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Assessment the efficacy of some herbes in the prevention of steroid induced hepatopathy in dog

Nehal Elhosiny[1], Abdel Raouf M. Mahmoud[2], Hatem M. Selim[2], Heba Gouda[2*]

[1]*Directorate of Veterinary Medicine, Ismailia Governorate, Egypt.*
[2]*Internal Medicine, Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt.*

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***Correspondence:**

Corresponding author: Heba Gouda
E-mail address: dr.hebagouda@gmail.com

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ABSTRACT

Medicinal plants play a crucial role in the treatment and prevention of steroid- induced hepatopathy. The purpose of the study was to evaluate the ability of some herbes to reduce the steroid- induced hepatopathy. Twenty mongrel dogs were included in the experimental study. Dogs were divided into four groups each group consisting of five animals; Group I received dexamethasone, Group II received dexamethasone and concentrated milk thistle (silymarin), Group III received dexamethasone and L-carnitine, Group IV received dexamethasone and liquorice powder. Clinical, blood samples, ultrasound were done on days 0, 7, 14 and 21. Haemato- biochemical analysis indicated a significant increase in hemoglobin (Hb) concentration and hematocrit value, a significant decrease in white blood cells count, a significant decrease in alkaline phosphatase (ALP) activity and a significant increase in total protein and albumin levels in silymarin treated Group II, L-carnitine treated Group III and liquorice treated Group IV compared to Group I. Ultrasonography revealed an increase in echogenicity of liver parenchyma in hepatopathic induced dogs and returned to nearly normal in Group II and Group III. In conclusion, the medicinal plants have a potential effect in treatment and prevention of steroid induced hepatopathy.

Introduction

Liver is the most vital complex organ in the body which plays a critical role in control of metabolism, synthesis of plasma protein, biliary secretion, bile acids and intestinal absorption of essential fatty acids (Kozat and sepehrizadeh, 2017). Glucocorticoids used as anti-inflammatory and immunosuppressive drugs in small animals. Administration of glucocorticoids for long period accompanied by several side effects as vomiting, diarrhea, loss of weight, increased susceptibility to infection, behavior change, loss of hair, and liver hepatopathy which is considered one of the most common pathological changes as a result of corticosteroid treatment (Elkholly *et al*., 2020). Medicinal plants play an important role in maintaining the health and treatment of many diseases in dogs especially liver diseases with low incidence of side effects (Ahmed *et al*., 2021). Because herbal medicine is from natural source, so there is a constant believe that it is a safe source of treatment (Stickel and Schuppan, 2007).

Silymarin (milk thistle) has been used since ancient times in the treatment of liver diseases, it consists of flavonoids silibinin, silidianin, silichristin, and isosilibinin, it has hepatoprotective effect and antioxidant effect to protect against fibrosis by glutathione depletion prevention. Silymarin prevent hepatic stellate cell action through inhibition of leukotriene forming in kupffer cell cultures (Stickel and Schuppan, 2007). Silymarin prevent ALP elevation and protect from liver injury, it protects liver from poisonous effect of some plants and mushrooms (Floersheim *et al*., 1978).

L-carnitine improve of the internal organs functions in many animal species, remove toxic products after some oxidation reactions, used to increase growth rate in aquafeeds, decrease ammonia toxic effect, help in decreasing high temperature stress factor, L-carnitine is synthesized in liver and also derived from lysine and methionine amino acids, it helps cardiac and skeletal muscles in oxidation process to produce energy from fatty acids consumption (Harpaz, 2005). L-carnitine have very important role in fat metabolism, it helps in activation of ketogenesis, gluconeogenesis, and the metabolism of valine, leucine, and isoleucine and suppression of triglycerides and cholesterol. About 95% of L-carnitine is stored in muscles and hepatocytes and about 4% in renal cells and 1% in plasma (Neumann *et al*., 2007). L- carnitine is used in diets of pet animals to protect from hepatic lipidosis as it helps in conversion of fat to energy and increase muscle mass of the body and increase burning of fat (Hatzade *et al*., 2020).

