INFLUENCE OF SILYMARIN EXTRACTED FROM *SILYBUM MARIANUM* SEEDS COMPARED TO LEGALON AGAINST NICKEL CHLORIDE INDUCED HEMATOLOGICAL AND BIOCHEMICAL CHANGES IN MALE RABBITS

Wasfi Dh. Abid Ali* , Abdul Razak N. Khudair**, Eman.A. AL-Masoudi**

*College of Nursing, , University of Basrah, Basrah, Basrah, Iraq.
**Department of Physiology ,College of Veterinary Medicine , University of Basrah, Basrah, Basrah, Iraq.

(Received 13 April 2015,Accepted 5 June 2015)

Keywords; Silymarin, Nickel, Rabbits

ABSTRACT

The object of this study is to evaluate the influence of silymarin extracted from *Silybum marianum* seeds from north of Iraq and traditional silymarin (legalon) against $NiCl_2$ toxicity in male rabbits. Forty male rabbits weighted (1.250-1.500 Kg) were randomly assigned into four equal groups treated for 35 days :Control group ,received normal saline ,1st group received 1mg/100g B.W $NiCl_2$,2ndgroup received 1mg/100g B.W $NiCl_2$ plus 0.1mg/100g B.W silymarin extrct,3rdgroup received 1mg/100g B.W $NiCl_2$ plus 0.1mg/100g B.W legalon for 35 days. $NiCl_2$ administration significantly decreased RBCs count,Hb,PCV,total protein,HDL and total body weight but WBCs showed insignificant change while increased serum glucose ,cholesterol,TG,LDL,ALT,AST,ALP, urea and uric acid. The study indicated that silymarin and legalon reversed the level of all studied parameters value near the normal, and revealed the crucial role of silymarin extract and legalon supplementation resulted in a remarkable protective effect against $NiCl_2$.

INTRODUCTION

Milk thistle is a plant with a long history in medicine, having been mentioned in every important medicinal record of herbs**.(1).** It was widely used as a medicinal plant in traditional European medicine, and its seeds have been used for over 2000 years for a variety of purpose (**2**), The milk thistle *Silybum marianum* is an annual or biannual herbaceous plant that widespread in American countries , Australia and Mediterranean climate(**3, 4).** Milk thistle also grow in north part of Iraq and north of Bagdad (**5**). Silymarin appears to act as an antioxidant not only because it acts as a scavenger of the free radicals that induce lipid

peroxidation, but also because it influences enzyme systems associated with glutathione and superoxide dismutase**. (6).**

The biosynthesis of flavonolignans in *Silybum marianum are* Flavonolignans which are formed by combination of flavonoid and lignan structures. This occur by oxidative coupling processes between a flavonoid and a phenylpropanoid, usually coniferyl alcohol (Oxidative coupling occurs between free radical generated from the flavanol taxifolin and the free radical generated from coniferyl alcohol. **(7).**

Nickel and its compounds have been reported to be potent carcinogenic and-or toxic agents in human beings and experimental animals **(8).** Nickel is a known haematotoxic, immunotoxic, neurotoxic, genotoxic,reproductive toxic, pulmonary toxic, nephrotoxic **(9)** , hepatotoxic and carcinogenic agent, also nickel had effect of its acute, subchronic and chronic doses on certain metabolically active tissues in human as well as animals(**10*).***

MATERIAL AND METHODS

Milke thistle seeds (*silybum Marianum)*from the north of Iraq(mussel), seeds were selected according to their condition where damaged seeds were discarded before seeds in good condition were cleaned,. Seeds were grounded using grinder prior to extraction.50 grams each time were defatted in a soxhlet apparatus, using normal hexane (boiling point of 40◦C) for 3 hours **.**The oil was separated by distillation, the remain defatted seed powder was transferred into a flask fitted with a condenser and 150 ml of absolute ethanol was added and stirred for 72 hr. at room temperature.. After filtration and concentration of the silymarine fraction under vacuum, the yellow residue was dissolved in 20 ml of toluene and evaporated for one hour., 20 ml of diisopropyle add to the crystals and reflexed for I hr. and cooled for I hr.The mixture evacuated and the remain crystals will collect and weighted. Stored in cold place. **(11,12).**

