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The effectiveness of the Astragalus root phenolic extracts on mice blood profile

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***Abstract*---**The aim of this study was to determine the effectiveness of Astragalus hamosus root phenolic extracts (APE) on blood profiles in albumin mice. Twenty male mice were divided into 4 groups, each with five mice, that were treated orally with a plant extract in three doses (0.3,0.5 and 1.3 mg.ml-1). The chemical compositions of (APE) were analyzed using Gas chromatography-mass spectrometry (GC–MS). In contrast to the results, Thymol a was the main active volatile monoterpenoid phenol ingredient in the phenolic compounds. Also, the results showed that the dose of 1.3 mg.ml-1 of (APE) was the powerful extract that reduced levels of cholesterol (CHO), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and triglycerides (TG), respectively, compared with the control treatment. Besides, the effective extract increased high-density lipoprotein (HDL) level with high significant differences compared with the control in serum mice blood. Also, the dose 1.3 mg.ml-1 increased the Monocyte count, granulocyte count, Hemoglobin with no changing in RBC, WBC level compared to the control treatment. In addition, of (APE) reduce Platelet count and Lymphocyte in the blood of the mice after four weeks. Also, the result in showed no significant differences between liver enzymes (ALT), (AST) and (ALP), Blood urea level in the serum compared with control treatment Moreover, 1.3 mg.ml-1 of the (APE) was the most effective concentration. Hence, this effect could be linked to its richness highest amount of phenolics compounds as Thymol by 94.20 %. Hence, this plant could be a significant source of medically important critical compounds.

***Keywords*---**hypolipidemic, Astragalus hamosus, phone extracts, blood serum, mice.

Introduction

The study of medicinal plants reveals locally important species that are often useful for discovering new bioactive products [1,2]. In line with this, several studies have been carried out on the characteristics of several species of Astragalus, the largest genus of the Fabaceae family that includes more than 3000 species and represents one of the most important legumes widely used in the food and pharmaceutical industries due to its strong antioxidant capacity, which is attributed to the presence of many bioactive secondary metabolites [3]. This genus is distributed in Mediterranean climatic regions in Europe and North Africa [4], where 114 species have been found in the IRAQ [5]. as well as ten species endemics to Morocco and Tunisia [6,7]. The members of the genus Astragalus have great interest as traditional drugs in several folk systems including Iraq. However, two species of pharmacological effects Astragalus grown in Iraq, *Astragalus hamosus* and *Astragalus tribuloides*.

These species are perennial plants, about 16 to 36 inches tall, that is native to the northern and eastern parts of IRAQ, as well as some aria of turkey and Iran. It has hairy stems with leaves made up of 12 to 18 pairs of leaflets. The root is the medicinal part of the plant, and is usually harvested from 4-year-old plants [8]. Phenolic compounds in plants may play attractants (flavonoids and carotenoids), defense response chemicals (tannins and phytoalexins), structural polymers (lignin), signal compounds (flavonoids and salicylic acid), UV screens (flavonoids), and antioxidant compounds. Phenolic constituents are essential in defense responses, like anti-inflammatory, anti-aging, anti-proliferative, and antioxidant activity, therefore, it is healthy to eat like plant foods which contain a great antioxidant constituent, which will reduce the occurrence of some acute diseases, for example cardiovascular, tumors, and diabetes illnesses, by the oxidative stress control [9].

Lipids are defined as biological substances which are generally hydrophobic in nature and in many cases soluble in organic solvents [10]. The main biological functions of lipids include energy storage, signaling, and acting as structural components of cell membranes [11]. Lipids are absorbed from the small intestine and emulsified by bile salts, while they are synthesized from cholesterol in the liver, stored in the gallbladder and secreted following the ingestion of fat [12]. The conditions associated with the increase or decrease of levels of lipids in blood serum are known as hyperlipidemia and hypolipidemia, respectively. Hyperlipidemia is an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters and phospholipids. It is also associated with increased levels of plasma very low-density lipoprotein and low-density lipoprotein, as well as reduced levels of high-density lipoprotein [13]. Hyperlipidemia is considered as one of the major risk factors causing cardiovascular diseases (CVDs) [14]. Hypolipidemia is a decrease in plasma lipoprotein caused by primary (genetic) or secondary (acquired) factors.