Glycyrrhizin is extracted from liquorice root (Glycyrrhiza glabra) and consists of glycyrrhizic acid, flavonoids, hydroxycoumarins, and beta-sitosterol, it was reported that it has good role in treatment of gastritis, bronchitis and chronic hepatitis. Glycyrrhizin protects from liver injury through inhibition of 11-beta-hydroxysteroid dehydrogenase, prostaglandin E2 production by macrophages, and initiate activation of Glutathione-S-transferases and catalase to make antioxidative effect (Stickel and Schuppan, 2007). Ethanolic extract of licorice root is rich in antioxidant, which act to reduce inflammation and activate healing process, and have several positive reactions on biochemical markers, Glyceric acid and glycerin help in treating gastrointestinal ulcers, as the licorice roots contain coumarin, flavonoids, essential oils, and plant sterols so it used to treat muscle spasms and swelling, bronchitis, arthritis, and rheumatism (Zhang *et al*., 2021).

Therefore, the aim of the present study was focused on evaluating the ability of some herbal to reduce steroid- induced hepatopathy in dogs.

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Materials and methods

Ethical approval

All the experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Zagazig University (Approval No. ZU-IACUC / 2 / F / 351 / 2023).

Animals and study design

Animals under investigation were classified into 20 mature mongrel dogs aging from 8-18 month and weighted from 10-20 kg. On admission, physical examination was done to all animals to check their health status. The animals were housed for 15 days to acclimatize and were monitored during this period. All dogs were vaccinated and treated with an appropriate anthelmintic (praziquantel [Drontal], 5 mg/kg PO) according to Elfadadny *et al*. (2021) before the experiment. All groups were reared in uniform nutritional and managemental condition and in the same boxes during the whole experimental periods, they were supplied with ad libitum diet (meat and chicken extract) and had free access to water.

Experimental protocol

Clinical examination was performed for all animals with closed daily observation to any clinical abnormalities including body temperature, heart rate, respiratory rate, and mucous membranes examination.

Animals grouping

The dogs were randomly divided into four groups (GI, GII, GIII and G IV); each consisted of five animals (3 males and 2 females) in GI, GII, GIII and (5 males) in G IV.

Group I: received dexamethasone at a dose of 1mg/kg(ADwia Pharma, Egypt) .

Group II: received dexamethasone (ADwia Pharma, Egypt) at a dose of 1mg/kg and concentrated milk thistle at a dose of 5 g per os and esomeprazole (Copad Pharma, Egypt) at a dose of 1mg/kg per os.

Group III: animals received dexamethasone at a dose of 1mg/kg and l carnitine (MEPACO-MEDIFOOD, Egypt) at a dose of 500 mg, 3 times per os daily and esomeprazole at a dose of 1mg/kg per os.

Group IV: received dexamethasone at a dose of 1mg/kg and liquorice powder at a dose of 2g per os and esomeprazole at a dose of 1mg/kg per os.

Blood Sampling technique

Blood samples were collected from cephalic vein of each dog by using hypodermic needle and divided into two parts, first part on heparinized tubes for hematological examination and the second part on plain tube left to coagulate at room temperature for collection of clear non hemolyzed serum for biochemical analysis.

Hematological and biochemical analysis

Whole blood samples were used for determination of Hb (g/dl), RBCs (10^6/ul), HCT%, WBCs (10^3/ul) and platelets by using full version automatic cell counter (Sysmex KX-2IN, Japan) according to the method described by Grindem (2011).

The clear non hemolyzed serum was obtained from tubes by Pasteur pipette and clarified by centrifugation at 3000 rpm for 20 minutes to remove residual red cells, then kept in sterile clean vials at -20^{0}C in deep freeze until analysis.

Serum samples were used for measurement of alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, total protein, albumin, globulin, creatinine, and urea levels which were determined spectrophotometrically by standard procedures using (spectrum diagnostic commercial kits).