Experimental animals

Forty male rabbits bought from the local market of Basrah city market of 5 to 6 month age and weight 1.250-1.500 kg caged in metallic cages and randomly divided into four group treated for 35 days . Ten male rabbits was served as control group and received 1 ml normal saline orally. First group: Ten male rabbits received 1mg /100gram B.W $NiCl_2$ orally ,Second group :Ten male rabbits received 1mg /100gram B.W

$NiCl_2$ fallowed by 0.1mg/100gram B.W extracted silymarin .Third group: Ten male rabbits received 1mg /100100gram B.W $NiCl_2$ fallowed by 0.1mg/100gram B.W Legalon (legalon forte MADUS GmbH, Colgen,Germany).

Collection of serum samples:

Blood samples were collected via heart puncture in glass centrifuge tubes by sterile disposable syringes then centrifuged for 15 min. at 3500rpm. Serum were separated and stored at –20^{o}C in deep freezer till further biochemical measurements.

The erythrocyte, the total leukocyte counted by Hemocytometer, the packed cell volume (PCV)measured by Hematocrit procedure and hemoglobin (Hb) concentrations were determined by sahli 's method.

Biochemical analysis:

Serum total protein, cholesterol, triglycerides, glucose ,HDL and LDL levels were determined automatically using visible Spectrophotometer (Apel PD 303)

The Statistical analysis

The results of the present study were analyzed by using one way analysis of variance (ANOVA) test. The statistical analysis was performed by using the program(spss). The data were expressed as a means $\pm$SE. ($P<0.05$) were considered to be significant for all data of this study.

RESULTS AND DISCUSSION

In general, Nickel is widely distributed metal that is industrially applied in many forms, it can accumulated to high level and become source of intoxication**(13)**. The present study investigated that RBCs count ,PCV and Hb were significantly ($p\leq0.05$) decreased in males rabbits that received oral $NiCl_2$, while WBCs showed insignificant change (table 1). (**14),(15)** showed that the level of hemoglobin, red blood cells and packed cell volume were significantly decreased and simultaneously the white blood cells after nickel supplementation.

(16) reported that reduction in RBC may be caused by the inhibition of erythropoiesis or by destruction of red cells. They also reported that these changes may be due to anemic condition and hemolysis caused by heavy metals. **(17)** concluded that changes observed indicate that hematological parameters can be used as an indicator of Ni stress ,The elevation of RBCs count ,PCV and Hb in the treated rabbits with $Nicl_2$ and silymarin extract or with

legalon indicated the ameliorative effects of silymarin extract and legalon . (**18**)found that RBCs ,WBCs

and PCV were markedly improved with silymarin treatment in male rats treated with CCL_4 or Aloxan (**19**).

Table(1)The effect of oral dosing of $NiCl_2$, $NiCl_2$ plus silymarein extract and $NiCl_2$ plus Legalon on RBCs count WBCs count ,HB and PCV count in male rabbits.(Mean ± SE).

Parameters / group	RBCs $\times10^6$cells/mm^3	WBCs $\times10^3$cells/mm^3	Hb g/dl	PCV%
Control Normal saline 1ml (0.9 NaCl)	9.650 ±0.6034 A	5.120±0.6604 A	11.500±0 .830 A	31.60±1.046 A
Group1 $NiCl_2$(1mg/100 g B.W)	5.270 ±0.3026 B	5.200±0.3261 A	7.360±0.2621 B	21.30 ±0 .761 B
Group 2 $NiCl_2$(1mg/100 g)+SNext.(0.1g/ 100gB.W)	9.470 ±0 .7460 C	5.490 ±0.5870 A	11.720+0.2426 AB	40.40±1.176 C
Group 3 $NiCl_2$(1mg/100 g)+legalon.(0.1 g/ 100gB.W)	9.650 ±0.7306 C	4.185±0 .6591 N.S	10.720 ±0.2426 C	40.50±1.213 C

N=5 SN =Silymarin Capital letters denote differences between groups,P≤0.05 vs. control