It is usually asymptomatic and diagnosed incidentally on routine lipid screening [15]. Hypolipidemia is generally uncommon, but secondary causes are relatively common as compared to the rare primary hypolipidemic disorders. The frequency of hypolipidemia depends on which plasma cholesterol level is used to define the

condition [16]. Moreover, many studies showed that elevated total or low-density lipoprotein (LDL) cholesterol in the blood are powerful risk factors for coronary heart disease [17], whereas high HDL-cholesterol: LDL-cholesterol ratio may protect against this disease [18]. The aim of this study was evaluating the biological effects of *A. hamosus* root phenolic extracts on mice blood serum parameters.

Materials and Methods

Collection of plant samples

Plant samples included root of *A. hamosus* was obtained from Mosul, Iraq. The sample was air dried in shade and grinded by a blender to give small-size pieces (2 mm), then stored in a glass container at room temperature in a dry dark place before being used in the extraction step.

Crude phenols extract

Crude Phenols were extracted according to Harborne [19]. 200 g of plant powder was divided into two equal parts, 300 ml of 1% hydrochloric acid was added to one part, and 300 ml of D.W. was added to the other, the two quantities were transferred to electrical blender for 5 minutes. Then the two mixtures were transferred to boiled water bath for 30-40 minutes, the two mixtures were cooled and filtered through muslin cloth, then transferred to a centrifuge with speed of 3000 rpm for 10 minutes. The two supernatants were mixed. Equal quantity of n-propanol was added to the mixture and sodium chloride was added until the solution was separated into two layers. The lower layer extracted in separating funnel with Ethyl acetate, and the solvent layer was collected and evaporated in a rotary evaporator at 40°C for (1-2) hours. The upper layer was evaporated in a rotary evaporator at 40°C for (1-2) hours the dried material of both layers was mixed and dissolved in 5ml of 96% ethanol, then transferred to oven 37 °C then the extract was kept in refrigerator until use.

Gas chromatography-mass spectrometry (GC–MS) analysis

GC-MS analysis was carried out on a GC - mass 5977A Series Agilent system auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column HP-5MS (30 mm×0.25 mm I.D) operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 uL was employed (split ratio of10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 60°C (isothermal for 2 min), with an increase of 10 °C/min, to 270°C, then 5°C/min to 290°C, ending with a 9 min isothermal at 310°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time is 60 min [20].

Preparation of plant extracts dose

Root phenolic extract 200 mg/Kg body weight of *Astragalus hamosus* [21] and 0.3, 0.5 and 1.3 $mg.ml^{-1}$ phenolic extracts [19] were prepared according to the following equation [22]:

$$\text{Concentration mg/ml} = \text{weight/ volume} \times 1000$$

Animals and Housing

Swiss albino mice (19 weeks old) have been used in this study. the have been housed in Makrolon cages under standard laboratory conditions (12 h light/12 h darkness, 21 ± 2°C). The animals have been given standard pellets diet and water ad libitum throughout the experimental period. All animals have been cared for in compliance with the internationally accepted guide for the care and use of laboratory animals, published by the US National Institutes of Health (NIH Publication No. 85-23) [23].

Laboratory Animals

Twenty albino male mice weighted 23-27 gram were attained from Al-Nahrain University Biotechnology Research Center. Their age at the start of experiments was 10-12 weeks. They were divided into groups, and each group was kept in a separate plastic cage (details of these groups are given in the section of experimental design). The animals were maintained at a temperature of 20-25°C. The animals were fed food of laboratory animal, and were given water.

