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Impacts of Feeding with *Lupinus albus* (White Lupin) and *Lupinus termis* (Egyptian Lupin) on Physiological Activities and Histological Structures of Some Rabbits' Organs, at Taif Governorate

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Abstract: Experiments were carried out on two hundred ten immature and intact male and female rabbits aged one month old, of local hybrid strains with body weight ranging between (1000-1100 gm). They were housed and maintained under standard animal house conditions The rabbits were randomly assigned into seven groups (30 rabbits each one) as follows; group 1= Control was fed with a complete standard laboratory rabbits feed pellets and water *ad libitum* daily; groups 2,3,4,5, 6 and 7= rabbits which were fed daily with grounded white *lupinus albus* seeds, grounded light red *lupinus albus* seeds, green white *lupinusalbus*, green red *lupinus albus*,, dry white *lupinus albus* and dry green red *lupinus albus* diets consecutively.All rabbits groups with the exception of rabbits' control, were received adequate and defined green, dry or seeds of *lupin* rations and water *ad libitum*. Determination of nutritional rations was done by recording daily average intakes of different *lupin* feeds. Body weight was undertook at the beginning and at the end of the experiments. Clinical signs of affected rabbits were investigated throughout trials periods.Blood samples were collected for hematological and biochemical studies. The most marked symptoms in the early and late acute phases were : a rise temperature, loss of appetite and/or anorexia, reduced food consumption, emaciation, progressive weakness, listlessness, dullness,convulsions and pale mucus membrane followed by mild jaundice and anaemia. In a few of affected rabbits, lacrimation and salivation were occurred lately in acute stage of poisoning. Deaths were observed in 7-12 days for rabbits groups 5, 3, 4, 2, 7 and 6 respectively. The toxic impacts of seeds, green or dry *lupinus albus* on rabbits' liver tissues led to high significant increase ($P \leq 0.01$) in T-Bilirupin and in ALT (SGPT) and AST (SGOT) activities for rabbits' groups 5,3,4, 2, 7 and 6 respectively when compared to the control group. On the other hand, rabbits Lupinosis caused by feeding on seeds, green or dry *lupinus albus* rations was detected by increasing of serum urea, but the levels of Creatinine, Total Protein and Albumin were significantly decreased ($P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.01$ consecutively) for rabbits groups 5, 3, 4, 2, 7 and 6 respectively when compared with control rabbits. The toxic effects of *lupinus albus* resulted in significant increase ($P \leq 0.01$) in WBC, NEU, LYM, MONO, EOS and BASO values for rabbits groups mentioned above. Moreover, RBC and HGB estimates were significantly decremented ($P \leq 0.05$ and $P \leq 0.01$ successively) in all rabbits' groups 5,3, 4, 2, 7 and 6 consecutively, in comparison with control group. The results achieved suggest that lupin feeding have negative effects on general health status and physiological activities and histological structures in affected rabbits.

Key words: Rabbits · Toxic Impacts · *Lupinus albus*. · *Lupinus termis* · Feeding · Symptoms · Poisoning · Hematologic Parameters · Biochemical Values

INTRODUCTION

Lupines are perennial or sometimes annual herbs with palmately compound leaves; their leaflets are narrow; their flowers are in terminal racemes, pea-like, often blue, but yellow or white in some species. They have pods up to 1 inch in length. The fruit is a pod about one inch long coating several somewhat flattened seeds. The seeds are cream-colored and irregularly circular and no more than 1/4 inch in diameter [1].

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Lupines are in the Legume family which is characterized by irregular, five-petaled flowers with a central "keel." Flowers are most often blue; occasionally white, red or yellow varieties are seen. Leaves: the leaf, a palmately compound leaf with five to seven elongated, ovoid leaflets, is somewhat distinctive [2, 3].

Both cultivated garden lupines and native lupines of the western rangelands are toxic. In rangelands, they occur from the dry, open ranges of the Great Plains to the Rocky Mountains [4].

Lupinine, a quinolizidine alkaloid, induces nicotinic effects in animals. Leaves, seeds and fruits all contain lupinine, which is retained in dried plants. Pods may concentrate the toxin, becoming a source of poisoning during the winter season when livestock are moved through infested areas or contaminated hay is fed.

Lupines are a major toxic problem in range sheep. Lupines are toxic when ingested at 1% or less of body weight [5]. Legumes represent, together with cereals, the main plant source of proteins in human diet. They are also generally rich in dietary fibre and carbohydrates [6]. Minor compounds of legumes are lipids, polyphenols and bioactive peptides [7]. Lupin is an economically and agriculturally valuable plant [3, 4]. Its seeds are employed as a protein source for animal and human nutrition in various parts of the world, not only for their nutritional value, but also for their adaptability to marginal soils and climates. Human consumption of lupins has increased in recent years [8]. Lupins (*Lupinus spp*.) belong to the *Genisteae* family, *Fabaceae* or *Leguminosae* [7, 9]. From the genus *Lupinus* more than 400 species are known, from which only four are of agronomic interest [1]: (*L. albus*L.: white lupin, *L. angustifolius*L.: blue or narrow-leafed lupin, *L. luteus*L.: yellow lupin and *L. mutabilis*L.: pearl or Tarrwi lupin) [1, 9, 10]. The first three species originate from the Mediterranean area, including Turkey, while *L. mutabilis* belongs to South America [10]. These species are known as sweet lupins due to their low levels (0.003%) of bitter-tasting and potentially toxic alkaloids [11] and, therefore, there is no risk of toxicity for animals and humans[12].