Table (2) showed that total protein was decreased significantly ($p\leq0.05$) all treated group for 35 day compared with control group , total protein showed significant difference changes($p\leq0.05$) between the group treated with $NiCl_2$ plus silymarin and that treated with $NiCl_2$ plus legalon. , **(20)** did not found any significant effect of nickel or zinc mixed with diet on the growth of rabbits as well as on total protein but **(21)** found nickel supplementation decreased serum total protein in rats.Glucose significantly increased ($p\leq0.05$) in treated group compared with control while the values of serum glucose concentration significantly ($p\leq0.05$) decreased in the group treated with $NiCl_2$ plus legalon and $NiCl_2$ plus legalon). Glucose significantly increased after oral administration of $NiCl_2$ this result showed agreement with **(22)** who pointed to the systemic effects of nickel exposure include Hyperglycemia, increase level of plasma glucagon and damage to the

pancreatic islet cells, other study on male and female rabbits indicated that nickel caused increase in blood and plasma level of glucose and male

showed fast recovery**(23**,**24,25**).**)**.(**26)** suggested that silymarin induces pancreatic function recovery demonstrated by insulin and glucagon in rats .

Table (2)The effect of oral dosing of $NiCl_2$, $NiCl_2$ plus silymarein extract and$NiCl_2$ plus Legalon on serum concentration of Total protein, body weight and glucose after 35 day of treatment on male rabbits.(Mean ± SE)

Parameters / Group	Total body weight gram	Total protein g/l	glucose mg/dl
Control Normal 1 ml saline (0.9 NaCl)	127.500±17.658 A	10.485 ± 0.477 A	115.069± 2.017 A
Group 1 $NiCl_2$(1mg/100g B.W	1137.500±17.682 B	4.499 ± 0.393 B	150.663 ± 6.058 B
Group 2 $NiCl_2$(1mg/100g)+SN ext.(0.1g/ 100gB.W)	1300.600±15.118 AC	7.4700 ±0.350 C	125.44 ± 1.161 C
Group 3 $NiCl_2$(1mg/100g)+legalon(0.1g/ 100gB.W)	1314.100±12.251 AC	9.26180 ±0.338 D	120.555 ± 2.196 A

N=10 ,SN= Silymarin Capital letters denote differences between groups,P≤0.05 vs. control

Nickel Chloride drenching caused statically significant (P≤0.05) increase in cholesterol, TG,LDL, and VLDL except fall in HDL in serum concentration of male rabbits in the group that treated with oral dosing $NiCl_2$ for 35 days in comparison with the control. Nickel sulfate and potassium dichromate treated rats showed a significant increase in serum low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) and triglyceride (TG) level as well as decrease in serum high density lipoprotein-cholesterol(HDL-C) level(table 3)**.**

(27), (28) , (29) supported the results of the study ,they pointed that nickel induced rise in serum TC, LDL-C, VLDL-C and TG and fall in serum HDL-C V may be due to changes in gene expression of some hepatic enzyme like HMG-CoA reductase (hydroxyl-methyl-glutaryl-CoA), which in turn depresses LDL-receptor gene expression these investigation emphasized thethe outcome of the present study study. The rise in serum triglyceride is possibly due to hypoactivity of lipoprotein lipase in blood vessels which breaks up TG **(30).**

(31)found that feeding standard laboratory diet did not respond to oral administration of silymarin by decrease of serum cholesterol, but the mild increase in HDL cholesterol was found .but Previous support or result, that orally administered silymarin, possesses a hypocholesterolemic effect in rats fed high cholesterol diet enriched with fat**(32).**

Table(3)The effect of oral dosing of $NiCl_2$, $NiCl_2$ plus silymarein extract and $NiCl_2$ plus Leganol on serum concentration of cholesterol,TG,HDL,LDL and VLDL after thirty five days of treatment on male rabbits (Mean ± SE).

Parameter / group	Cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl	LDL mg/dl	VLDL
Control Normal 1 ml saline (0.9 NaCl)	81.721± 0.752 A	62.176±0.314 A	48.318 ± 0.493 A	19.115±0.250 A	12.435±0.062 A
Group 1 $NiCl_2$(1mg/100g B.W	138.777 ±6.460 B	134.37±12.92 B	37.965± 0 .851 B	47.038±3.056 B	26.875±3.056 B
Group 2 $NiCl_2$(1mg/100g) + SN ext.(0.1g/100gB. W)	74.659± 3.202 AC	58.645±1.936 AC	45.932 ± 0.528 B	17.074± 0.751 AC	11.729±0.387 AC
Group 3 $NiCl_2$(1mg/100g) +legalon.(0.1g/ 100gB.W)	85.609± 2.412 AC	66.098 ±1.26 AC	47.036± 0 .743 B	16.724±1.281 AC	13.219±0.252 AC

