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castus Leaves) Herbal against
Nephrotoxicity on
Experimental Rats**

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Protective Effect of Chastberg (Vitex agnus-castus Leaves) Herbal against Nephrotoxicity on Experimental Rats

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Abstract:

Oxidative damage is involved in the pathogenesis of various nephrotoxicity. In the present study, the protective effect of *Vitex agnus-castus* leaves was investigated against cisplatin (CIS) induced nephrotoxicity in rats. Thirty albino male rats were classified into five groups as followed, the first group as control group while the four groups injected with CIS, other were the second group received only CIS, as groups 3, 4 and 5 were administrated 5%, 10%, and 15% *Vitex agnus-castus* leaves) respectively for 28 days.

The results revealed that CIS intoxication impaired kidney function tests. Serum livers of AST, ALT and ALP levels were elevated by CIS administration. Treatment with *Vitex agnus-castus* at three different levels attenuated these adverse effects and biochemical alterations caused by CIS especially, at the highest level 15%. Nephrotoxicity by CIS resulted in significant elevation of serum triglycerides, total cholesterol, LDL-C, VLDL-C and decreasing in serum HDL-C. Moreover, kidney function tests as serum urea, uric acid and creatinine were found to be increased and the treatment with *Vitex agnus-castus* leaves at all treated levels (especially, at the highest level 15%) significantly reversed these adverse alterations. CIS caused significant increased in acetyl cholinesterase (AChE) and significant decreased in total antioxidant and superoxide dismutase activity.

Treatment with *Vitex agnus-castus* has strong radical scavenging activity and antioxidant activity at all level. Therefore, the results of this study show that *Vitex agnus-castus* leaves can be proposed to protect nephrotoxicity induced by CIS in rats. The results also revealed that the nephrotoxicity effect of *Vitex agnus-castus* may be attributed to its antioxidant and free radical scavenger effects.

Key words: Cisplatin, liver function, serum lipid profile, acetyl cholinesterase, antioxidant activity.

Introduction:

Thus, many herbal products are used as traditional medicine for the treatment of nephrotoxicity the world (**Chen et al., 2011**). Herbal products can improve not only glucose metabolism but also improve lipid metabolism, antioxidant status, and capillary function (**Webster, 2011**).

Vitex agnus-castus, also has many common names including vitex, chaste tree, chasteberry, Abraham's balm, lilac chastetree, or monk's pepper is a native of the Mediterranean costal region and central Asia and belongs to *the Botanical family Verbenaceae* (**Hogner et al., 2013**). *Vitex agnus-castus* contain flavonoids (vitexin, casticin), agnuside, p-hydroxybenzoic acid, alkaloids, diterpenoids and steroidal hormone precursors (**Hoberg et al., 2000**). It was used in ancient Greece and Rome as anaphrodisiac (diminish sexual desire) and traditionally used as digestive aid, sedative, anti-infective, treat acne, insect repellent (**Hajdu et al., 2007**), antihistaminic, anti inflammatory and antioxidant (**Johann et al., 2016**) as well as treatment of several female disorders such as endometriosis, abnormal menstrual cycles, menopausal conditions, insufficient lactation and acne (**Choudhary et al., 2009 and Johann et al., 2016**).

Cisplatin (CIS) is an inorganic, divalent, water soluble, platinum-containing compound. CIS is a highly efficient anti-neoplastic drug commonly used as a first-line therapy for treatment of various solid tumors such as: stomach cancer, ovarian cancer, lung cancer, bladder cancer and germ cell tumors (**Bergs et al., 2007**). The anticancer effect of CIS is mediated by apoptosis and DNA-crosslinks with subsequent cytotoxic lesions in malignant cells (**Benedetti et al., 2013**). However, its clinical use is associated with dose and duration-dependent nephrotoxic side effect (**Saad et al., 2009**).

The present study is carried out to develop chemical composition and functional properties of *Vitex agnus castus* leaves. Also the effect of *Vitex agnus castus* leaves on kidney functions, liver functions, serum lipid profile, antioxidant capacity and acetylcholinesterase activity will be also the scope of this investigated.

Materials and Methods:

Materials:

Vitex agnus-castus was obtained from the Crops Intensification Research Section Field Crops Research Institute, Agricultural Research Center. Cairo, Egypt.

Cisplatin solution[®] (Platinol AQ) was obtained from Sigma Chemical Co. (St Louis, Mo, USA).