Lupines (*Lupinus spp*) cause 2 distinct forms of poisoning in livestock—lupine poisoning and lupinosis. The former is a nervous syndrome caused by alkaloids present in bitter lupines; the latter is a mycotoxic disease characterized by liver injury and jaundice, which results mainly from the feeding of sweet lupines. Lupinosis is important in Australia and South Africa and also has been reported from New Zealand and Europe. There is increasing use of sweet lupines, either as forage crops or through feeding of their residues after grain harvest, as strategic feed for sheep in Mediterranean climate zones. Sheep and occasionally cattle and horses, are affected and pigs are also susceptible [13-15]. The causal fungus is *Phomopsis leptostromiformis*, which causes *Phomopsis* stem-blight, especially in white and yellow lupines; blue varieties are resistant. It produces sunken, linear stem lesions that contain black, stromatic masses and it also affects the pods and seeds. The fungus is also a saprophyte and grows well on dead lupine material (e.g, haulm, pods, stubble) under favorable conditions. It produces phomopsins as secondary metabolites on infected lupine material, especially after rain. Clinical changes are mainly attributable to toxic hepatocyte injury, which causes mitotic arrest in metaphase, isolated cell necrosis and hepatic enzyme leakage, with loss of metabolic and excretory function.Early signs in sheep and cattle are inappetence and listlessness. Complete anorexia and jaundice follow and ketosis is common. Cattle may show lacrimation and salivation. Sheep may become photosensitive. In acute outbreaks, deaths occur in 2-14 days.In acute disease, icterus is marked. Livers are enlarged, orange-yellow and fatty. More chronic cases show bronze- or tan-colored livers that are firm, contracted in size and fibrotic. Copious amounts of transudates may be found in the abdominal and thoracic cavities and in the pericardial sac. Feeding of moldy lupine material, together with clinical signs and increased levels of serum liver enzymes, strongly indicate lupinosis [15]. Quinolizidine alkaloids are found in various plants including those belonging to the *Lupinus* genus although the nature and level of these alkaloids is highly variable between species [16, 17]. While they are not the only alkaloids found in lupins, they are the major concern in relation to human and animal health. The levels of alkaloids in seeds or meal can be reduced through a de-bittering process involving soaking or washing with water. This is commonly practised in Europe where high alkaloid lupins, so-called ‘bitter lupins’, are grown. The level of alkaloids in these lupins after the de-bittering process is reported to be approximately 500mg/kg. In Australia, lupin varieties with low alkaloid content, so-called ‘sweet lupins’, have been developed through plant-breeding programs. Data indicates that the mean alkaloid content of marketable sweet lupin seed is on average 130-150mg/kg. Lupin alkaloids may be found

in any derivative of the seed or plant, including flours and meal that can be used to prepare pastas, pastries and dairy product substitutes [5]. Traditionally, lupin seeds of *Lupinus albus* following debittering have been used in the Middle East and Europe to make snack foods. In Europe, lupin seeds are known as lupini beans. Lupins are also used in traditional fermented foods such as tempe,miso and soy sauces in Indonesia and Japan. More recently, lupin derivatives are increasingly being introduced into food for human consumption through the use of lupin flour derived from low alkaloid varieties. Little data is available on the metabolism and excretion of alkaloids in animals or in humans. In one human study, orally administered lupanine was excreted unchanged in the urine with a half-life of 6-7 hours.The majority of the acute studies have been performed on the common lupin alkaloids, sparteine and lupanine. Both have moderate acute oral toxicity in rodents although sparteine is the more toxic [5]. The symptoms observed suggest the alkaloids cause toxicity through neurological effects leading to loss of motor co-ordination and muscular control [14]. The effects are generally reversible. In 3-month feeding studies in rats, there was little evidence of toxicity, even at the high dose levels. At a dose level of 505 mg/kg bw/day, haematological changes were the only significant effect that could be linked to treatment. The no-observable-effect level (NOEL) for these studies was in the range 90-105 mg/kg bw/day. A two-generation reproduction study in rats revealed no adverse effects on fertility, in a study to investigate the neurotoxicity of lupin alkaloids, sparteine and lupanine when administered by intravenous route inhibited ganglionic transmission of the sympathetic nervous system [5, 13, 14]. Lupanine also suppressed the effects of pre-ganglionic stimulation of the pneumo-gastric nerve in the parasympathetic nervous system. This study may provide some insight into the mechanism of acute toxicity. There are no chronic studies available that specifically examine the toxicity of lupin alkaloids, however, rats fed a low alkaloid lupin seed-based diet did not show any evidence of toxicity after 2 years. Human acute toxicity studies were restricted to anecdotal reports of poisoning cases. General toxic symptoms included malaise, nausea, respiratory arrest, visual disturbances, ataxia, progressive weakness and coma. On the basis of the data available, the acute lethal dose for humans is approximately 30 mg/kg bw, which is considerably lower than the lethal dose levels reported in rodents. The only data available on human chronic toxicity are the reports of traditional use of lupini beans in Europe, which indicate a daily dose of 0.35 mg/kg can be tolerated in adults without adverse effects. On the basis of this limited data, however, it is not appropriate to consider this dose level as the safe level for all individuals in the population. The only data available on the levels of alkaloids in lupini beans is anecdotal - there seems to be no published information available [5]. Also, the information applies only to adults, not children and it is likely that the adult population has developed a certain amount of tolerance to these alkaloids. The limited metabolism data available, however, suggests that the alkaloids are rapidly excreted unchanged which would reduce the likelihood of chronic toxicity. If a safety factor of 10 is applied to account for the uncertainties in the data and particularly to take into account likely human variation, the provisional tolerable daily intake (PTDI) for humans is 0.035 mg/kg/day or 35 µg/kg/day. There are no dietary survey data available from which to determine food consumption levels of lupin alkaloids since lupins currently have very limited use in foods. Based on conservative assumptions regarding the potential for use of lupin flour in wheat based products and the typical concentration of alkaloids in lupins harvested for human consumption, the data indicates that consumers of products such as pasta, pastry and cakes and biscuits would be likely to have a daily exposure to lupin alkaloids of 2 µg/kg bw/day at the 95% ile of consumption. The level of exposure to alkaloids from home use of lupin seeds is difficult to assess. The European experience suggests that lupin seeds in the home are most likely to be consumed as a snack food. Low alkaloid varieties of lupin seeds contain approximately one quarter of the alkaloid content of debittered European lupini beans and thus are unlikely to cause symptoms of toxicity for the majority of the population. However, given the paucity of data, it is not possible to state that ingestion of these lupins will be without adverse effects for all individuals in the population. There is little information available on the effect of heating or cooking on the stability of lupin alkaloids although they are known to be soluble in water as shown by debittering processes [5].