N=10 ,SN= Silymarin Capital letters denote differences between groups,P≤0.05 vs. control

Supported the result of this study **(33)** confirmed the anti-hyperlipidimic effect of silymarin on rabbits by evaluating the effect of silymarin alone on 20 patients with hyperlipidaemia**.(34)** showed that silymarin produce significant reduction in triglyceride, cholesterol, LDL, VLDL but significant elevation in HDL level. The administration of silymarin reduces plasma levels of cholesterol and low-density lipoprotein (LDL) cholesterol in rats, whereas silibinin does not reduce plasma levels of cholesterol in normal rats (**35**). Improvements of silymarin might be due

to their ability to lower serum total cholesterol and low- density lipoprotein cholesterol levels as well as slowing the lipid peroxidation process by enhancing antioxidant enzyme activity (**36).** A significant positive effects were found in men on plasma cholesterol and LDL-cholesterol levels .On the other hand, a significant decrease in HDL-cholesterol level was found**(37).**

Table(4)The effect of oral dosing of $NiCl_2$, $NiCl_2$ plus silymarein extract and $NiCl_2$ plus Legalon on serum concentration of AST,ALT and ALP after thirty five day of treatment on male rabbits.(Mean ± SE).

Parameters / group	AST U/L	ALT U/L	ALP U/L
Control Normal 1 ml saline (0.9 NaCl)	9.6160 ±0.34092 A	13.8780 ±0.558 A	25.828 ±0.84077 A
Group 1 $NiCl_2$(1mg/100g B.W	41.6780 ±0.891 B	53.5070 ±1.239 B	62.793 ± 0 .951 B
Group 2 $NiCl_2$(1mg/100g)+silymarein ext.(0.1g/ 100gB.W)	11.8160 ± 0 .3409 C	16.3780±00.55 C	29.165 ± 0 .616 C
Group 3 $NiCl_2$(1mg/100g)+legalonl.(0.1g/ 100gB.W)	13.8160 ± 0 .339 D	17.2780 ± 0.558 D	31.128 ± 0.840 D

N=10 ,SN= Silymarin Capital letters denote differences between groups,P≤0.05 vs. control

The outcome of the present study revealed a significant(P≤0.05) increased in ALT,AST an ALP on males group that treated with $NiCl_2$compared with control group. Groups that received $NiCl_2$plus Silymarin or legalon(0.1mg/100gram B.W).also data showed that there were significant(P≤0.05) differences changes between both treated groups of $NiCl_2$plus Silymarin or legalon. Nickel intoxication caused a significant increase in the activities of GOT, GPT and ALP, probably due to hepatocyte membrane damage resulting in increased release and leakage out of these enzymes from the liver cytosol into the blood stream which gives an indication on the hepatotoxic effect of this metal (**38, 39**).

The mean levels of ALT, AST, ALP in rats that received Methotrexate plus silymarin were significantly lower than those animals received only Methotrexate –induce liver damage**(40, 41**). Agreement with this study the markers of liver inflammation, i.e. serum ALT and AST levels, were significantly higher in nickel-exposed workers compared with the control group**(9**). Studies results emphasized the present study when they showed significantly increased activity of serum ALT and AST enzymes following nickel treatment, which are indicative of damage to the liver parenchyma(**42 ,43**) .**44**) concluded Silymarin also stabilized cellular membrane structure and regulated the levels of AST, ALT, ALP, CK,

and LDH activity. **(45)** showed significant protection of silymarin against anti-tuberculosis drugs induced hepatotoxicity, as evidenced by marked reduction of the raised serum markers of hepatic function. **(36)**Treatment with milk thistle seeds extracts was found to return the ALT ,AST and ALP level back to normal and urea was significantly elevated, their results shows similar trends as our results.

Table(5)The effect of oral dosing of $NiCl_2$, $NiCl_2$ plus silymarein extract and $NiCl_2$ plus Leganol on serum concentration of urea and uric acid after thirty five day of treatment on male rabbits .(Mean ± SE).