Rats and diet:

Thirty male albino rats (Sprague Dawley Strain) were obtained from National Research Center, Dokki, Egypt. Casein, cellulose, all vitamins and minerals were purchased from El-Gomhoryia Company For Trading Chemicals and Medical Appliances. Cairo. Egypt. Kits were obtained from Biodiagnostic Cairo. Egypt.

Methods:

Chemical Composition of *Vitex agnus-castus*:

Moisture, protein, crude fibers, fat content and ash contents were determined according to the method described in the **A.O.A.C (2000)**. Total carbohydrates were calculated by difference. Total phenolic was determined according to (**Singleton et al., 1999**). Total flavonoid was determined according to (**Zhishen et al., 1999**).

Experimental Design:

Thirty male albino rats (*Sprague Dawleey strain*) weighing $120 \pm 5g$ were kept in individual stainless steel cages under hygienic conditions and fed one week on basal diet for adaptation in *ad libitum*. The basal diet in the preliminary experiment of diet prepared according to (**Reeves et al., 1993**). After a period of adaptation (one week), the rats were divided into two main groups, the first group (n = 6 rats) fed on basal diet, as a normal control group, while the second group (n = 24 rats) were a single intra-peritoneal injection of cisplatin in a dose of 12 mg/kg body weight according to (**Atssahin et al., 2006**).

The second main group was fed on basal diet and injected with cisplatin, and divided into four subgroups (6 rats each). The first subgroup was fed on basal diet as positive control (group 2). Subgroups

3, 4 and 5 were fed basal diet containing 5%, 10% and 15% vitex agnus-castus powder leaves respectively. During the experimental period (28 days), the feed intake was calculated daily and the body weight gain was recorded weekly (**Chapman et al., 1950**).

Feed efficiency ratio (FER) was calculated as follow: FER = weight gain (g)/ feed intake (g). At the end of the experiment, the animals were fasted overnight, then the rats were anaesthetized sacrificed, and blood samples were collected into clean centrifuge tubes to obtain the serum which used for biochemical analyses.

Biochemical Analysis:

Serum uric acid, urea nitrogen and creatinine were determined by the methods of **Fossati, et al. (1980)**, **Patton and Crouch, (1977)** and **Bohmer and Heirli (1972)** respectively. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by **Reitman and Frankel (1957)**, while alkaline phosphatase (ALP) was assayed by **Kind and King, (1954)**. Serum total cholesterol, triacylglycerol, high density lipoprotein and low density lipoprotein were determined by the methods of (**Allian et al., 1974**, **Fossati and Principe, 1982** and **Friede Wald et al. 1972**), respectively, Kidneys were separated from each rat and weighted to calculate kidneys weight to body weight %. Superoxide dismutase (SOD) activity, and total antioxidants capacity (TAC), were determined according to **Nishikimi et al. (1972)** and **Cao et al. (1993)** respectively. Acetylcholinesterase (AChE) activity was determined according to **Knedel and Boottger (1967)**.

Statistical Analysis:

The obtained results of biological evaluations were statistically analyzed according to statistical analysis system SAS User's Guide, (**SAS, 1999**). LSD at 5% level of significance was used to compare between means according to **Snedecor and Cochran (1980)**.

Results and Discussion:

Table (1) described the chemical composition of *V. agnus-castu* which the high in carbohydrate, protein, moisture and crude fiber were 68.9, 15.4, 8.2 and 7.6 %, respectively. While was low in fat and ash 4.8 and 2.7% respectively. Data in Table (2), showed that T. phenol and flavonoide were high levels in 15% *V. agnus-castus* powder. While, *V.*

agnus-castus at levels of (5&10%) recorded to lowest levels of T. phenol and flavonoide compared to 15%. *V. agnus-castus* powder.

The results showed that there were significant decreases in the mean body weight, food intake and food efficiency ratio (FER) in CIS group (+ve) compared with normal control group. After CIS fed with treated groups 5%, 10% and 15% *V. agnus-castus* the in body weight, food intake and food efficiency ratio were significantly increased when compared with CIS group ($P < 0.05$) (Table 3).

A marked increase in weight gain and the more improvement in nutritional results appeared in treated with 15% *V. agnus-castus* group. These results may be attributed to loss of appetite, caused by cisplatin disturbance in the gastric enzymes secretions. These data are similar to that of **Venugopal et al. (2013)**. This is in agreement with the previous studies done by **Hanaa (2017)** who reported that *V. agnus-castus* leaves contain important polyphenolic compounds such as high level of gallic, pyrogallol, 4-amino-benzoic, catechol, caffeine and caffeic compounds.