The available data on lupin alkaloids is limited and does not allow a full characterisation of the risk of exposure to humen. It is of particular concern that the available data indicates that humen are more susceptible to the toxicity associated with lupin alkaloids than other

species. The traditional use of debittered lupini beans in Europe as a snack food has been reported to be without adverse effects at a dose level of 0.35 mg/kg/day in adults. On the basis on this data, a tolerable level of exposure for humen has tentatively been established at 35 µg/kg/day using an uncertainty factor of 10 in order to account for the variability in the human population. The major potential source of exposure to lupin alkaloids is the use of lupin flour from low alkaloid varieties of lupins to substitute for a small percentage of wheat flour. The available information on potential exposure via lupin flour suggests that at current levels of use, human and beef cattle exposure will be well below this tolerable level of exposure [2, 5, 17-19]. There is also potential for exposure to these alkaloids through the use of lupin seeds as a snack food. At present, this practice is uncommon and confined to a subpopulation of southern European immigrants. Given the uncertainty regarding the toxicity of lupin alkaloids, there may be cause for concern if this practice were to become commonplace, particularly if lupini beans (The large seeded bitter cultivars of *L. albus*) were to be widely marketed. The consumption of the lupini bean in Australia and New Zealand is currently not a concern since the current consumers understand the de-bittering process required [5, 17, 18]. While the low alkaloid varieties grown in Western Australia have approximately one quarter the alkaloid content of debittered lupini beans, on the basis of the data available, it is not possible to state that ingestion of the low alkaloid varieties will be without adverse effects for all individuals in the population. In order to characterise further the potential human risk associated with lupin alkaloids, additional research is required to establish the basis for the observed toxicity in humen [5,17,18].

Quinolizidine alkaloids are found in various plants including those belonging to the *Lupinus* genus (Family *Leguminaceae*) [1,7,9,10]. lupin alkaloids are considered poisonous at high levels - recognized to be 1-2% alkaloid concentration in the plant [16]. Levels and combinations of alkaloids are highly variable between plant species - the alkaloid profiles of domesticated *Lupinus* species revealed that these alkaloids are not restricted to the *Lupinus* genus, being also found in several members of the pea family. Quinolizidine alkaloids are not the only alkaloids found in lupins but they represent the greatest concern. There are almost 70 different quinolizidine alkaloids found in various *Lupinus* species. The notable alkaloids of interest in human and animal health are:

- Lupanine (A ketonic derivative of sparteine) and its isomers;
- Sparteine - mainly isolated from broom but also present in severa species of Lupinus;
- Anagyrine - a teratogenic alkaloid, which is not present in the major cultivated species for human consumption [2, 5, 9, 12, 17].

Lupins are often referred to as either bitter or sweet. Bitter lupins, such as the lupine beans consumed in Europe, have high concentrations of alkaloids (Mainly sparteine), which make them bitter to the taste and a debittering process is required before consumption. Sweet lupins, such as those grown in Western Australia, have low levels of alkaloids (Mainly lupanine). Other alkaloids that are of concern belong to the group of piperidine alkaloids [17]. These are found in a range of other plant genera (eg. *Piper* spp), but are also peculiar to the lupin species *L. formosus* and *L. arbustus* which are pasture species found in the USA. These alkaloids are suspected to be teratogenic as well as causing general toxicity [17] but are not found in domesticated species. However, previous studies reported a marked pathogenesis of acute lupinosis in sheep. The main events in the pathogenesis of acute lupinosis of sheep, as revealed by biochemical and pathological studies, have been elucidated. Inappetence or anorexia, accompanied by mild pyrexia, ushered in the intoxication, often after only 2 or 3 days. Plasma bilirubin increased significantly on an average of 5'3 days, followed by the plasma enzymes glutamic-oxalacetic transaminase, lactic dehydrogenase and glutamic dehydrogenase on an average of 6'5 days. These increases, indicating cell damage, occurred irrespective of whether toxic feed intake had ceased or was continuing and the level remained elevated for the duration of trials of up to 38 days during which normal appetite for non-toxic feed was not regained. Acute liver damage with fatal results occurred in only one sheep. Nine others had varying degrees of less acute or subacute lupinosis characterized by minor necrosis and more prominent granular degeneration of liver cells. Interference with the hepatic conjugation and excretion of plasma bilirubin, accompanied by an increase in intravascular erythrocyte damage, is a further event in the pathogenesis of lupinosis [18]. Although several investigations have tried to identify the factors that predict the outcome after lupines poisoning in animals, little is known about the

mpacts of lupinosis at the time of poisoning, in regard to the clinical characteristics of lupinosis, the histo-pathological and hematologic picture changes observed after poisoning i.e., in the acute stage of affection.

