Research Article

Adverse Histo-Physiological Damages of Increasing Consummation of Puma (Super Fat) on Female Rats

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Abstract | The using of nutritional supplements is an issue reflected on human health because its intake randomly, Puma (super fat) is a one of nutritional supplements which used for weight increasing. So, the aim of the present study was to estimate dangerous effects of Puma (super fat) on kidney and liver functions and histopathological alterations of these organs. The total number of animals in present experiment was thirty two of adult female rats, It were divided into two groups (16/each), the first group which gave distilled water as control group while the second group was divided into three subgroups (A, B and C) which treated with nutritional supplement Puma (super fat) at (250 mg/kg body weight) for three periods (one, two and three months), respectively. The dose of Puma was given for rats by gavage. At the end of experiment period for each group, blood was collected and serum obtained in purpose of chemical examinations were included functions of kidney as (urea and creatinine) and liver enzymes (ALT, AST and ALP). After that, all rats were dissected then kidney and liver were eradicated for histopathological examinations. The results indicated to puma administration led to significant increase (P≤0.05) in urea and creatinine concentrations with significant decrease (P≤0.05) in ALT, AST and ALP these changes in functions of kidney and liver enzymes were elevated by increasing consumption of Puma (super fat). Histological sections of studied organs were manifested lesions in tissues by receiving of puma, it involved many histopathological changes (mild, middle and very severe injuries) in renal structure as hemorrhage, congestion, inflammation and structural disorders such as breakdown, death and absence of glomeruli with increasing of Bowman's space. while these histological changes in liver were included congestion, hemorrhage, hypertrophy as well as necrosis of the hepatocytes. Conclusion: It was concluded in this study eating of Puma for long times causes renal-hepatic damages.

Keywords | Puma, (Super fat), Histopathological changes, Urea, Liver enzymes, Rats, Nutritional supplements, Biochemical indices, Traditional products

Received | Dcember 30, 2023; **Accepted** | January 29, 2024; **Published** | May 27, 2024
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Citation | Al-Badry FAM (2024). Adverse Histo-physiological damages of increasing consummation of puma (super fat) on female rats. Adv. Anim. Vet. Sci., 12(7):1335-1340.
DOI | https://dx.doi.org/10.17582/journal.aavs/2024/12.7.1335.1340
ISSN (Online) | 2307-8316

INTRODUCTION

Nutritional supplements are animal or botanical compounds which consist of vitamins, minerals, enzymes and amino acids, it were a traditional products as capsules, powders, drinks and tablets (Terjung *et al*., 2000). It were important for health when using at appropriate levels that contained vitamin C and calcium ion that necessary for bone health, as it a rich source with folic acid which help cardiovascular patients. Dietary supplements were used for raising of weight by composed of proteins or it characterizing to releasing energy by quick absorption and oxidation, these two types were called protein and carbohydrate supplements respectively (Dangin *et al*., 2003).

Puma (Super fat) is one of these dietary supplements as tablets which contributed to body and face increasing, the elevated weight was occurred after nutrition supplementation intake is associated with high amount of fat in body which act as unhealthy weight because increases of fat weight non muscular weight (Fiorino and Brooks, 2009).

The treatment with body weight fattened by consumers at irregular doses heavily causes health problems as gynecomastia, disorder of mood, lesions of liver and kidney and myocardial hypertrophy (Sousa *et al*., 2016). Also, it was observed that the excessive exposure of nutritional supplements lead to infertility problems with testicle shrinkage, prostate cancer, ovarian defects (Donohoe *et al*., 2010). Also, these had side effects resulted from it's effect on nervous system causing depression in addition to toxic effects in all body systems by lipid peroxidation and generation of ketonic bodies (Tirosh *et al*., 2011). The aim of the current designed study was to estimation the physiological and histopathological effects of nutrition supplement Puma (super fat) on kidney and liver functions in female rats laboratory and investigate sever renal- hepatic alterations at different periods after Puma consummation.

MATERIALS AND METHODS

Animals and experiment design

Thirty two of adult female rats had been used, it aged (9-11 weeks) and weighted about (190-210 grams). Animals were housed and treated in animal house of biology department in Thi-Qar university. Laboratory conditions were controlled at temperature (21±3˚C) and 12:12 hours light: Dark cycle. Rats were divided into two groups. First group (Control group) which it administrated distilled water, the second group (treated group) which divided into three subgroups (A, B and C), all these subgroups treated with Puma (super fat) (250 mg/kg body weight) for (one, two and three months), respectively.