The effect of *V. agnus-castus* at levels of 5, 10 and 15% on kidney function measured in cisplatin-induced nephrotoxicity in rats was presented in Table (4). From such these results it could be that there was significant $P \leq 0.05$ improvement in uric acid, urea and creatinine with *V. agnus-castus* levels. While indicated that it has favourable effect in bringing down the severity induced by cisplatin as in the positive control group. Uric acid urea, urea and creatinine level were significantly higher in control (+ve) group compared to the normal control group. Urea and creatinine are waste products of protein metabolism that need to be excreted by the kidney, therefore a marked increase in serum urea and creatinine as noticed in this study confirms an indication of functional damage to the kidney. Therefore, significant increases in urea and creatinine levels noticed in this study are a classical sign that the kidney was adversely affected by CIS administration (**Saad et al., 2009 and Venugopal et al., 2013**).

Current findings show that the administration of *V. agnus-castus* at levels of 5, 10 and 15% decreases uric acid, urea and creatinine all treated rats groups compared with CIS treated group. We conclude that *V. agnus-castus* levels may be a promising compound for reducing cisplatin-toxic side effects including nephrotoxicity.

Table (5) presented that, positive control rat group showed significant increase in AST, ALT and ALP level compared to negative control group. The groups treated with *V. agnus-castus* at levels of (5, 10 and 15 %) reduced the level of AST and ALT with respect to (+ve) control rat group. In similar study of **Olaleye and Rocha (2008)** reported that CIS generally are considered hepatotoxic, which associated with a decreased in serum enzyme liver and steatosis. In another instance, hepatocellular liver injury was described associated to cisplatin.

While groups treated with *V. agnus-castus* at levels of 10 and 15% showed significant decrease in AST, ALT and ALP compared with treated group with (5%) *V. agnus-castus*. Similar finding was observed by **Hanaa (2017)** who noticed hepatoprotective potential of *V. agnus-castus* due to its content of gallic, pyrogallol, 4-amino-benzoic, catechol, caffeine, caffeic, vanillic and p-conmaric are reported from its leaves which might be due to the presence of vitamin C, A and E which has hepatoprotective effect.

Serum lipid profile analysis (Table 6) revealed that CIS group (+ve) has significantly $P \leq 0.05$ the highest levels of triacylglycerols, total cholesterol, LDL-C and VLDL-C while having the lowest level of HDL-C which indicate the negative effect of CIS group (+ve). On the other hand, treated groups with 5% and 10% *V. agnus-castus* has significantly the lowest levels of triacylglycerols, total cholesterol and VLDL-C while having the highest level of HDL-C although the LDL-C level was similar to that in treated group with 15 % *V. agnus-castus* and the control group (insignificant difference). The results indicate the great effect of *V. agnus-castus* leaves in control of dyslipidemia. This effect may be due to certain chemical constituents such as polyphenols or terpens in vitex leaves which possess good oxygen radical scavenging potential (**Mu and Porsgaard, 2005**). Flavonoids can dramatically lower cholesterol levels and the rate of formation of oxidized (LDL) (**Johann et al., 2016**). These results are in harmony, with those obtained by (**Hesham et al., 2008**). The antioxidant effect of *V. agnus-castus* plays a significant role in amelioration of CIS-induced renal damage, but the lipid-lowering effect of statins may also be involved in this mechanism.

In Table (7), the positive control group showed a significant decrease in superoxid dismutase (SOD) and total antioxidants, while a

significant increase in acetyl cholinesterase (AChE) compared to (-ve) control group. It is potential that increased reactive oxygen species (ROS) production in the serum may be responsible for this damage of the organ as reflected by the change in the levels of SOD and activities of AchE in the study.

Treatment with *V. agnus-castus* levels at (5, 10 and 15%) showed also significant increase total antioxidants. While insignificant decrease in AchE compared to (+ve) control group. These results confirm the antioxidant effect of *V. agnus-castus* leaves extract. As mentioned previously, the free radical scavenging activity of *V. agnus castus* may be due to certain chemical constituents such as polyphenols or terpenes which possess good oxygen radical scavenging potential (Mu and Porsgaard, 2005). Thus, it was judged that co-administration of *V. agnus-castus* powder leaves with cisplatin may be useful to minimised the negative effects of cisplatin nephrotoxicity, and oxidation on male rats.