MATERIALS AND METHODS

Two hundred ten immature and intact male and female rabbits aged one month old, of local hybrid strains with body weight ranging between (1000-1100 gm) obtained from Alfier Rabbits Breeding Center, Liya region, Taif governorate, KSA, were used for these experiments. They were housed and maintained under standard animal house conditions (Temperature:23± 2ºC; photoperiod: 24 h light; humidity: 45-50%). The rabbits were randomly assigned into seven groups (30 rabbits each one) as follows; group 1= Control was fed with average standard laboratory rabbits feed pellets (which were contained Protein 18.0-18.5%, Fibers 13-14%, Fat 2.0-2.5% and Energy 2600-2800 k calorie/kg) (185gm) and water *ad libitum* daily; group 2 = rabbits which were fed with grounded white *lupinus albus* (White Lupin) seeds (94 gm) daily; group 3 = rabbits fed with grounded light red *lupinus albus* [*Lupinus termis* (Egyptian Lupin)] seeds (83 gm) daily ; group 4= rabbits which were rationed with green white *lupinus albus* (137 gm) daily ; group 5 = rabbits were foraged with green red *lupinus albus* (129 gm) daily, group 6 = were fed with dry white *lupinusalbus* (147 gm) daily and finally group 7 = rabbits foraged with dry green red *lupinus albus* (135 gm) daily. All rabbits groups with the exception of rabbits' control, were received adequate and defined green, dry or seeds of *lupinus albus* rations and water *ad libitum*. Determination of nutritional rations was done by recording daily average intakes of green or seeds of *lupinus albus* feeds.

Body weight was determined at the beginning and at the end of the experiments. Clinical signs of affected rabbits were investigated throughout trials periods. Blood samples were collected from the ear-vein using heparinized capillary tubes and/ or vacuumized tubes. Part of the blood was collected on EDTA for hematological studies [i.e., White blood corpuscle (WBC), Neutrophils (NEU), Lymphocytes (LYM), Monocytes (MONO), Eosinophils (EOS), Basophils (BASO), Red blood corpuscle (RBC), Haemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width [RCDW(RDW)], Platelets (PLT) and Mean platelet volume (MPV)] and the other part collected to separate the serum by centrifugation for 10 min. at 5000 rpm and the supernatant serum was immediately separated for biochemical analysis in relation to renal panel parameters (i.e., Sodium, Potassium, Chloride, Carbone dioxide, Urea (Blood Urea Nitrogen[BUN], Creatinine, Total Protein and Albumin) and liver parameters (i.e., Alkaline Phosphatase (Alk Phos = ALP), Total Protein, Albumin, T-Bilirubin, Alanine Transaminase [ALT (Serum glutamic-pyruvic transaminase (SGPT)] and Aspartate Transaminase [AST (Serum glutamic-oxaloacetic transaminase (SGOT)]. All haematological and biochemical tests were applied according to Bauer and John [20].

Statistical Analyses: Data were analyzed using Student (t) test. Data at ($P \leq 0.05$) were considered significant.

RESULTS

Marked clinical sings of impacted rabbits were approximately the same during the first three days of the early acute stage, but ranged from severe condition as concerns rabbits related to groups 5 and 3 consecutively to moderate condition in regard to the rabbits groups 4, 2, 7 and 6 respectively in comparison with control group. The most marked symptoms in the early and late acute phases depending upon the duration and virulence of poisoning with *lupinus albus* feeds, which were : a rise temperature, loss of appetite and/or anorexia, reduced food consumption, emaciation due to significant decrease of body weight ($P \leq 0.01$) while the rabbits control group (1) fed with standard laboratory rabbits feed pellets manifested significant increase in body weight ($P \leq 0.01$) (Table. 1), progressive weakness, listlessness, dullness, convulsions, pale mucus membrane followed by mild jaundice and anaemia. In a few of infected rabbits, lacrimation and salivation were occurred lately in acute stage of poisoning. In acute poisoning with lupinosis outbreaks, deaths were observed in 7-12 days, whereas poisoning cases were established in 7, 8, 9 and 12 days for rabbits groups 5, 3, 4, 2, 7 and 6 consecutively.

The toxic impacts of seeds, green or dry *lupinus albus* on rabbits' liver tissues led to high significant increase ($P \leq 0.01$) in T-Bilirupin and in ALT (SGPT), AST (SGOT) activities for rabbits' groups 5,3,4, 2, 7 and 6 respectively when compared to the control group. Nevertheless, the feeding rabbits groups on seeds or

Table 1: Effect of Seeds, green and dry *lupinus albus* rations intakes on body weights

	Body weight (gm)#			
Groups	Initial	Final	Weight loss (gm)	Food intake# gm/day
1	1050±0.74	1554±0.09	504±0.22****	185±0.65
2	1050±0.74	610±0.19ns	440±0.25ns	94 ±0.34ns
3	1050±0.74	571±0.63ns	479±0.17ns	83±0.18ns
4	1050±0.74	592±0.24*	458±0.12*	137±0.33*
5	1050±0.74	556±0.13*	494±0.43*	129±0.40**
6	1050±0.74	625±0.23*	525±0.39***	147±0.24**
7	1050±0.74	617±0.12ns	433±0.28ns	135±0.16ns

- where n=6. Data were expressed as mean ± S.E.M. * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$) and ns (not significant) when groups v/s 2,3,4,4,5,6 and 7. **** = Weight gain (gm) -# = Body weights and Food intakes were calculated as mean ± S.E.M

Table 2: Effect of Seeds, green and dry *lupinus albus* rations intakes on liver functions parameters.