Samples were collected on days 0, 7, 14 and 21 of dexamethasone administration and at the same times of herbal treatment.

Ultrasonography

Real time ultrasound system with linear probe 5,7 MHZ (Pie medical ultrasound imaging system, Toshiba just vision 200 imaging system) and coupling gel were used. Ultrasound examination was done on days 0, 7, 14 and 21 of dexamethasone injection and at the same times of herbal extract treatment.

Statistical analysis

Data of the present study were analyzed using two way factorial design ANOVA procedures according to Snedecor *et al*. (1989) for testing of significance among the studied groups. Means separation and pairwise comparisons were done by Duncan's Multiple Range test according to Duncan (1955). Statistical analyses were conducted by SPSS for windows (SPSS version 25). Results are considered significant at probability level of 0.05 for each ($P \leq 0.05$).

Results

Clinical findings

In Group I, till the end of the first week, the dogs were observed alert, active with normal appetite and weight. On day 7, the dogs had a decrease in body weight and changes in hair and hair loss. On day 14, there was a severe decrease in body weight from 1-2 kg, changes in stool color from dark brown to black, sever alopecia with appearance of some skin ulcers.

In Group II, III, the dogs appeared alert, active, with a gradual increase in appetite and body weight till day 21 with no changes in hair , while in Group IV, the dogs appeared alert, active and there was a slight decrease in body weight on day 21 with no changes in hair.

Hematological findings

The hematological indices are summarized in Table 1. There were significant decreases in Hb (g/dl) and HCT % and a significant increase in WBCs in Group I on day 14. After herbal treatment, there were significant increases in Hb (g/dl) and HCT % and a significant decrease in WBCs in Silymarin treated Group II and l-carnitine treated Group III on day 21.

Biochemical findings

The biochemical findings are summarized in Table 2. Serum biochemical analysis revealed a significant decrease in ALP in silymarin treated Group II, l-carnitine treated Group III and liquorice treated Group IV on day 21 compared to Group I. There was a significant increase on day 14 and a significant increase in total protein and albumin in silymarin treated Group II, l-carnitine treated Group III and liquorice treated Group IV on day 21 compared to the Group I where there was a significant decrease on day 7.

Ultrasonographic examination

Ultrasonographic examination at day zero of the experiment revealed normal sagittal scan of the liver parenchyma that appeared homogenous moderately echoic interrupted by anechoic gall bladder (GB) and short, highly echogenic paired parallel lines that represent the portal vein (PV)

Table 1. Haematological parameters alterations all over the experimental study (means and standard error for fixed effects and interactions of treated groups under different times.