Table (1): Chemical composition of *V. agnus-castus* leaves (g/100g dry weight basis).

Variables Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrates (%)	C. Fiber (%)
<i>V. agnus-castu</i>	8.2	15.4	4.8	2.7	68.9	7.6

Values are the means of 3 independent determinations.

Table (2): Total phenol and flavonoids of *V. agnus-castus* levels

Variables Levels	T.phenol mg/g	Flavonoids mg/g
5%	2.52	2.16
10%	3.69	2.90
15%	4.37	3.56

Values are the means of 3 independent determinations.

Table (3): Effect of *V. agnus-castus* levels on Feed intake, Body weight (%) and FER of rats groups.

Parameters Groups	Feed intake g/day/rat	Body weight gain %	FER
Normal control	15.667±0.817 ^a	21.359±1.549 ^a	0.994±0.013 ^a
Positive control	13.383±0.585 ^c	9.616±0.971 ^d	0.753±0.083 ^c
5% <i>V. agnus-castus</i>	14.667±0.605 ^b	13.988±1.496 ^c	0.954±0.102 ^b
10% <i>V. agnus-castus</i>	14.750±0.758 ^b	16.523±1.246 ^b	0.943±0.086 ^b
15 % <i>V. agnus-castus</i>	14.950±0.561 ^b	20.537±1.113 ^a	0.992±0.088 ^a

Mean values in each column having different superscript letters denote significant difference. $P \leq 0.05$

Table (4): Effect of *V. agnus-castus* levels on kidneys function of rats groups.

Parameters Groups	Uric acid mg/dl	Urea Nitrogen mg/dl	Creatinine mg/dl
Normal group	1.93±0.042 ^d	31.99±2.981 ^d	0.94±0.054 ^d
Positive control	2.87±0.062 ^a	82.98±3.577 ^a	4.24±0.407 ^a
5% <i>V. agnus-castus</i>	2.10±0.104 ^b	61.90±2.382 ^b	2.855±0.130 ^b
10% <i>V. agnus-castus</i>	1.91±0.126 ^{bc}	52.36±4.148 ^c	2.58±0.107 ^c
15% <i>V. agnus-castus</i>	1.51±0.070 ^c	50.26±3.338 ^c	2.34±0.205 ^c

Mean values in each column having different superscript letters denote significant difference. $P \leq 0.05$

Table (5): Effect of *V. agnus-castus* levels on liver enzymes activity of rats groups.

Parameters Groups	AST Activity (Iu/l)	ALT Activity (Iu/l)	ALP Activity (Iu/l)
Normal group	52.80±2.010 ^e	24.21±1.678 ^e	65.26±3.936 ^e
Positive control	94.47±5.568 ^a	53.86±3.127 ^a	113.28±6.488 ^a
5% <i>V. agnus-castus</i>	70.63±4.741 ^b	39.76±3.013 ^b	90.33±3.642 ^b
10% <i>V. agnus-castus</i>	63.82±4.271 ^c	32.83±3.431 ^c	76.64±5.649 ^c
15% <i>V. agnus-castus</i>	56.92±3.022 ^d	27.63±2.104 ^d	68.96±2.622 ^d

Mean values in each column having different superscript letters denote significant difference. $P \leq 0.05$

Table (6): Effect of *V. agnus-castus* levels on serum lipid profile of rats groups.

Parameters Groups	TC mg/dl	TG mg/dl	LDL-c mg/dl	HDL-c mg/dl	VLDL-c mg/dl
Normal control group	75.88± 3.856 ^c	45.16 ± 2.288 ^c	18.152± 0.718 ^c	48.30 ± 2.825 ^a	9.07 ± 0.457 ^c
Positive control	125.62 ± 3.884 ^a	75.24 ± 4.151 ^a	84.177± 1.182 ^a	26.20± 2.831 ^e	15.85± 0.830 ^a
5% <i>V. agnus-castus</i>	105.47± 4.028 ^b	54.24± 3.083 ^b	59.584± 1.766 ^b	33.08± 2.767 ^d	12.84± 0.617 ^{bc}
10% <i>V. agnus-castus</i>	89.29± 4.054 ^c	51.94± 3.456 ^c	42.067± 0.937 ^c	36.96 ±2.755 ^c	11.29 ± 0.692 ^c
15 % <i>V. agnus-castus</i>	81.14± 3.644 ^d	47.88± 2.795 ^d	35.076± 2.386 ^d	40.60± 1.748 ^b	10.48± 0.559 ^d