	Parameters					
Groups	ALK PHO (U/L)	Protein Total (g/L)	Albumin (g/L)	T-Bilirubin (umol/L)	ALT (SGPT) (U/L)	AST (SGOT) (U/L)
1	110±0.14.	72±1.85	40±2.04	1.3±0.17	33±4.02	17±1.07
2	107±0.28	44±1.77	25±2.16	22.1±0.27	60±4.09	35±1.44
3	118±0.25	48±1.79	28±3.01	24.3±0.33	65±3.24	39±1.19
4	115±0.44	46±2.03	27±3.27	23.2±0.45	63±3.33	37±1.09
5	119±0.32	51±1.98	30±.2.45	26.5±0.51	67±4.09	41±1.18
6	100±0.30	38±2.09	21±2.66	18.3±0.77	55±3.12	29±1.39
7	103±0.39	40±1.88	23±2.58	20.2±0.56	57±3.62	32±1.25

-where n=6. expressed as mean ± S.E.M. All horizontal columns values with the exception of the horizontal column (1) [Group 1] are (Not significant) when group1 v/s groups 2,3,4,4,5,6 and 7

Table 3:Effect of Seeds, green and dry *lupinus albus* rations intakes on kidney functions parameters.

	Parameters							
Groups	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Carbon Dioxide (mmol/L)	Urea (Bun) (mmol/L)	Creatinine (µmol/L)	Protein Total (g/L)	Albumin (g/L)
1	144±1.44	3.9±0.02	99±1.75	18±0.13	3.9±0.02	57±0.08	72±0.12	40±0.05
2	137±1.65	5.2±0.04	111±2.01	17.3±0.09	9.0±0.01	37±1.03	44±0.03	25±0.02
3	140±1.35	5.5±0.06	114±1.81	19.5±0.11	9.7±0.04	40.1±1.67	48±0.14	28±0.04
4	139±1.55	5.3±0.03	112±1.65	18.7±0.03	9.2±0.03	39±2.01	46±0.17	27±0.07
5	146±1.87	5.7±0.01	115±1.88	20.1±0.06	10.9±0.07	43±0.54	51±0.23	30±0.15
6	130±1.13	4.81±0..08	103±2.3	15.2±0.01	8.6±0.05	32±0.16	37±0.19	21±0.01
7	134±1.89	4.97±0.02	108±2.10	15.9±0.02	8.83±0.10	34±0.23	40±0.27	23±0.03

- where n=6. Data were expressed as mean ± S.E.M.

All horizontal columns values with the exception of the horizontal column (1) [Group 1] are (Not significant) when group1 v/s groups 2,3,4,4,5,6 and 7

green *lupinus albus* feeds recorded no significant changes in serum ALK PHOS, but still recorded significant decreased ($P \leq 0.05$) serum Total Protein and Albumin when compared with control rabbits (Table 2).

On the other hand, rabbits Lupinosis caused by feeding on seeds, green or dry *lupinus albus* rations was detected by increasing of serum urea. In all rabbits' groups fed on seed, green or dry *lupinus albus* food, Creatinine, Total Protein and Albumin levels were significantly decreased ($P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.01$ consecutively) for rabbits groups 5, 4, 3, 2, 7 and 6 respectively when compared with control rabbits as illustrated in table (3). Also, the present study results revealed that the rabbits' groups fed on seed, green or dry *lupinus albus* feeds established no significant changes in serum Sodium and Carbon Dioxide estimations, but just detected slight significant increased serum Potassium and Chloride levels in comparison with control rabbits (Table 3).

The haematological parameters i.e., White blood corpuscle (WBC), Neutrophils (NEU), Lymphocytes (LYM), Monocytes (MONO), Eosinophils (EOS), Basophils (BASO), Red blood corpuscle (RBC), Haemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular hemoglobin

Table 4: Effect of Seeds, green and dry *lupinus albus* rations intakes on Haematological parameters.

	Groups						
Parameter	1	2	3	4	5	6	7
WBC (X 10^3/µl)	5.64±0.21	6.09±0.17	7.1±0.24	6.88±0.29	8.4±0.12	5.72±0.27	5.80±0.23
NEU(%)	3.51±0.15	4.01±0.18	4.99±0.26	4.23±0.24	6.50±0.12	3.89±0.19	3.98±0.11
LYM(%)	0.924±0.20	1.33±0.27	2.01±0.40	1.77±0.32	2.28±0.36	1.86±0.30	1.92±0.24
MONO(%)	0.922±0.16	1.02±0.20	1.30±0.22	1.22±0.41	1.85±0.31	1.29±0.42	1.35±0.29
EOS(%)	0.054±0.24	0.055±0.41	0.062±0.23	0.058±0.26	0.069±0.35	0.061±0.31	0.069±0.28
BASO(%)	0.123±0.25	0.287±0.30	0.301±0.32	0.299±0.34	0.310±0.40	0.285±0.38	0.297±0.36
RBC (X 10^6/µl)	6.70±0.23	3.88±0.12	3.46±0.20	3.79±0.46	3.35±0.47	3.96±0.17	4.09±0.22
HGB (g/dl)	12.2±0.31	7.16±0.18`	6.9±0.23	7.10±0.51	6.2±0.46	7.39±0.53	7.44±0.47
HCT (%)	44.5±020	26.3±0.29	24.1±0.22	25.2±0.33	23.5±0.56	28.6±0.57	29.2±0.37
MCV (fl)	67.4±0.90	48.9±0.73	47.2±0.88	48.1±0.66	46.7±0.77	50.3±0.80	52.2±0.74
MCH (pg)	21.4±0.21	18.1±0.19	17.6±0.23	17.9±0.32	17.1±0.29	18.2±0.36	18.9±0.41
MCHC (g/dl)	32.9±0.54	24.1±0.58	23.1±0.72	23.8±0.69	22.9±0.77	25.8±0.83	26.2±0.84
RDW(%) (RCDW(%)	15.1±0.22	12.10±0.28	12.4±0.27	12.7±0.29	12.1±0.34	12.47±0.22	12.75±0.39
PLT (X 10^3/µl)	175.±30.44	140.±26.23	133.±22.42	137.±25.62	130.±28.52	145.±33.44	149. ±24.39
MPV (fl)	4.69±0.10	2.97±0.07	2.74±0.15	2.86±0.18	2.63±0.20	3.02±0.19	3.14±0.23