	Treatments / Times	Group I	Group II	Group III	Group IV	Times Main effects
Hb (g/dl)	Zero day	10.20±1.08^{ab}	10.53±0.96^{ab}	11.20±0.91^{ab}	9.67±0.65^{ab}	10.40±0.42^{a}
	7 days	9.77±1.74^{ab}	11.77±0.42^{a}	11.73±0.18^{a}	10.30±0.92^{ab}	10.89±0.51^{a}
	14 days	8.43±1.22^{b}	10.67±0.19^{ab}	10.83±0.67^{ab}	9.17±0.47^{ab}	9.78±0.44^{a}
	21 days	8.30±1.00^{b}	11.00±0.58^{ab}	11.90±0.30^{a}	10.47±1.18^{ab}	10.61±0.51^{a}
	Treatments Main effects	9.25±0.63^{c}	10.99±0.30^{ab}	11.42±0.28^{a}	9.90±0.39^{bc}	10.41±0.24
RBCs (10^6/µl)	Zero day	4.38±0.51^{ab}	4.16±0.35^{ab}	4.77±0.46^{ab}	4.22±0.21^{ab}	4.38±0.19^{a}
	7 days	3.90±0.68^{ab}	4.06±0.14^{ab}	4.40±0.16^{ab}	6.02±2.16^{a}	4.59±0.55^{a}
	14 days	3.36±0.53^{b}	3.82±0.08^{ab}	4.19±0.32^{ab}	3.57±0.17^{b}	3.73±0.17^{a}
	21 days	3.50±0.50^{b}	4.23±0.26^{ab}	4.75±0.18^{ab}	3.64±0.46^{b}	4.08±0.21^{a}
	Treatments Main effects	3.81±0.28^{a}	4.07±0.11^{a}	4.53±0.15^{a}	4.36±0.56^{a}	4.20±0.16
HCT (%)	Zero day	29.63±3.35^{ab}	31.00±2.74^{ab}	33.20±2.94^{ab}	28.73±1.79^{ab}	30.64±1.28^{a}
	7 days	28.13±5.53^{ab}	33.90±1.26^{ab}	34.17±0.59^{a}	29.73±2.77^{ab}	31.48±1.57^{a}
	14 days	24.20±4.06^{b}	31.13±0.72^{ab}	31.53±2.12^{ab}	26.33±1.46^{ab}	28.30±1.40^{a}
	21 days	24.15±2.85^{b}	30.43±2.90^{ab}	35.57±0.92^{a}	27.90±4.85^{ab}	30.00±1.88^{a}
	Treatments Main effects	26.75±1.98^{c}	31.62±0.99^{ab}	33.62±0.92^{a}	28.18±1.34^{bc}	30.11±0.76
WBCs (10^3/µl)	Zero day	13.70±3.44^{bcdef}	7.20±2.17^{f}	9.67±0.77^{ef}	10.90±2.91^{def}	10.37^{b}±1.29
	7 days	25.43±8.10^{a}	19.80±1.31^{abcde}	22.33±0.77^{abc}	15.57±5.17^{abcdef}	20.78^{a}±2.34
	14 days	20.87±2.52^{abcd}	19.23 ±1.03^{abcde}	22.83±0.88^{abc}	23.53±2.46^{ab}	21.62^{a}±0.95
	21 days	22.85±6.25^{abc}	9.10±1.42^{ef}	12.30±0.12^{cdef}	15.13±4.11^{abcdef}	14.12^{b}±1.98
	Treatments Main effects	20.52±2.70^{a}	13.83±1.85^{b}	16.78±1.80^{ab}	16.28±2.13^{ab}	16.78±1.08
Platelets (10^3/µl)	Zero day	43.33±3.18^{d}	84.00±13.11^{cd}	96.00±16.62^{bcd}	86.33±27.42^{cd}	77.42±9.60^{b}
	7 days	215.00±13.53^{ab}	208.67±41.09^{ab}	232.00±20.55^{a}	252.33±57.98^{a}	227.00±16.83^{a}
	14 days	206.67±43.33^{ab}	137.67±35.55^{abcd}	62.67±15.34^{cd}	66.33±16.86^{cd}	118.33±22.00^{b}
	21 days	86.00±36.00^{cd}	174.67±51.09^{abc}	67.67±12.73^{cd}	151.67±86.38^{abcd}	123.09±28.02^{b}
	Treatments Main effects	142.45±26.80^{a}	151.25±21.33^{a}	114.58±21.96^{a}	139.17±31.90^{a}	136.74±12.65

Means carrying different superscripts within the same raw or same column are significantly different at ($p<0.05$)