Experimental Protocol Design
Blood collection

Blood samples were collected from direct heart puncture at the end experiment. The blood sample was rocked slightly and centrifuged at 3000 rpm for 5 minutes to obtain serum specimens; the serum was then stored in the freezer at -21 ºC until analyzed [24].

Biochemical analysis

Spectrophotometer was used to measure different parameters in this study. Previously described methods were used to determine total cholesterol [25] and Triglycerides [26]. Levels of HDL and LDL cholesterol were determined using methods reported by other studies [27, 28], respectively, while VLDL concentration was estimated using a previously described equation [29].

Statistical analyses

Statistical analyses were performed by using SPSS (version 25) [30]. Analysis of variance ANOVA was used to compare the significant differences between means. P value of less than ($P \leq 0.05$) was accepted to indicate statistical significance for each test.

Result and Discussion

GC-MS is frequently applied to characterize the chemical complexity of analytical samples based on its separation and identification capacity. The phenolic compounds of *A. hamosus* root extracts were presented in table 1. At the same time, the highest amount of phenolics was 94.20 % as (Thymol), while the lowest phenolic was 1.09% as (3-metheylbensyl alcohol, tert-butyl 90681 1000364-16-7 12 dimethylsilyl ether) compounds observed in roots, respectively. In most cases phenolic structures are derived from aromatic amino acids such as phenylalanine and tyrosine. Detectable phenolic structures comprise monophenols such as thymol (an aromatic monoterpene), phenylethanoids (e.g., tyrosol), and the coumarins (e.g., umbelliferon) (Figure 1) [31]. However, due to relatively higher molecular weight of glycosylated polyphenols, the result in table 1 showed that the thymol was the main compound in root phenolic compounds.

As, the Thymol is a natural volatile monoterpenoid phenol, that is the main active ingredient of oil extracted from many species such as *Thymus vulgaris*, *Ocimum gratissimum* , *Carum copticum*, It is a versatile molecule with a wide variety of practical applications such as medical, dentistry, veterinary, food, and agrochemicals, among others. Its pharmacological applications have been the most investigated and reported, focusing on its prominent antimicrobial, antioxidant, anti-inflammatory, cicatrizing activities. Furthermore, it is noteworthy that the research on its agricultural applications has increased, highlighting its uses as a natural agrochemical and preservative to safeguard foods from pathogenic microorganisms both in sowing and storage, which could have a beneficial effect on human health and the environment. Research has also been reported on its activity as an insecticide, acaricide, and animal repellent. This review summarizes important aspects of thymol such as its bioavailability, synthesis, and biological activities, with special interest in practical applications [32]

Table 1
The GC-Mass analysis for total crude Phenols in roots of *A. hamosus*

No.	QCompounds names	Compounds structures	RT	Peak area%
1	3-Methylbenzyl alcohol, tert-butyl 90681 1000364-16-7 12 dimethylsilyl ether	C14H24OSi	5.489	1.09
2	Thymol	C10H14O	16.953	94.20
3	Thymol	C10H14O	19.454	1.10
4	Hexadecanoic acid, methyl ester	C17H34O2	24.365	1.10
5	trans-13-Octadecenoic acid ester	C19H3602	26.623	2.51