WBC: White blood corpuscle, NEU: Neutrophils, LYM: Lymphocytes, MONO: Monocytes, EOS: Eosinophils, BASO: Basophils, RBC: Red blood corpuscle, HGB: Haemoglobin, HCT: Hematocrit , MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, RDW: Red cell width, PLT: Platelets, MPV: Mean platelet volume, , where n=6. Data were expressed as mean ± S.E.M. All columns values with the exception of the column (1) [Group 1] are (Not significant) when group 1 v/s groups 2,3,4,5,6 and 7.

concentration (MCHC), Red cell distribution width [RCDW(RDW)], Platelets (PLT) and Mean platelet volume (MPV) recorded significant alterations in all rabbits' groups fed on seed, green, or dry *lupinus albus* feeds (Table 4). The toxic effect of *lupinus albus* led to significant increase ($P \leq 0.01$) in WBC, NEU, LYM, MONO, EOS and BASO values which play an important function in initiating body immunity. Moreover, RBC and HGB which play a pivotal role in carrying nutrient and oxygen to the body cells were significantly decremented ($P \leq 0.05$ and $P \leq 0.01$ successively) in all rabbits' groups fed on seed, green or dry *lupinus albus* feeds i.e., rabbits' groups 5,3,4, 2, 7 and 6 respectively, when compared with control group (Table 4).

DISCUSSION

Legumes represent, together with cereals, the main plant source of proteins in human diet [2]. They are also generally rich in dietary fiber and carbohydrates [6]. Minor compounds of legumes are lipids, polyphenols and bioactive peptides [7].

Lupin is an economically and agriculturally valuable plant [3, 4]. Its seeds are employed as a protein source for animal and human nutrition in various parts of the world, not only for their nutritional value, but also for their adaptability to marginal soils and climates. Human consumption of lupins has increased in recent years [8]. Lupins (*Lupinus* spp.) belong to the *Genisteae* family, *Fabaceae or Leguminosae* [7, 9]. From the genus *Lupinus* more than 400 species are known, from which only four are of agronomic interest [1]: (*L. albus* L.: white lupin*, L. angustifolius* L.: blue or narrow-leafed lupin*, L. luteus* L.: yellow lupin and *L. mutabilis* L.: pearlor Tarrwi lupin) [1, 9, 10]. The first three species originate from the Mediterranean area, includingTurkey, while *L. mutabilis* belongs to South America [10]. These species are known as sweet lupins due to their low levels (0.003%) of bitter-tasting and potentially toxic alkaloids [11] and, therefore, there is no risk of toxicity for animals and humen [12].

Lupins (*Lupinus* spp.) affections of cattle, sheep, horses, chicken and pigs have been regarded as veterinary medical oddities since their role in the etiology of clinical disease often remaining obscure. Otherwise, marked difference in toxicity and pathogenicity among livestock of the same and different species have been demonstrated [17-22].Feeding of animals on Lupins (*Lupinus* spp.) rations has not necessarily associated with a clinical disease; indeed, the disease may be depend upon the Lupins (*Lupinus* spp.) intake, species of animal, quantities of toxic compounds involved in seeds, green and/or dry lupins rations, toxicity severity and animal resistance and immunity.The pathogenesity of some of these rations was established by experimental feeding of various animals including cattle, sheep, horses, chicken and pigs [17-19, 22] and rats [16, 22]. Concerning clinical symptoms after toxicity with Lupins (*Lupinus* spp.) rations, a mild temporary to severe impacts on general condition was observed in acute stage, with

reproducing the disease or appearance of hepatic lesions and signs.In our trials on rabbits feeding with different Lupins (*Lupinus* spp.) rations, the most manifested symptoms in the early and late acute phases depending upon the duration and virulence of poisoning with stated *lupinus albus* feeds were : a rise temperature, loss of appetite and/or anorexia, reduced food consumption, emaciation due to significant decrease of body weight ($P \leq 0.01$), while the rabbits control group (1) fed with standard laboratory rabbits feed pellets, a marked significant increase in body weight ($P \leq 0.01$) (Table. 1), was recorded. In a few of infected rabbits, lacrimation and salivation were occurred lately in the acute stage of poisoning (i.e., groups 5, 3 and 4 sequencely). In acute poisoning with lupinosis outbreaks, deaths were observed in 7-12 days, whereas poisoning cases were established in 7, 8, 9,10,11 and 12 days for rabbits groups 5, 3, 4, 2, 7 and 6 consecutively. The present clinical findings in agreement with Hungerford [13] and Gardiner and Parr [18]. However, Blood *et al*. [14] mentioned that the green lupins are usually safe to feed, but the dried mature plants can be toxic in several ways; -A nervous syndrome caused by alkaloids in seeds. The toxicity varies between varieties of lupins and within the variety in different years depending on the climate and - an hepatic syndrome - lupinosis - caused by fungi (e.g., *Phomopsis leptostromiformis* and *P. rossiana*) growing on the plant, which the degree of infestation determines the crop's toxicity and - intermittent photosensitization due to previous hepatic injury and- myopathy. In the nervous form of the disease convulsive episodes occur in which there is staggering, falling, clonic convulsions, dyspnea and frothing at the mouth, the signs often appearing only with exercise. There is no liver damage and the mortality rate varies from very low to as high as 50%. Lupinosis is the commoner of the two syndromes. It is characterized clinically by anorexia, depression, loss of body weight and jaundice. Photosensitization is not uncommon. Death may occur within a few days of first illness or be delayed for months, affected animals standing immobile for long periods or wandering aimlessly, often dying from misadventure [14]. The toxic impacts of seeds, green or dry *lupinus albus* feeds on rabbits' liver tissues led to high significant increase ($P \leq 0.01$) in T-Bilirupin and in ALT (SGPT) and AST (SGOT) activities for rabbits' groups 5,3,4, 2, 7 and 6 respectively when compared to the control group. The present work was in coincidence with Gardiner and Parr [18] who could establish increasing plasma bilirubin and liver enzymes activities in sheep affected with acute lupinosis and in agreement with Pastuszewska *et al*. [22], who recorded increasing plasma bilirubin and liver enzymes activities in growing chicken, rats and pigs fed on low- and high- germine yellow lupin seesds. Nevertheless, the feeding rabbits groups on seeds or green *lupinus albus* feeds recorded no significant changes in serum ALK PHOS, but still recorded significant decreased ($P \leq 0.05$) serum Total Protein and Albumin when compared with control rabbits group (1) (Table 2).