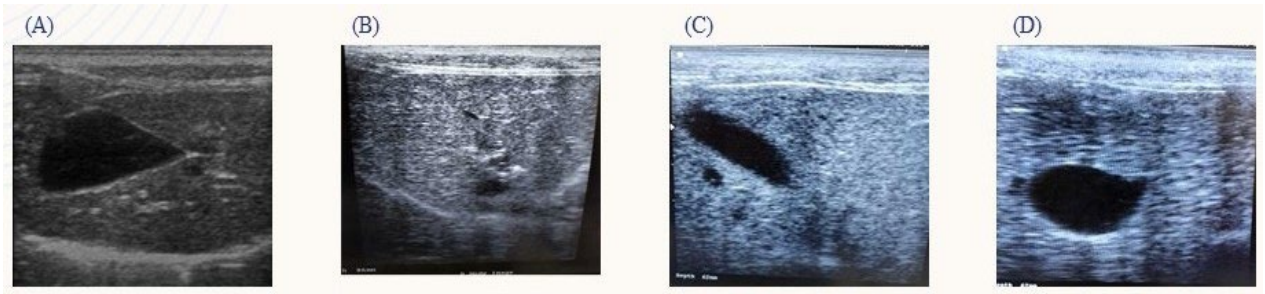

Fig.1. A) Normal sagittal scan of the liver parenchyma that appeared homogenous moderately echoic interrupted by anechoic gall bladder (GB) and short, highly echogenic paired parallel lines that represent the portal veins (PV) at day 0. B) Liver ultrasonography on day 7 of dexamethasone injection notice uniformly increase in echogenicity of hepatic parenchyma (appeared nearly equivalent). C) Liver ultrasonography on day 14 of dexamethasone injection notice uniformly moderate increase in hepatic parenchyma with necrotic foci. D) Liver ultrasonography on day 21 of dexamethasone treatment notice uniformly marked increase in hepatic echogenicity.

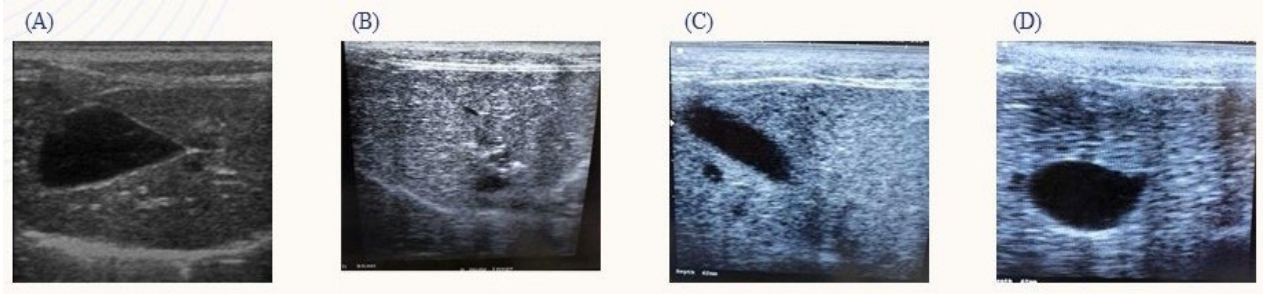

Fig. 2. A) Ultrasonographic picture of Silymarin treated Group II on day 21 showing moderate echoic hepatic parenchyma and anechoic gall bladder and average wall thickness which look to be nearly normal texture. B) Ultrasonographic picture of liver in l-carnitine treated group III on day 21 showing slight increase in echogenicity and anechoic gall bladder compared with day 0. C) Ultrasonographic picture of liver in liquorice treated group IV on day 21 showing more increase in echogenicity of liver parenchyma compared with silymarin treated group II and l-carnitine treated group III.

Table 2. Biochemical parameters findings all over the experimental study (means and standard error for fixed effects and interactions of treated groups under different times.