Biochemical parameters

It involved functions of kidney and liver enzymes which required serum. Serum was collected by centrifuged the blood at 2500 rpm for 15 minutes. Concentration of urea and creatinine were measured according to Tietz (1999), Wills and Savory (1981) by kits (Biomerieux, Biolabo/ France), while liver enzymes involved (ALT, AST and ALP). The first enzymes were assessed by kits (Atlas medical/England) according to (Reitman and Frankel, 1957). ALP kits (Biomerieux/ France) was used for evaluation ALP (Belfield and Goldberg, 1971).

Histological examination

Both kidney and liver were dissected out for histological study. It was fixed in formalin 10% for 48 hours, the method Muhammad-Azam *et al*. (2019) was used for preparing the histological sections of these organs. Samples were processed by ascending concentrations of ethanol that included (50%,70%, 80%, 85%, 90%, 95%, 100%) for dehydration, after that it cleared with xylene, then paraffin wax was used for embedding and preparation of block. Sections (5 µ thickness) were supplied and stained with hematoxylin and eosin for examination by light microscope. (Figure 1).

Histopathological scoring of kidney and liver

The degree of histological injury of kidney and liver was scored depending on the grading system according to Al Asmari *et al*. (2017). Magnitude of injury was included four stages which it graded (0-4) upon microscopical results, it involved hemorrhage, congestion, inflammation and structural disorders such as breakdown, death and absence of glomeruli with dilation of Bowman's space while the changes of liver were included congestion, hemorrhage, hypertrophy as well as necrosis of the hepatocytes, these were shown in Table 3.

Statistical analysis

The data present in tables were acted mean ± standard deviation of studied parameters, it used for comparing among groups and its analysis by SPSS (Version 21) for determination the significance which it occurs at (P≤0.05) (Bryman and Cramer, 2012).

RESULTS AND DISCUSSION

The results in Table 1 detected to administration of Puma (super fat) at all exposure periods (one, two and three months) caused a significant increase (p≤0.05) in functions of kidney compared with control group. Also, the concentration of urea and creatinine were increased significantly (p≤0.05) among groups that treated with Puma (super fat) by raising period of intake it.

Table 1: Effect of Puma (Super fat) in kidney functions (n=8) (Mean ± Standard deviation).

Creatinine (mg/dL)	Urea (mg/dL)	Group
0.72±0.00 [d]	43.12 ± 0.51 [d]	Control group
0.77±0.00 [c]	49.01 ±0.93 [c]	Puma group (One month)
0.81 ± 0.01 [b]	53.30± 1.48 [b]	Puma group (Two months)
0.86± 0.01 [a]	60.15 ± 2.23 [a]	Puma group (Three months)
0.00	0.65	L.S.D.

The current results in Table 2 showed a significant decrease in liver enzymes (P≤ 0.05) (ALT, AST and ALP) in Puma super fat groups when compared with control group, this

decline in enzymes was increased significantly (P≤ 0.05) as a result of elevated receiving of Puma (Super fat).

Table 2: Effect of Puma (Super fat) in liver enzymes (n=8) (Mean ± Standard deviation)

ALP IU/L))	AST (IU/L)	ALT IU/L))	Group
143.21±1.66[a]	23.88±1.00[a]	23.42± 1.11[a]	Control group
139.14±1.00[b]	20.33±0.35[b]	20.11± 1.37[b]	Puma group (One month)
105.15±2.00[c]	18.91±0.74[c]	17.83± 0.51[c]	Puma group (Two months)
93.71± 0.87[d]	11.71± 0.61[d]	10.00± 0.70[d]	Puma group (Three months)
0.71	0.45	0.40	L.S.D.

Different letters indicate to significant difference (P≤ 0.05) among groups

Table 3: Scoring system of tissue injury.

Score	Description	Status
0	Normal structure of tissue without any change	Normal
1	Tissue injury at less 25 %	Mild injury
2	Tissue injury at (25 %- 50%)	Middle injury
3	Tissue injury at (50 %-75%)	Sever injury
4	Tissue injury at more than 75 %	Very severe injury

Effect of puma on histological changes of organs

Results of scoring system were recorded histopathological injury of kidney and liver in control and infected group with Puma, it were included control group without any changes (normal, score:0), Puma group one month (mild injury, score: 1), Puma group two month (sever injury, score: 3), Puma group one month (very severe injury, score: 4), these summarized in Table 4.