On the other hand, rabbits Lupinosis caused by feeding on seeds, green or dry *lupinus albus* rations was detected by increasing of serum urea. In all rabbits' groups fed on seed, green or dry *lupinus albus* feeds, Creatinine, Total Protein and Albumin levels were significantly decreased ($P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.01$ consecutively) for rabbits groups 5, 4, 3, 2, 7 and 6 respectively when compared with control rabbits as illustrated in Table (3). These results are supported by the findings of Helal *et al*. [21], who detected and observed diabetic rats treated with aqueous extracts of *Lupinus albus* were susceptible to these extracts with clinical symptoms and with some changes regarding biochemical and hematological parameters especially that concerned with liver and kidney functions estimations. In addition, it has been reported by Gardiner and Parr [18] that the acute lupinosis of sheep appeared to be chiefly responsible for the elevation of plasma enzymes, suggesting irreversible changes in the liver cell permeability. Not only do enzymes and other cytoplasmic elements leak out of affected cells, but plasma constituents including bilirubin and serum iron appear to traverse the membrane barrier to initiate the formation of toxic granules in tile necrobiotic hepatic cells. Interference with the hepatic conjugation and excretion of plasma bilirubin, accompanied by an increase in intravascular erythrocyte damage, is a further event in the pathogenesis of lupinosis. Previous studies have shown that the red cell damage is at least partly associated with release of copper from the liver cell stores, representing a form of copper poisoning [18]. Moreover, various clinical studies on the effects of lupinosis in cattle and sheep revealed that visibly affected cattle were always acutely affected and many of clinical signs were associated with concomitant nutritional stress and acetonaemia. Jaundice illustrates the basic hepatotoxic nature of the disease. Marked fatty infiltration of the liver with bile ductule cell proliferation and generalized lobular fibrosis were found in most cases of bovine lupinosis, whilst necrotic and granular degeneration of hepatocytes commonly found in

affected sheep, was less frequent and relatively unimportant. Chronic changes occurred in cattle which were only mildly affected clinically or which recovered from the less severe acute or sub-acute stages. Unlike sheep, cattle affected with chronic lupinosis showed only signs of unthriftines [19].

In regard to kidney functions parameters, rabbits Lupinosis caused by feeding on seeds, green or dry *lupinus albus* rations was detected by increasing of serum urea. In all rabbits' groups fed on seed, green or dry *lupinus albus* feeds, Creatinine, Total Protein and Albumin levels were significantly decreased ($P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.01$ consecutively) for rabbits groups (5, 4),(3, 2), (7 and 6) respectively when compared with control rabbits as illustrated in Table (3). However, the present study results revealed that the rabbits' groups fed on seed, green or dry *lupinus albus* feeds established no significant changes in serum Sodium and Carbon Dioxide estimations, but just detected slight significant increased serum Potassium and chloride levels in comparison with control rabbits (Table 3).

The reported results coincide with the recorded findings of Helal *et al*. [21] who found significant increase in creatinine and urea levels in diabetic rats untreated with *lupines albus* extracts. In addition, these increasing in some liver and kidney functions parameters might be due to affections of liver and kidney, then the increasing of liver and renal enzymatic activities [22].

Regarding the haematological parameters, WBC, NEU, LYM, MONO, EOS, BASO, RBC, HGB, HCT, MCV, MCH, MCHC, [RCDW(RDW)], PLT and MPV, our experiments were reported significant changes in all rabbits' groups fed on seed, green, or dry *lupinus albus* rations (Table 4). The toxic effect of *lupinus albus* led to significant increase ($P \leq 0.01$) in WBC, NEU, LYM, MONO, EOS and BASO which play an important function in initiating body immunity. Moreover, RBC and HGB which play a pivotal role in carrying nutrient and oxygen to the body cells were significantly decremented ($P \leq 0.05$ and $P \leq 0.01$ successively) in all rabbits' groups fed on seed, green or dry *lupinus albus* feeds i.e., rabbits' groups 5,4,3, 2, 7 and 6 respectively, when compared with control group (1) (Table 4).

In agreement with Bulter *et al*. [16] study on male and female rats; A 90-day feeding investigation of lupin (*Lupinus angustifolius*) flour (55.4 g/100 g diet) that had been spiked to provide dietary concentrations of 250, 1050 or 5050 mg lupin alkaloids/kg diet, the dose-related reduction in red blood cell count and haematocrit (HCT) occurred in both sexes after 45 days and the mean cell volume (MCV) was decreased in all the male treatment groups. The induction in HCT and MCV persisted in the males until termination of the study when decreased haemoglobin levels were also observed in the top-dose males. At the other extreme, the relative liver weights of female rats showed a dose-related increase. As pointed out by a study of Bulter *et al*. [16], altered foci of liver parenchymal cells were seen in five females receiving dietary levels of 5050 mg/kg, in one female fed 250 mg/kg and in one male from each of the 250 mg/kg and 1050 mg/kg treatment groups. No foci were seen in the control group. Basophilic foci are uncommon in young rats suggesting that the low incidence in this study is compound related [16].