Mean values in each column having different superscript letters denote significant difference. $P \leq 0.05$

Table (7): Effect of *V. agnus-castus* levels on antioxidant parameters and acetyl cholinesterase of rats groups

Parameters Groups	Total antioxidants mmol/L	(AchE) U/L	(SOD) mg/L
Normal control group	4.65±0.42 ^a	121.93±4.61 ^d	1.73±0.12 ^a
Positive control	1.12±0.15 ^c	204.710±10.75 ^a	0.26±0.09 ^d
5% <i>V. agnus-castus</i>	2.77±0.15 ^b	163.30±6.58 ^b	0.99±0.02 ^c
10% <i>V. agnus-castus</i>	2.94±0.06 ^b	158.13±6.75 ^c	1.40±0.07 ^b
15% <i>V. agnus-castus</i>	3.65±0.6 ^b	133.04±5.16 ^d	1.52 ± 0.2 ^b

Mean values in each column having different superscript letters denote significant difference. $P \leq 0.05$

AchE: Acetyl cholinesterase
dismutase

SOD: Superoxide

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التأثير الوقائي لأوراق عشبة كف مريم ضد التسمم الكلوي على فئران التجارب

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٢- قسم الاقتصاد المنزلي - كلية التربية النوعية - جامعة بور سعيد - مصر

الملخص:

تهدف هذه الدراسة الى معرفة التأثير الوقائي لأوراق عشبة كف مريم للوقايه من الضرر الناتج من عمليات التأكسد الحاده للكلى فى الفئران نتيجة حقنها بالسيبيلاتين. وقد اجريت هذه الدراسة على ثلاثين من ذكور الفئران البيضاء وتم تقسيمها الى خمس مجاميع (الضابطه، المتناوله السيبيلاطين فقط و ٥% و ١٠% و ١٥% من أوراق عشبة كف مريم) لمدة ٢٨ يوما. وقد اوضحت النتائج ان التسمم بالسيبيلاتين قد اثر تأثيرا سلبيا على وظائف الكبد حيث ارتفع معنويا سيرم كل من AST,ALT,ALP، وذلك بالمقارنه بالمجموعة الضابطة. وكذلك اسفرت المعالجة عشبة كف مريم على الثلاث مستويات المختلفة الى تحسن معنوى ملحوظ فى كل هذه العوامل السابقة وخصوصا على المستوى الأعلى ١٥%. كما ادى تسمم الكلوي بالسيبيلاتين الى ارتفاع معنوى لسيرم كل من الدهون الثلاثية، الكوليستيرول الكلى، الكوليستيرول المنخفض الكثافة بينما كان هناك انخفاض معنوى لسيرم الكوليستيرول العالى الكثافة وكذلك اظهرت النتائج وجود زياده معنوية فى مستويات اختبارات الكلى لكل من سيرم اليوريا، الكرياتينين وحمض اليوريك، وبالمقارنه بالمجاميع المعالجة بعشبة كف مريم على الثلاث مستويات المختلفة لوحظ انخفاض معنوى لكل هذه العوامل السابقه مع ارتفاع معنوى فى مستوى الكوليستيرول العالى الكثافة HDL. وكانت احسن النتائج للمجموعة المتناوله ١٥% من عشبة كف مريم.

كما اثبتت النتائج زيادة معنوية فى مستوى أستيل كولين AchE وكذلك انخفاض معنوى فى نشاط الانزيمات المضادة للاكسدة فى السيرم SOD، للفئران المصابة بالسيبيلاتين وان المعالجة بكف مريم لديها تأثير قوى ككاسحات للشقوق الحرة وكذلك كمضادات اكسدة لكل المستويات الثلاثة المتناولة وخصوصا على المستوى الأعلى ١٥% يليه ١٠% ثم ٥% من كف

مريم والتي عكست كل هذه التغيرات السلبية حيث ادت المعالجة بكف مريم الى ارتفاع معنوى فى مستوى نشاط مضادات الاكسدة و SOD، وانخفاض معنوى فى مستوى أستيل كولين. لذلك توصى الدراسه بضرورة تناول عشبة كف مريم لما لها من تأثير فعال فى حماية الكلى من التسمم حيث تعمل كمضادات للأكسدة مما ينعكس على الصحة العامة.