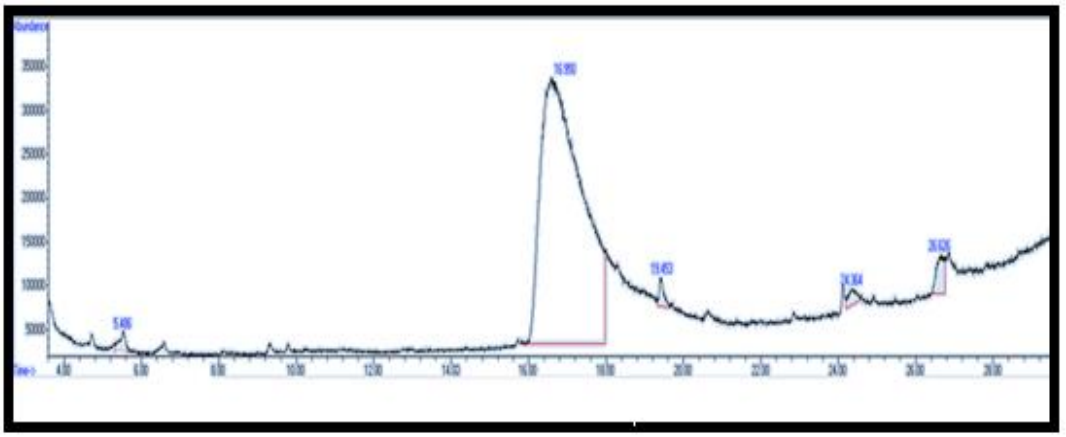

figure 1. The upper figure shows a chromatogram cut from the analysis of a total crude Phenols compounds in roots of *A. hamosus* extract.

The effects of *A. hamosus* root phenolic extracts on the blood lipid profile

The administration of *A. hamosus* root phenolic extracts revealed a positive effect on lipid profile. So far, the study of the bioactivity of thymol, a major constituent *of A. hamosus* phenols in the animal blood profile has received little attention. In addition, *A. hamosus* phenols significantly decreased the levels of CHO, TRI, HDL, VLDL, LDL, while here was highly significant increased HDL level after oral administration extract for four weeks (figures 2). Hypolipidemia is a decrease in plasma lipoprotein caused by primary (genetic) or secondary (acquired) factors. It is usually asymptomatic and diagnosed incidentally on routine lipid screening. Hypolipidemia is generally uncommon, but secondary causes are relatively common as compared to the rare primary hypolipidemic disorders. The frequency of hypolipidemia depends on which plasma cholesterol level is used to define the condition. Moreover, many studies showed that elevated total or low-density lipoprotein (LDL) cholesterol in the blood are powerful risk factors for coronary heart disease, whereas high HDL-cholesterol: LDL-cholesterol ratio may protect against this disease [9].

Many studies suggested that *A. hamosus* root phenolic extracts have a beneficial effect on lipid. This effect could be due to their phenolic compounds, which could be reacted alone, or in synergy to revealed a positive effect on lipid profile [31]. Moreover, the capacity of their phenolic compounds improves antioxidant activity. Thymol as one of the major constituents of *A. hamosus* crude phenols (table 1) presents a wide range of functional possibilities in pharmacy and the food industry [33]. Besides thymol, thyme contains high concentrations of monoterpene phenols like carvacrol, p-cymene, 1,8-cineole, linalool, borneol, camphor, β-caryophyllene, thymol methyl ether and carvacrol methyl ether, which could have influenced the thymol absorption [34]. Thymol a volatile monoterpenoid phenol that was the main active ingredient, which may be responsible for the observed variations of hypolipidemic activity.