	Treatments / Times	Group I	Group II	Group III	Group IV	Times Main effects
ALT (IU/L)	Zero day	19.67±2.33^{e}	20.00±1.53^{e}	28.33±4.67^{e}	37.67±10.84^{e}	26.42±3.41^{c}
	7 days	116.70±5.96^{bc}	64.47±6.84^{de}	46.67±10.68^{e}	42.33±3.71^{e}	67.54±9.44^{b}
	14 days	154.47±7.73^{b}	120.00±11.55^{bc}	134.33±35.53^{bc}	124.67±23.78^{bc}	133.37±10.38^{a}
	21 days	225.50±17.50^{a}	99.33±9.24^{cd}	128.67±24.36^{bc}	147.33^{b}±11.20	143.36±15.21^{a}
	Treatments Main effects	120.32±22.82^{a}	75.95±11.95^{b}	84.50±17.19^{b}	88.00^{b}±15.90	91.59±8.64
ALP (IU/L)	Zero day	96.96±8.16^{c}	137.10±13.23^{c}	135.37±20.99^{c}	166.23±12.53^{c}	133.92±9.65^{c}
	7 days	302.70±34.49^{bc}	228.33±17.23^{c}	281.03±42.88^{bc}	254.33±88.04^{bc}	266.60±23.97^{bc}
	14 days	540.50±18.19^{b}	142.17±11.51^{c}	274.97±104.12^{bc}	534.63±244.68^{b}	373.07±76.79^{ab}
	21 days	1623.00±384.00^{a}	191.50±21.94^{c}	207.27±43.05^{c}	201.03±41.96^{c}	458.67±181.75^{a}
	Treatments Main effects	551.50±175.98^{a}	174.78±13.32^{b}	224.66±31.60^{b}	289.06±71.26^{b}	304.86±48.97
Total bilirubin (mg/dl)	Zero day	0.57±0.03^{a}	0.66±0.08^{a}	0.69±0.06^{a}	0.58±0.07^{a}	0.63±0.03^{a}
	7 days	0.69±0.08^{a}	0.70±0.12^{a}	0.67±0.09^{a}	0.60±0.06^{a}	0.67±0.04^{a}
	14 days	0.66±0.03^{a}	0.50±0.06^{a}	0.50±0.06^{a}	0.80±0.31^{a}	0.62±0.08^{a}
	21 days	0.64±0.03^{a}	0.52±0.07^{a}	0.55±0.03^{a}	0.73±0.19^{a}	0.61±0.05^{a}
	Treatments Main effects	0.64±0.03^{a}	0.59±0.04^{a}	0.60±0.03^{a}	0.68±0.08^{a}	0.63±0.03
Direct bilirubin (mg/dl)	Zero day	0.14±0.03^{bc}	0.10±0.03^{c}	0.43±0.03^{a}	0.32±0.14^{ab}	0.25±0.05^{a}
	7 days	0.25±0.03^{abc}	0.16±0.03^{bc}	0.16±0.03^{bc}	0.12±0.02^{bc}	0.17±0.02^{ab}
	14 days	0.22±0.03^{bc}	0.09±0.01^{c}	0.08±0.01^{c}	0.27±0.17^{abc}	0.17±0.04^{ab}
	21 days	0.16±0.005^{bc}	0.12±0.01^{bc}	0.13±0.01^{bc}	0.14±0.02^{bc}	0.13±0.01^{b}
	Treatments Main effects	0.20±0.02^{a}	0.12±0.01^{a}	0.20±0.04^{a}	0.21±0.05^{a}	0.18±0.02
Indirect bilirubin (mg/dl)	Zero day	0.42±0.02^{ab}	0.56±0.05^{a}	0.26±0.02^{b}	0.26±0.09^{b}	0.38±0.04^{a}
	7 days	0.44±0.07^{ab}	0.54±0.09^{a}	0.51±0.06^{ab}	0.48±0.04^{ab}	0.49±0.03^{a}
	14 days	0.44±0.04^{ab}	0.42±0.03^{ab}	0.42±0.06^{ab}	0.53±0.15^{a}	0.45±0.04^{a}
	21 days	0.49±0.03^{ab}	0.40±0.06^{ab}	0.43±0.02^{ab}	0.59±0.16^{a}	0.48±0.05^{a}