Table 4: Percentage of histological injury for all experimental groups.

Score	Injury of liver	Injury of kidney	Group
0	%0	%0	Control group
1	%20	%19	Puma group (One month)
3	%67	%65	Puma group (Two months)
4	%81	%83	Puma group (Three months)

Histopathological changes

Histological study of kidney was found histological damages by Puma (Super fat) exposure, it involved hemorrhage among tubules, congestion, inflammation and structural disorders such as breakdown, death and absence of glomeruli with increasing of Bowman's space. Also, Puma was led to histological lesions in liver, that included necrosis, fibrosis, congestion of central vein. hemorrhage as well as hypertrophy of hepatocytes with increasing of sinusoids (Figures 2-9).

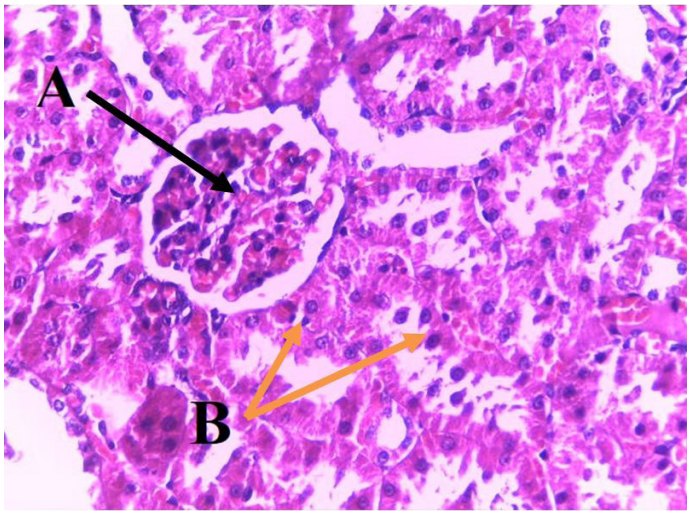

Figure 1: Section in kidney of control group showing cortex of kidney consist of glomerulus (A) renal tubules in medulla (B) (H&E) (100 X).

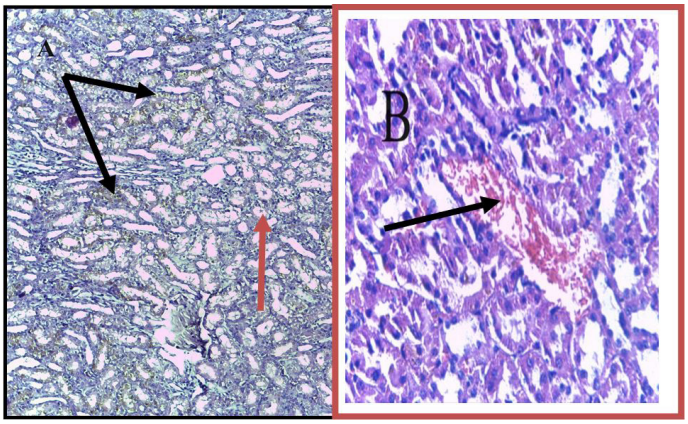

Figure 2: Section in kidney of Puma super fat group (One month) showing, (A): hemorrhage (black arrow) natural renal tubules (red arrow). (B): Section in kidney of Puma group (Two months) showing congestion of blood vessel (black arrow) stained by H&E, (A) (40 X) (B) (100 X).

The functions of kidney which studied in the current study were included urea and creatinine, these were elevated significantly (p≤0.05) after administration of Puma (super fat) at all exposure periods (one, two and three months) compared with control group. Also, the concentrations of urea and creatinine were increased significantly (p≤0.05) among groups that treated with Puma (super fat) by raising period of intake it. That raising in these parameters may be associated to effect Puma fattened on kidney, this result is accordance with Akande and Banjoko (2011) who reported to disturbances in functions of kidney belong to structural damages in kidney tissue. while the elevated of kidney functions did not agree with Abudal-Kadhum *et al*. (2016) who noted no significant differences in urea and

creatinine concentration when dietary supplements intake.