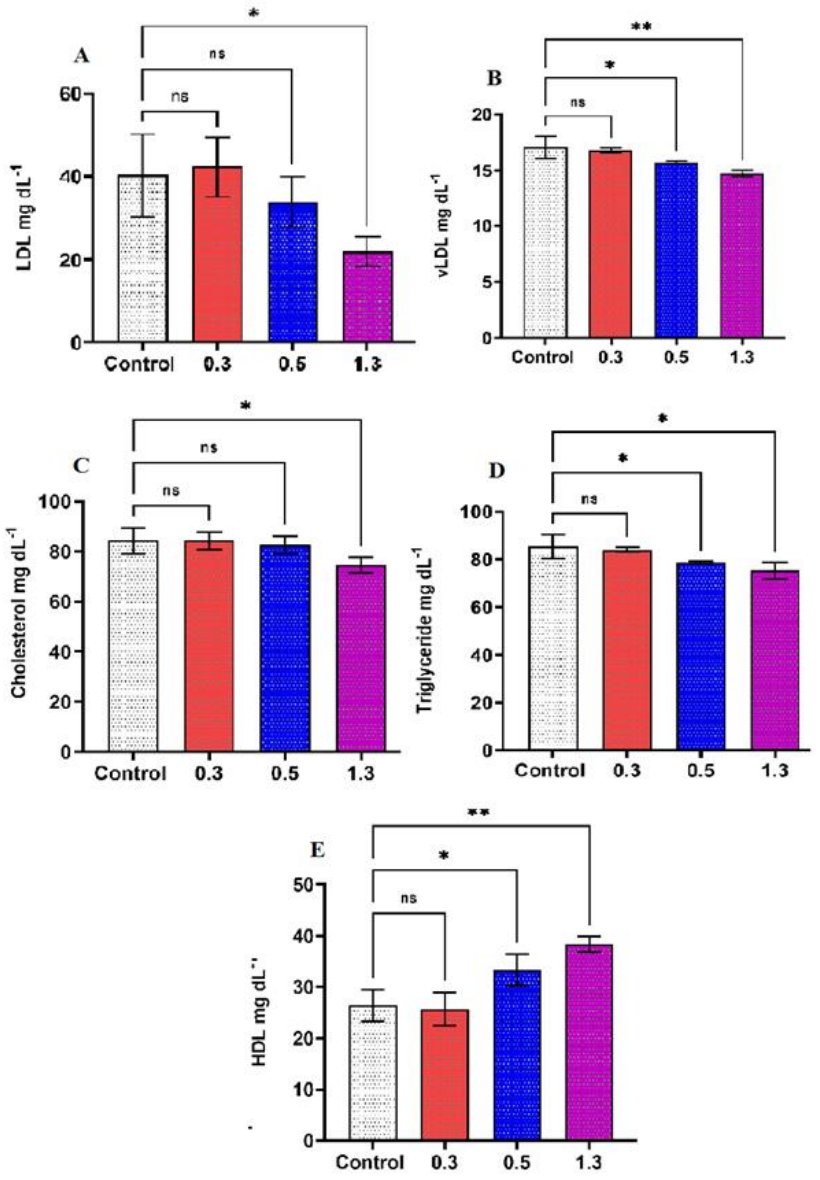

Figure 2: The effectiveness of the Astragalus root phenolic extracts on mice blood lipid profile after oral administration extract for four weeks .(A) LDL, (B) VLDL, (C) Cholesterol, (D) Triglyceride and (E) HDL. ** High significant differences, * Low significant differences, ns no significant differences

Effectiveness of *A. hamosus* root phenolic extracts on blood serum

The result in figure 3 inducted that 1.3 mg.ml-1 of A. hamosus extracts dose increased significantly the Monocyte count and Granulocyte count, However, due to relatively result the RBC, Hb, WBC level which were not significant differences compared to the control treatment. In addition, of A. hamosus extracts reduced platelet count and Lymphocyte in the blood of the mice after oral administration

extract for four weeks. Taken together, these data identify that blood monocytes are well-characterized precursors the effectiveness of the body's immune system. Monocyte count and Granulocyte count changes are better predictor of survival compared with the control treatment. there was a significant increase in immature monocytes cells. [35]