	Treatments Main effects	0.44±0.02^{a}	0.48±0.03^{a}	0.40±0.03^{a}	0.47±0.06^{a}	0.45±0.02
Total Protein (g/dl)	Zero day	4.90±0.09^{bcd}	6.18±0.50^{ab}	6.53±0.57^{a}	5.21±0.07^{abcd}	5.70±0.26^{a}
	7 days	4.71±0.10^{cd}	5.92±0.47^{abc}	5.91±0.55^{abc}	5.28±0.19^{abcd}	5.45±0.22^{a}
	14 days	5.19±0.86^{abcd}	4.33±0.50^{d}	5.66±0.29^{abcd}	4.70±0.41^{cd}	4.97±0.28^{a}
	21 days	5.34±0.67^{abcd}	5.20±0. 26^{abcd}	5.13±0.38^{abcd}	5.00±0.06^{bcd}	5.15±0.15^{a}
	Treatments Main effects	5.01±0.23^{b}	5.41±0.29^{ab}	5.81±0.25^{a}	5.05±0.12^{b}	5.32±0.12
Albumin (g/dl)	Zero day	1.67±0.03^{bc}	2.33±0.58^{abc}	2.13±0.30^{abc}	1.42±0.17^{c}	1.89±0.18^{a}
	7 days	1.77±0.20^{abc}	2.47±0.09^{abc}	2.13±0.03^{abc}	1.93±0.07^{abc}	2.08±0.09^{a}
	14 days	1.62±0.18^{bc}	2.97±0.23^{a}	2.11±0.92^{abc}	2.77±0.22^{ab}	2.37±0.27^{a}
	21 days	2.70±0.10^{ab}	2.47±0.26^{abc}	2.65±0.53^{ab}	1.97±0.28^{abc}	2.42±0.18^{a}
	Treatments Main effects	1.87±0.14^{b}	2.56±0.16^{a}	2.26±0.24^{ab}	2.02±0.17^{ab}	2.18±0.10
Globulin (g/dl)	Zero day	2.51±0.30^{bcde}	3.84±0.75^{ab}	4.40±0.81^{a}	3.79±0.24^{abc}	3.63±0.32^{a}
	7 days	2.08±0.43^{cde}	3.45±0.55^{abcd}	3.77±0.52^{abc}	3.35±0.23^{abcd}	3.16±0.27^{ab}
	14 days	1.95±0.44^{de}	1.36±0.70^{e}	3.54±0.79^{abcd}	1.84±0.33^{de}	2.17±0.35^{c}
	21 days	2.04±0.03^{de}	2.73±0.52^{abcde}	2.49±0.14^{bcde}	3.03±0.33^{abcde}	2.62±0.18^{bc}
	Treatments Main effects	2.15±0.17^{b}	2.85±0.39^{ab}	3.55±0.34^{a}	3.00±0.25^{a}	2.90±0.16
Creatinine (mg/dl)	Zero day	0.61±0.08^{abcde}	0.76±0.12^{abc}	0.67±0.05^{abcd}	0.49±0.01^{de}	0.63±0.04^{a}
	7 days	0.68±0.03^{abcd}	0.73±0.18^{abcd}	0.75±0.03^{abc}	0.56±0.04^{bcde}	0.68±0.05^{a}
	14 days	0.53±0.02^{bcde}	0.86±0.08^{a}	0.78±0.04^{ab}	0.57±0.02^{bcde}	0.68±0.05^{a}
	21 days	0.48±0.07^{de}	0.56±0.12^{bcde}	0.52±0.06^{cde}	0.40±0.06^{e}	0.49±0.04^{b}
	Treatments Main effects	0.58±0.03^{bc}	0.73±0.06^{a}	0.68±0.04^{ab}	0.50±0.03^{c}	0.63±0.02
Urea(mg/dl)	Zero day	27.27±4.94^{b}	25.30±3.49^{b}	29.67±3.38^{b}	19.67±4.81^{b}	25.48±2.12^{b}
	7 days	23.33±2.85^{b}	32.33±1.67^{b}	26.67±1.45^{b}	21.00±2.52^{b}	25.83±1.59^{b}
	14 days	18.23±0.37^{b}	38.00±5.77^{b}	39.33±2.85^{b}	35.33±2.03^{b}	32.73±2.94^{b}
	21 days	32.75±2.35^{b}	61.00±14.05^{a}	68.67±13.17^{a}	32.67±16.37^{b}	50.23±7.83^{a}
	Treatments Main effects	24.73±2.10^{b}	39.16±5.24^{a}	41.08±5.82^{a}	27.17±4.25^{b}	33.21±2.49