In growing chicken, rats and pigs, the hematological, biochemical and histological effects of feeding varied levels of low-and high-gramine yellow lupin seeds (LG and HG, respectively) and of synthetic gramine added to the diets in amounts ranging from 0.15 to 1.2 g per kg were investigated by Pastuszewska *et al*. [17] in one experiment on growing chicken and in two experiments on growing rats, while the comparison of LG and HG lupin and the effect of 0.5 g gramine per kg of LG diet were determined in a growth-balance experiment with pigs. The authors' findings mentioned that the response to HG lupin and gramine concentration varied among the species, the rats being more affected than chicken; no adverse effects of HG lupin or gramine were found in growing pigs. The common reaction of rats and chicken to the high levels of gramine (Native or synthetic) was the decrease of feed intake and body gain. The increase of the relative weight of liver or kidney, changes in hematological parameters and liver enzymes were found only in rats. The estimated NOAEL (no-observed-adverse-effect level) of gramine was about 0.3 g/kg diet for rats, 0.65 g for chicken and at least 0.5 g for growing pigs [17].

Previously, the incidence of Lupinosis in sheep has been investigated by Neil *et al*. [23], who pointed out that the increase in the incidence of lupinosis was related to a change in grazing practice; some graziers now have breeding herds where previously lupins were not grazed throughout the year. According to Neil *et al.* [23] observation, the disorder seemed to be caused not by seed but by stalks or small stems; it sometimes appeared on young pasture. Where lupinosis occurred there was generally more land under lupins and grazing was heavier. Death often took place from 1 to 3 weeks after the animals were put on lupin pasture but might not occur until after 16 weeks. Young sheep were generally more susceptible.

In mild cases recovery followed removal from lupin pasture. Lupines, depending on alkaloid composition and concentration, are toxic and/or teratogenic to livestock [23]. Most toxicoses resulting in death from lupine ingestion have been reported in sheep and occurred extensively in the late 1800's

and early 1900's [17, 24]. During this period sheep ranchers relied on lupine as important forage for summer grazing and winter hay. Much of the wild hay cut in Montana had lupine as an important component and it often contained 50% or more lupine. In the winter of 1898-1899 in the Judith Basin area of Montana, 3,600 sheep of a band of 7,000 died from eating lupine hay. Between 1897 and 1900 over 2,800 sheep from a flock of about 12,000 died after grazing lupine [17, 24, 25]. In Livingston, MT, 1,900 sheep of 3,000 died in 1 flock after eating *Lupinus cyaneus* seed pods. In most field-grazing cases the losses were attributed to eating lupine pods before seed shatter occurred. *Lupinus sericeus, L cyaneus* and *L leucophyllus* were reported to cause most of the poisoning. Even though no alkaloid analysis was done at that time we now know that *L sericeus* and *L leucophyllus* contain predominantly quinolizidine alkaloids [25]. Alkaloid analysis of *L cyaneus* is apparently not Known. Chesnut and Wilcox [24] reported that cattle and horses readily ate lupines during immature stages whereas sheep generally did not eat lupine in spring and summer but grazed it in early fall when in seed pod stage; they readily ate it in hay.

Cattle losses from lupine were not reported, although 4 horses were reported poisoned by lupine and 3 of those died (Chesnut and Wilcox, (1901) [24]. The lupines commonly associated with crooked calf disease are those containing the quinolizidine alkaloids, specifically anagyrine [25] and include the species *L sericeus, Lcaudatus* [26,27], *L laxiflorus* [2,3,28] and *L sulphureus* [29]. *Lupinus latifolius* was implicated in dog and human malformations when milk from goats eating this lupine was ingested by mothers during early pregnancy [30].

While most lupines predominantly contain quinolizidine alkaloids, a few contain high levels of toxic piperidine alkaloids and some contain both [31]. The toxic and teratogenic effects of 2 piperidine alkaloid-containing lupines, *L formosus* and *Larbustus,* have been demonstrated [32-34]. Ammodendrine and N-methylammodendrine possess similar toxicity [35] and both are believed to be teratogenic in cattle and goats [23,33,34]. Based on structural characteristics, N-acetylhystrine is believed to possess teratogenic activity and is more toxic than either ammodendrine or N-methylammodendrine [35]. Other piperidine alkaloids from *Conium maculatum* and *Nicotianaglauca* are structurally similar and have been demonstrated to cause identical birth defects in sheep, goats, cattle and pigs [35-37]. Clinical signs of toxicoses in cattle are similar with all these piperidine-containing plants and include protrusion of the nictitating ocular membranes, irregular gait, modest tremors, excessive salivation, muscular weakness and occasional temporary and involuntary sternal recumbency after exertion [32, 36]. Death usually results from respiratory failure. Lupine induced fetal toxicity (Reduction in fetal activity and death) has been observed by ultrasound in cattle and goats [33, 34, 38]. As well, the occurrence of congenital deformities in cattle fed on different lupin rations might have been due to the poisoning by chemical constituents found in most *lupinus albus* feeds [39]. Whatever, Lupin-derived protein ingredients have to provide both adequate nutritional and useful technological functionality to the foods in which they are incorporated in order to meet the needs of animal feeding programs, consumers and the food industry [40]. In respect of animal feeding, defining of composition, quantities and biochemical analysis of various green and dried *Lupinus* rations, ranges, seasons and animal species fed on lupin must be achieved periodically.

For more complex implementation of lupin products to bakery and other human food and animal forages products, the further research activities and reciprocal cooperation among lupine cultivators, processors and human food and animals foodstuffs manufacturers is necessary.

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