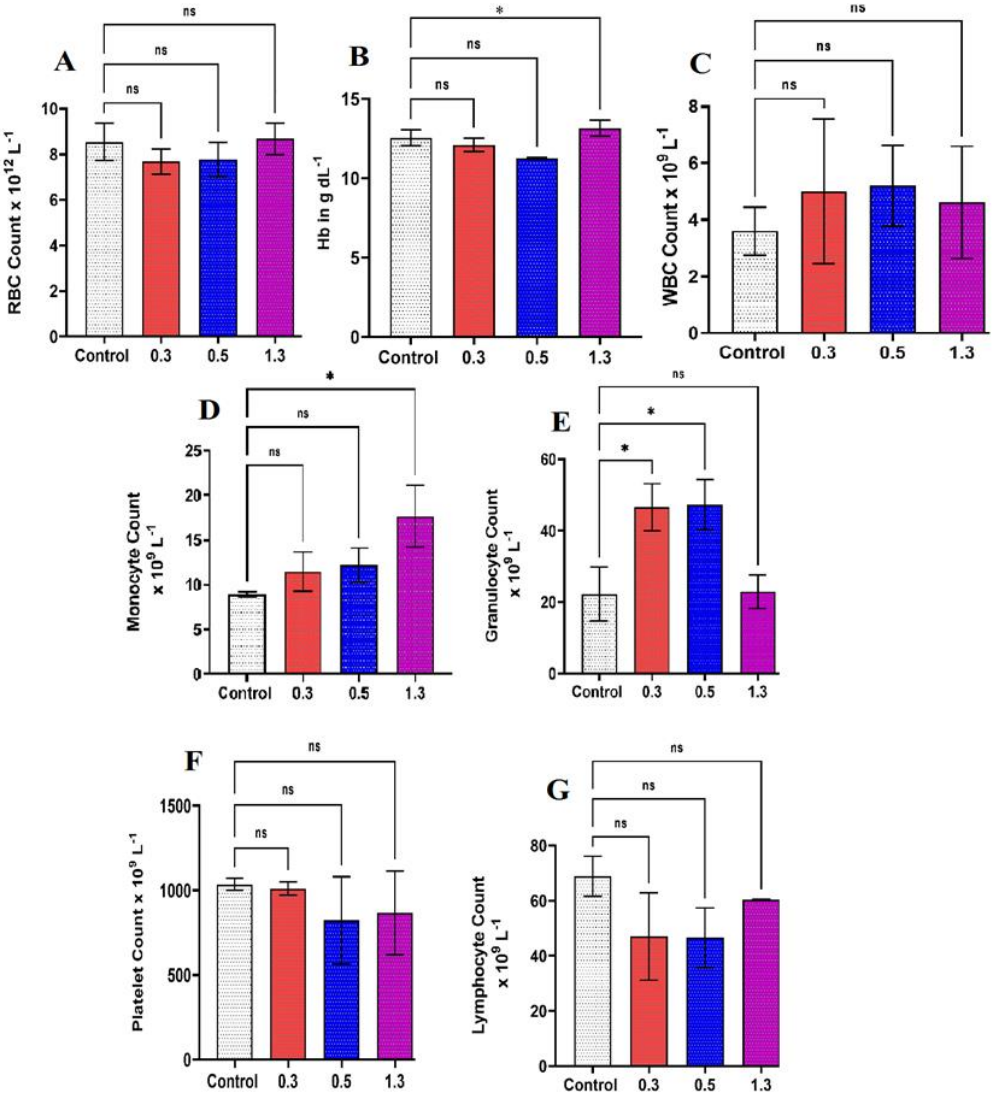

Figure 3: The effectivness of A. hamosus root phenolic extracts on the (A) RBC, (B) Hb, (C) WBC, (D) Monocyte count, (E) Granulocyte count, (F) Platelet count and (G) Lymphocyte. (**) High significant differences, (*) Low significant differences, (ns) No significant differences,

Effectiveness of *A. hamosus* root phenolic extracts on liver and kidney functions

As a result, compared with the control treatment, the parameters associated with the liver enzymes (ALT), (AST) and (ALP) levels were not statistically significant, also with blood urea. However, *A. hamosus* root phenolic have significant effect on and creatinine compared to the control group (figure 4). The use of dietary or medicinal plant based natural compounds to disease treatment has become a unique trend in clinical research. Kidney disease is a worldwide public health problem that affects millions of people worldwide. Plants have been shown to be potential therapeutic agents to protect the kidney against many diseases. Also, the result in figure 4 showed no significant differences between liver enzymes (ALT), (AST) and (ALP) levels in blood serum. [36]. As in shown in figure 4 (E), seen that no significant differences between Blood urea level in the serum compared with control treatment. On the other hand, Creatinine level increased especially after being orally with 1,3 mg.ml^{-1} with *A. hamosus* extracts for four weeks (Figure 4 (D)) [37].