Regarded of liver enzymes, the current study revealed to significant decrease (P≤ 0.05) in liver enzymes (ALT, AST and ALP) in all groups treated with Puma super fat when compared with control group, this decline in enzymes was increased significantly (P≤0.05) as a result of elevated receiving of Puma (Super fat). That decrease may be due to presence of caffeine in Puma super fat, this accordance with Abara *et al.* (2007) who found reduction of liver enzymes by caffeine. Also, this decline is identical with Kumar and Anandan (2007) who explained decreasing of ALT and AST by exposure for glutamin supplement, while that did not agree with Waldron *et al.* (2002) who mentioned to administration of nutritional supplements led to high liver enzymes.

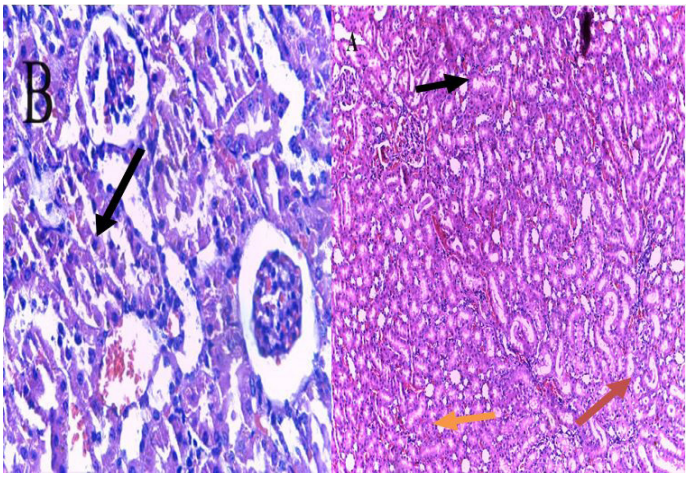

Figure 3: Section in kidney of Puma group (Two months) showing (A): hemorrhage among renal tubules (black arrow). (B): glomerular atrophy (black arrow) hemorrhage (red arrow) dilatation of Bowman's capsule (orange arrow), stained by H & E, (A) (40 X), (B) (100 X).

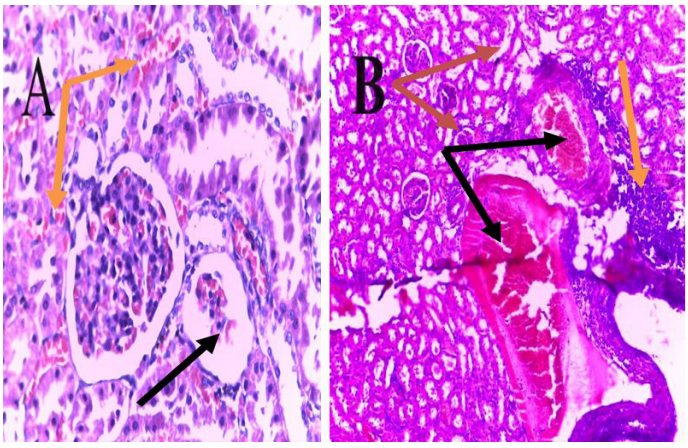

Figure 4: Section in kidney of Puma group (Three month) showing (A): absence of glomerulus (black arrow) severs bleeding (orange arrow). (B) sever congestion (black arrow) infiltration of inflammatory cells (orange arrow) shrinkage of glomerulus (red arrow) with autolysis stained by H&E, (A) (100 X) (B) (40 X).

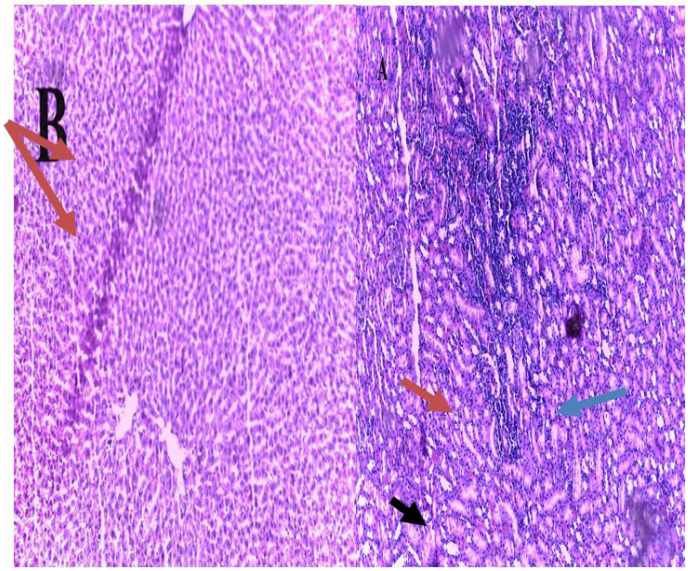

Figure 5: Section in the kidney of Puma group (Three months) showing (A): sever infiltration of inflammatory cells (red arrow). (B): Section in the liver of control group showing central vein (red arrow) hepatocytes (blue arrow) sinusoids (black arrow), stained by H & E, (A), (B) (40 X).

