm 0.4$ a
ACC extract	$64.4 \pm 3.0$ ab	$80.0 \pm 2.3$ ab	$55.6 \pm 3.4$ b	$8.5 \pm 0.5$ b
Tamsulosin	$112.6 \pm 2.7$ c	$124.6 \pm 3.3$ c	$27.6 \pm 1.2$ c	$19.9 \pm 0.5$ c
ACC extract + Tamsulosin	$87.4 \pm 2.9$ d	$116.4 \pm 2.9$ c	$45.6 \pm 1.2$ a	$10.8 \pm 0.4$ d

a, b, c, d: Small letters refer to the significant differences ( $p < 0.05$ ) between groups at the horizontal arrows.

Hormonal investigation revealed in Table 3 were the results of treatment with Tamsulosin recorded significant decrease ( $P < 0.05$ ) in activities of FSH ( $0.23 \pm 0.03$ ), LH ( $0.26 \pm 0.05$ ) and testosterone ( $0.94 \pm 0.09$ ) as compared to the control and other treated groups.

While group of animals treated with ACC and the group of animals co- treated with ACC+ Tamsulosin recorded significant decrease ( $p < 0.05$ ) in the activities of LH ( $0.42$

$\pm 0.03$ ), ( $0.42 \pm 0.03$ ) and testosterone hormone ( $2.4 \pm 0.2$ ), ( $2.37 \pm 0.17$ ) respectively as compared control group, but activities of these hormones when compared with Tamsulosin treated group recorded significant increase ( $P < 0.05$ ). On other hand, FSH level in both treated groups did not record any significant differences ( $P > 0.05$ ) as compared control group.

Table 3: Effect of aqueous extract of Citrullus Colocynthis seeds, Tamsulosin and ACC+ Tamsulosin on the level of FSH, LH and Testosterone in the serum of male mice.

Groups	FSH mIU/ml	LH mIU/l	Testosterone ng/ml
Control	$0.56 \pm 0.05$ a	$0.66 \pm 0.05$ a	$4.2 \pm 0.4$ a
ACC extract	$0.58 \pm 0.05$ a	$0.42 \pm 0.03$ b	$2.4 \pm 0.2$ b
Tamsulosin	$0.23 \pm 0.03$ b	$0.26 \pm 0.05$ c	$0.94 \pm 0.09$ c
ACC. extract + Tamsulosin	$0.50 \pm 0.03$ a	$0.42 \pm 0.03$ b	$2.37 \pm 0.17$ b

a, b, c: Small letters refer to the significant differences ( $p < 0.05$ ) between groups at the horizontal arrows.

### DISCUSSION

The study aimed to investigate the ameliorative effects of ACC administration on some physiological and biochemical parameters in the serum of male albino mice treated with Tamsulosin. The treatment of animals with tamsulosin for long period 5 weeks showed adverse effects on hepato-renal function tests (Table 1). Suggesting treatment of mice for long time by tamsulosin could create hepato-renal damages. Two amino acids of hepatic enzymes structure, aspartic and glutamic acids play role for transferring ammonia in urea cycle, so any disturbance in their metabolism leads to dangerous influences on liver and other vital cells of the body (Abolfazl, N., 2014). Damages of hepatic and renal tissues reflected by elevation levels of hepatic enzymes and renal function test fractions in the serum as a result of oxidative stress or drug intoxication of animals (Najdat et al, 2020). On other hand, the deleterious effects of tamsulosin treatment appeared on lipid profile fractions

and glucose concentration in the serum (Tab. 2). Dyslipidemia considered one of the great factors for the development of coronary artery diseases (Cai; 2017; Farhan, 2019). Also the adverse effects of the drug could significantly cause damage to the vital cells of the pancreas and consequently elevated glucose level (Hassan et.al; 2019)

Furthermore, administration of tamsulosin significantly declined activities of FSH, LH and testosterone (Table 3). These results compatible with other studies, suggesting hormonal synthesis and release balance interrupted either at the hypothalamo-pituitary- gonads axis improvement (Atika; 2014) or at the gonads level (Najdat et.al; 2020a). Anti-androgenic drugs block androgenic receptors competitively therefore hinders testosterone synthesis at the cellular level (Chiao; 2002). Decrement of testosterone level in rats and human beings recorded after oral administration of bicalutamide (Hashimoto 2010). While slightly decline documented by (Han, et.al;

## Effect of *Citrullus Colocynthis* Seeds Extract on Some Physiological and Biochemical Changes in Male Mice Treated with Anti-Androgenic Drug

2018) but no any decline by (Morgante et,al; 2001) studies. Most of biochemical parameters were normalized by co- treatment of animals with ACC (Tab. 1). Therefore, supplementation of animals with ACC and Tamsulosin therapy attenuated the deleterious influences of Tamsulosin. Treatment of rats with flaxseeds oil exposed to lead acetate improved many biochemical parameters included urea, uric acid and creatinine ( Ahmed et, al; 2011), suggested to the protective effects of oil against degradation of proteins, nucleic acids and creatinin (Palanisamy et al, 2012). On other hand, TC, HDL and glucose levels also ameliorated during ACC supplementation in the group treated with Tamsulosin (Table.2).

The hypolipidemic effects and lowering atherogenic indices with significant reduction of liver cholesterol contents achieved in rats treated with Gundelia oil (Sharaf et,al; 2004). On other hand, 500 mg/kg B.W of crude *Citrullus colocynthis* administration to albino rats resulted highly significant decline in cholesterol level in serum, with no evident decrease in triglyceride (Nabil et, al; 2007).

Deleterious alterations of hormonal activities in the serum of mice treated with Tamsulosin were ameliorated when treated the animals with the plant extract as revealed in ACC + T group (Table 3). These results indicated to the improvement effects of ACC extract against adverse effects of Tamsulosin drug, through either antioxidant capacity provided by the active ingredients of the extract (Fereshteh et, al; 2017), or through enhancement capability of the extract on the gonads (Fouzia et, al; 2015 ) or on the hypothalamo-pituitary axis ( Solomon et,al; 2010 ).

Stimulatory effects of damask rose extract on FSH, LH and testosterone levels were investigated in male rats when treated with 200 and 400 mg/Kg B.W (Hosseini et, al; 2016). While alcoholic extract of *Citrullus Colocynthis* streptozotocin-induced diabetic recorded significant decrease in FSH level and increment %10 in LH level, with non-significant alterations in testosterone level (Fereshteh et,al; 2014).

Finally, we concluded from the results of the study the aqueous extract of ACC have protective and ameliorative effects for most of biochemical enzymes, lipid fractions, glucose and hormonal activities against adverse effects of Tamsulosin administration.

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## *Effect of Citrullus Colocynthis Seeds Extract on Some Physiological and Biochemical Changes in Male Mice Treated with Anti-Androgenic Drug*

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