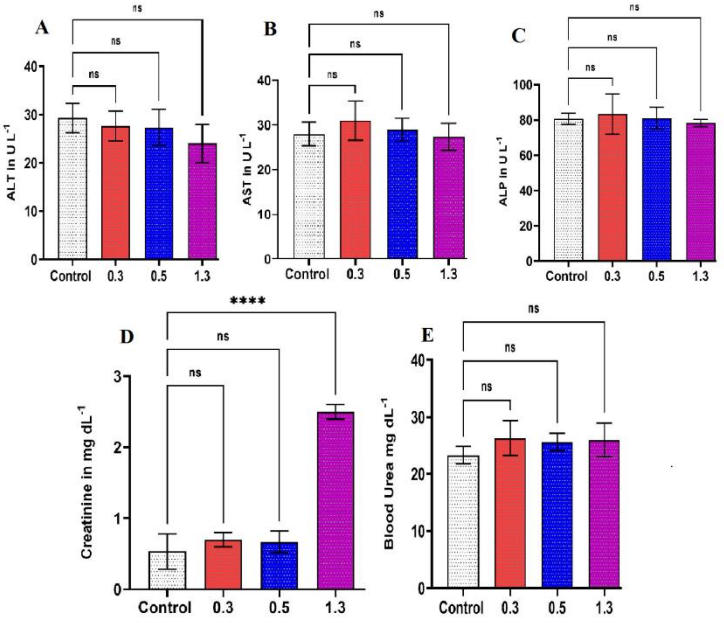

Figure 4: The effect of *A. hamosus* root extract on liver enzyems . A (ALT), B (AST) and C (ALP) , with D (Creatinie) and E (Blood urea)
(**) High significan differences, (*) Low significan differences,(ns) No differences

Serum biochemical indicators reflect the physiological and metabolic status of mice. ALT, AST and ALP play important roles in liver metabolic function which increased the immune ability, hepatic and plasma antioxidative enzyme activities [36]. Besides, large quantities of these three enzymes would be released into the blood when the liver damaged [37]. This study found no significant differences between ALT, AST and ALP levels in the blood serum of the treated and untreated mice after oral administration extract for four weeks. Although, many studies

have shown that the root portion of the *astragalus* could significantly decrease the activity of ALT, AST and ALP and increased the immune ability, and was agreed with Hiam *et. al.,* [38], when they study the potential effects of *Astragalus membranaceus* on the bluegill sunfish blood serum (figure 4). This study indicated that *A. hamosus* root phenolic extracts could significantly improve the immunity of treated mice with high dose of the extract which led to increase the hemoglobin, monocyte account and Creatinine level (figure 3), which may improve the kidney metabolism, with no obvious effect on the blood urea treated groups.

Conclusion

Many studies have shown that *Astragalus* extract has biological functions such as improving immune function, anti-oxidation, anti-stress, anti-virus, improving intestinal structure, and promoting food and growth [39]. Oral consumption of *A. hamosus* root extract has been widely prescribed in traditional medicine system [40]. Finally, the hypolipidemic activities of the *A. hamosus* root reported here show the potential of usage in reduction of hyperlipidemia disease. This makes crude phenolic extract of *A. hamosus* root is the good plant product therapeutically and commercially. this effect could be due to their phenolic compounds, which could be reacted alone, or in synergy with other secondary compounds. Consequently, further studies need to be carried out to find out the mechanism of action of these bioactive compounds present in these phenolic compounds.

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