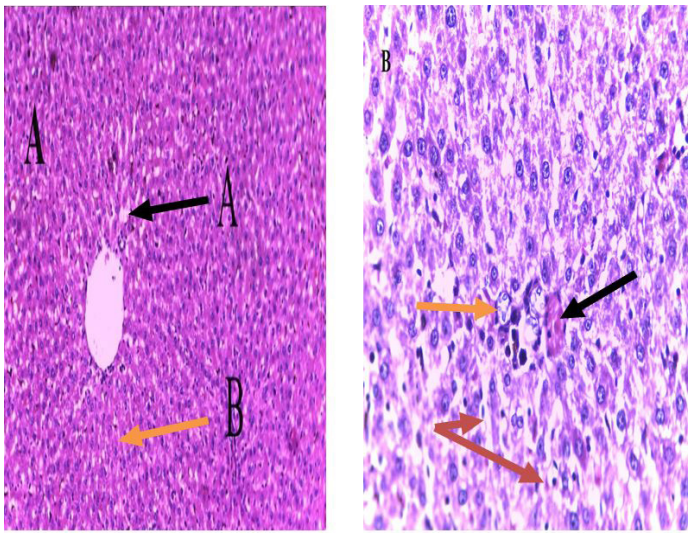

Figure 6: Section in the liver of Puma (Super fat) group (One month) showing (A): simple enlargement of sinusoids (Black arrow) natural tissue (orange arrow). (B) Puma group (Two months) showing congestion (black arrow) hypertrophy of hepatocytes (orange arrow) enlargement of sinusoids (red arrow) stained by H&E, (A) (100 X) (B) (40 X).

Histological study of kidney was found histological damages by Puma (Super fat) exposure, it involved hemorrhage among tubules, congestion, inflammation and structural disorders such as breakdown, death and absence of glomeruli with increasing of Bowman's space. Also, Puma was led to histological lesions in liver, that included necrosis, fibrosis, congestion of central vein, hemorrhage as well as hypertrophy of hepatocytes with increasing of sinusoids. All these changes were observed may be linked

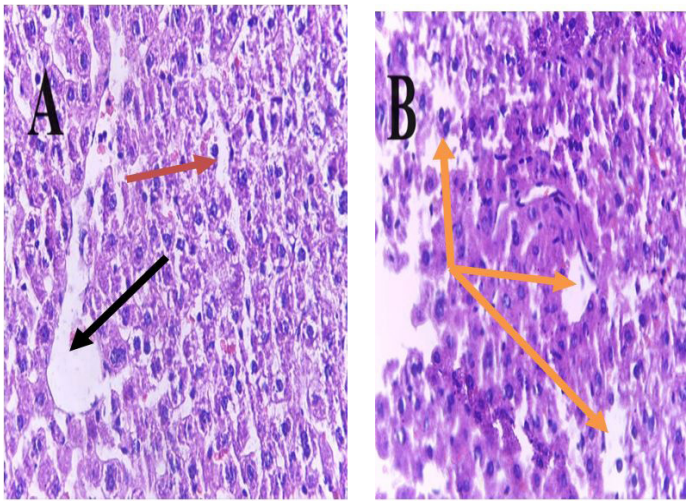

Figure 7: Section in the liver of Puma group (Two months) showing (A) dilation of central vein (black arrow) sinusoids (red arrow). (B): necrosis (orange arrow) stained by H&E, (A) (100 X) (B)(40X).