Parameters / group	Urea Mg/ dl	Uric acid mg/dl
Control Normal 1 ml saline (0.9 NaCl)	41.044 ±0.681 A	2.8440 ± 0.115 A
Group1 $NiCl_2$(1mg/100g B.W	60.041 ±0.475 B	10.725 ±0.393 B
Group 2 $NiCl_2$(1mg/100g)+SN ext.(0.1g/100gB.W)	47.124 ±0.845 C	4.780 ± 0.154 B
Group 3 $NiCl_2$(1mg/100g)+legalon.(0.1g/100gB.W)	44 .004 ± 0.527 AD	2.954 ±0.306 AC

N=10 ,SN= Silymarin Capital letters denote differences between groups,P≤0.05 vs. control

Table (5) indicate significant ($p \leq 0.05$) increase in urea and uric acid in $NiCl_2$ treated group compared with the control while the values were significantly($p \leq 0.05$) decreased in groups treated with $NiCl_2$ plus Silymarin and that which received $NiCl_2$ plus Legalon. The increased serum urea and serum uric acid were certainly as a result of kidney tissue damage and dysfunction in nickel and cobalt chloride treated rat **(46).**

(47)pointed that hyperuricemia is associated with renal disease, but it is usually considered a marker of renal dysfunction as ðhuman body cannot metabolized nickel ,so nickel salts considered as a industrial health hazard so kidney became major organ of nickel toxicity**(48).** To investigate the beneficial role nickel induced nephrotoxicity, Nickel administration increased the levels of serum urea, uric acid **(49),**this emphasizes the present investigations that serum urea and uric acid were significantly increased when male rabbits received $Nicl_2$

Conclusion

The results described here clearly confirmed the anti-hyperlipidemic Hepatoprotective and ameliorative effects on some blood parameterand of silymarin extract from *silybum mariamume* against nickel chloride toxicity.

تأثير مستخلص السليمارين من بذور نبات شوكة مريم المقارن بعقار اللجالون ضد كلوريد النيكل الحاث للتغيرات الدموية والكيموحيوية في ذكور الارانب

وصفي ظاهر عبد علي*، عبد الرزاق نعيم خضير**، إيمان عبود المسعودي**

*كلية التمريض ،جامعه البصره ،البصره ،العراق.

** فرع القسلجة والادوية ،كلية الطب البيطري ، جامعة البصرة،البصره ،العراق.

الخلاصة

تضمنت الدراسة تأثير مستخلص السليمارين من بذور شوكة مريم في شمال العراق والمستحضر التجاري اللجالون ضد التسمم الذي يسببه كلوريد النيكل في ذكور الارانب. اربعون ارنب ذكر تتراوح أوزانها(١.٢٥٠-١.٥٠٠ كيلو جرام) وزعت عشوائياً الى اربعة مجاميع متساوية تم معالجتها لفترة خمس وثلاثون يوماً :مجموعة السيطرة وجرعت ا ملليلتر من المحلول الملحي(NaCl0.9%) ، المجموعة الاولى جرعت ١ ملغم /١٠٠غرام من وزن الجسم كلوريد النيكل، المجموعة الثانية جرعت ١ ملغم /١٠٠غرام من وزن الجسم كلوريد النيكل مع١.٠ملغم/ ١٠٠غرام من وزن الجسم من مستخلص بذور السليمارن، المجموعة الثالثة جرعت ١ ملغم /١٠٠غرام من وزن الجسم كلوريد النيكل مع١.٠ملغم /١٠٠غرام من وزن الجسم مادة الجالون لفترة خمس وثلاثون يوماً. تناول كلوريد النيكل خفض معنويا ,Hb, PCV , RBCscount total protein ,total body weight و HDL, لم يظهر , WBCs count تغيراً معنوياً ، بينما ارتفع معنوياً كل من uric acid, ALP, ,AST, ALT, LDL, TG, cholesterol ,glucose و urea في مصل الدم .تشير نتائج الدراسة ان كل من مستخلص السليمارين و اللجالون عكس مستويات مؤشرات الدراسة نحو المستويات القريبة من المستويات الطبيعية لمجموعة السيطرة وكشف الدور الحاسم لمستخلص السليمارين واللجالون ضد تأثيرات كلوريد النيكل.

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