Means carrying different superscripts within the same raw or same column are significantly different at ($p<0.05$)

(Figure 1A). While in (Group I) there was increase in hepatic echogenicity at day 7, 14 (Figure 1B, C) which become nearly equal in echogenicity to spleen at day 21 (Figure 1D). While in (Group II) at day 21 show moderate decrease in echogenicity which look to be nearly normal texture (Figure 2A). While in (Group III) at day 21 liver picture revealed slight increase in echogenicity and anechoic gall bladder compared with day 0 (Figure 2B). While in (Group IV) there was increase in echogenicity of liver parenchyma compared with (Group II) (Group III) (Figure 2C).

Discussion

The prolonged use of Glucocorticoids, even at low dosages, can lead to the development of serious side effects especially liver hepatopathy which is a life threatening condition in dogs (Abdou *et al*., 2013).

Many people rely on herbal medicine to treat liver diseases which found that it has a great effect on liver functions as silymarin which protect liver from fibrosis and glycyrrhizin used to treat chronic cases of liver hepatitis (Stickel and Schuppan, 2007).

The clinical signs of anorexia, decrease body weight, alopecia, skin ulcers in steroid induced hepatopathy (Group I) were in accordance with Gouda (2010) and Eman (2018). After herbal treatment as in group II, III, these signs disappeared, which agree with Pradhan and Girish (2006) who reported that silymarin has antioxidative, anti-inflammatory, immunomodulatory, Anti lipid and liver-regenerating properties. Also, these results go in hand with Gogulski *et al*. (2020) that recommended that silymarin supplementation may lessen clinical signs, reduce liver enzyme activities, and improve the patient's general condition.

There was a significant decrease in Hb and HCT and a significant increase in WBCs in Group I on day14. These results agree with Gouda (2010) but were disagreement with Eman (2018) that reported no significant changes in haematological indices. Regarding our results, after herbal treatment there was a significant increase in Hb and HCT and a significant decrease in WBCs in silymarin treated Group II and l-carnitine treated Group III on day 21.

There was a significant increase in ALP and a significant decrease in total protein and albumin in Group I on day 14 and these results agreed with Gouda, (2010) and Abdou *et al*. (2013) as a result of increased membrane permeability and hepatocellular injury (Hinson *et al*., 2010 and Eman, 2018).

Our experiment revealed a significant decrease in ALP and a significant increase in total protein and albumin in silymarin treated Group II in agreement with Kotacurk *et al*. (2016). Our results also regarded there was a significant decrease in ALP and a significant increase in total protein and albumin in l-carnitine Group III and liquorice treated Group IV on day 21 compared to Group I where there was a significant decrease on day 14 and these results were in accordance with Yang *et al*. (2017) and Shalaby *et al*. (2004) who evaluated the anti-inflammatory activity of liquorice in male rats after 4 weeks of food intake and observed a significant decrease in the total cholesterol, triglyceride levels as well as in the levels of serum liver enzymes.

Ultrasonography was selected as the gold standard diagnostic tool for identifying liver diseases in dogs. In the current research, findings revealed that ultrasonography is a good tool for assessing the efficacy of medicinal plants to reduce the steroid induced hepatopathy in dogs. According to the ultrasonographic findings in the present study, in Group I, there was an increase in hepatic echogenicity on days 7, 14 which become nearly equal in echogenicity to spleen at day 21. These results agreed with Nyland *et al*. (2015). After treatment with silymarin and L-carnitine, there was a decrease in echogenicity of liver which appear to be nearly normal texture compared with liquorice treatment and Group I.

Conclusion

The present study confirmed that the prolonged use of glucocorticoid causes serious adverse effects on hepatic parenchyma and medicinal plants especially silymarin and l-carnitine play a great role in treatment and prevention of steroid- induced hepatopathy and can be used as supportive therapy for liver diseases.

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Conflict of interest

The authors confirm that they have no conflict of interest.

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