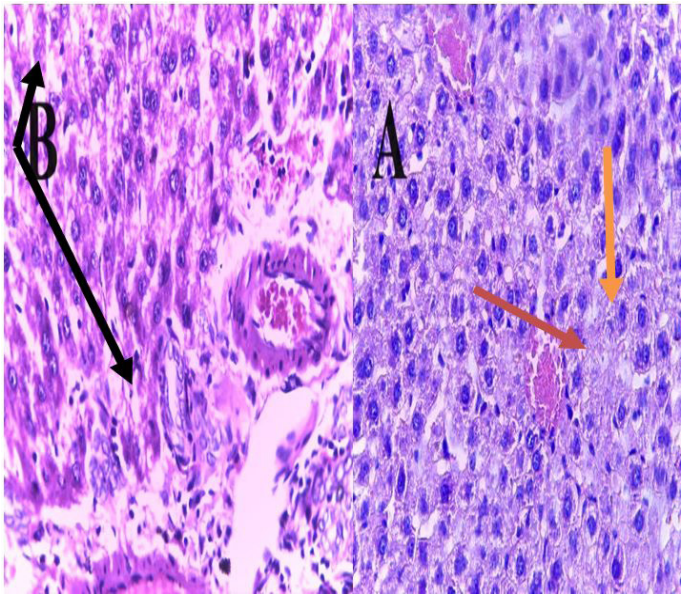

Figure 8: Section in the liver of Puma group (Three months) showing (A): congestion of central vein (black arrow). (B): fibrosis (red arrow) congestion (orange arrow) stained by H&E, (A), (B) (100X).

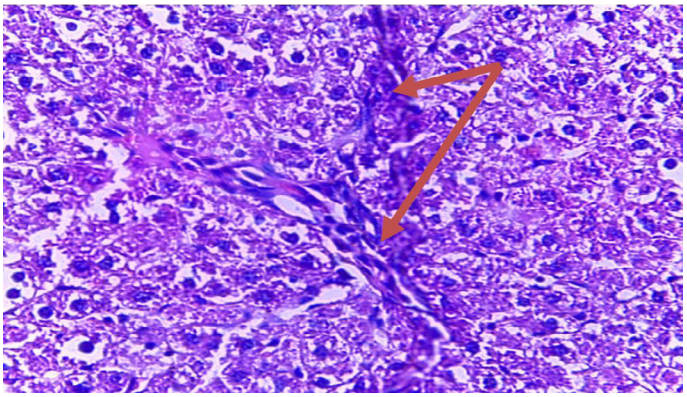

Figure 9: Section in the liver of Puma group (Three months) showing inflammation (red arrow) (H&E) (100X).

to intake of Puma fattened at irregular and large doses, this accordance with Taner *et al*. (2010) who indicated to damages of kidney are occur when treatment with supplements. Blach (2010) reported to important role of nutritional supplements components which cause renal damages and histological lesions of kidney.

The histological lesions were observed in liver may be related to lipid peroxidation that caused by elevated intake of Puma supplement, this is identical with Al-Moutaery *et al*. (2003) who indicated to some components of nutritional supplements as caffeine were stimulated lipid peroxidation led to damages of nucleic acid and proteins of cells subsequently necrosis occurrence. Congestion of central vein was showed clearly may be belong to caffeine of Puma super fat, that is similar to Abd El-Ghany *et al*. (2012) who noticed congestion by caffeine. Also, another reason of hepatic damage may be due to increasing administration of Puma supplement that affected on liver enzymes, Pratt and Kaplan (2001) assessed the changes in ALT, AST considered indicator of liver damage and refer to necrosis and breakdown of hepatocytes.

CONCLUSIONS AND RECOMMENDATIONS

In the current study, it was concluded that exposure for Puma (Super fat) for three various periods (one, two and three months) lead to kidney and liver injury. In addition to accentuation of that by elevated of urea and creatinine with decline of ALT, AST and ALP. Further, the histopathological changes which indicated to renal-hepatic injury. We recommend not excessive using of nutritional supplements because they have significant effects on the body. We also recommend conducting other studies on the various types of supplements on the market that increase weight and explaining their effects on the body.

ACKNOWLEDGMENTS

I would like to introduce my thanks to university of Thi-Qar for the all support and assistance during the achievement of the present research.

List of abbreviations

ALT, Alanine transaminase; AST, Aspartate transaminase; ALP, Alkaline phosphatase; SPSS, Statistical package for social sciences; LSD, Least significant difference; H & E, Hematoxylene and eosin.

NOVELTY STATEMENT

The current study highlights potential damages caused by puma (super fat) administration on physiological and

histological levels.

AUTHOR'S CONTRIBUTION

Author performed all the laboratory tests that belong to current work as well obtaining and treatment of animals. In addition to statistical analyses of data.

Ethical approval

Based on the guidelines of the animal interest committee, All experimental executions weref confirmed by the ethics committee in university of Thi-Qar /college of education for pure sciences (Accordance with No. 7/30/1776 in 31/1/2023).

Conflicts of interest

The author declares no conflict of interest.

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