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Toxicity Assessment of Anthocyanin Pigment Extracted from Pistacia lentiscus Fruits for Use as a Natural Food Colorant

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ABSTRACT

This paper studied the effects of a natural pigment (anthocynan-rich pigment) which was extracted on the physiological and biochemical condition of female Wistar rats. The three groups conducted were exposed to either 25 or 12.5 mg/kg daily dose orally administered within three weeks respectively. Indicators of the weight of the body, the indicators of liver functional and kidney functional indicators and the structure of the tissue were monitored. The rodent group administered the lower dose reported a significant increase in weight, as well as lack of biochemical imbalances. Conversely, sinusoidal dilatation and vascular congestion of the liver tissue were observed in the higher dose group albeit the values of liver and kidney functions were within the acceptable limits. These findings indicate the safety of the extract at low dose, but warn of possible hepatic effects with greater exposure, indicating a critical control of dose before pre- or post-food or pre-medical intake.

Keywords: Natural pigments, Anthocyanins, Pistacia lentiscus, Toxicity, Histopathology

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تقييم مدى سُمية صبغة الأنثوسيانين المستخلصة من ثمار البَطوم، لاستخدامها في تطبيقات الغذاء كملوّن غذائي

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الملخص:

هدفت هذه الدراسة إلى التحقيق في تأثير صبغة طبيعية غنية بالأنثوسيانين، مستخلصة من ثمار نبات *Pistacia lentiscus*، على الحالة الفسيولوجية والكيميائية الحيوية لإناث جرذان ويستر. شملت التجربة ثلاث مجموعات، حيث تلقّت مجموعتان منها جرعات فموية يومية مقدارها 12.5 ملغم/كغم و25 ملغم/كغم، على التوالي، لمدة ثلاثة أسابيع. تم تتبع مؤشرات مثل وزن الجسم، ووظائف الكبد والكلى، والفحص النسيجي للأنسجة. أظهرت المجموعة المعالجة بالجرعة المنخفضة زيادة ملحوظة في الوزن دون اضطرابات كيميائية حيوية. في المقابل، أظهرت مجموعة الجرعة المرتفعة تغيرات نسيجية خفيفة في الكبد، شملت اتساع الجيوب الكبدية واحتقان الأوعية الدموية، رغم أن قيم وظائف الكبد والكلى بقيت ضمن الحدود المقبولة. تشير هذه النتائج إلى أمان المستخلص عند الجرعات المنخفضة، مع احتمالية حدوث تأثيرات كبدية عند التعرض لجرعات أعلى، مما يبرز أهمية ضبط الجرعة قبل استخدامه في التطبيقات الغذائية أو الطبية.

الكلمات المفتاحية: الأصباغ الطبيعية، الأنثوسيانين، *Pistacia lentiscus*، السمية، الفحص النسيجي

INTRODUCTION

Anthocyanins contain derivatives of glycosylated anthocyanidins and mostly constitute the large array of colors possessed by plant tissues, which run in shade of red, orange, shades of blue and purple. Being the water-soluble flavonoids, they have basic

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flavylium cation core and are typically glycosylated with one or more units of sugar with a hydroxyl (-OH) or methoxyl (-OCH3) group. In spite of the fact that 31 anthocyanidins have been well investigated, over 500 anthocyanins have been described, differing due to their hydroxylation, methylation, glycosylation, and the nature of acyl group attached to the sugar unit. Out of them, six principal forms cyanidin, pelargonidin, peonidin, petunidin, delphinidin, and malvidin are commonly detected in an extensive variety of fruits and vegetables, such as berries, cherries, skin of grapes, eggplants, purple sweet potatoes, the bract of banana, and red cabbage (Suresh *et al*., 2022).

The number of positive properties involving anthocyanins has increased in past years making them one of the most sought after substances in terms of health maintenance. Nonetheless, low bioavailability in the body is one of the greatest restrictions of their full therapeutic capability in the human body. To deal with this problem several technology methods are being formulated to concentrate anthocyanins by separating it with other constituents of the food matrix. Even with these encouragements, it is not clear whether isolated anthocyanins may confer the same health benefits as when they are taken as part of whole food. The review covers research done on the absorption and metabolism of anthocyanins in the body with some differences being pointed out between extracted sources and whole food sources. Laboratory experiments, animal studies and clinical trials conducted in humans indicate that anthocyanin has more benefits when consumed along with whole foods as compared to when consumed in their solitary form (Kumkum *et al.*, 2024).

Cyanidins are in the category of natural products polyphenols that are flavonoids. They occur prominently in vegetables and fruits and they are abundant in all parts of plants such as in leaves, petals, flowers, and fruits that are red in colour. A number of vegetables which include blackberry, cranberry, grapes, cherry, apples, raspberry, peaches, plums, beans and red cabbage and red onions have cyanidins (de Araaujo *et al.*, 2021).

Phytonutrients have been identified as extraordinary bioactive molecules that are highly represented in most plant foods in the search of optimal health and longevity. One of the main group of flavonoids as well as the phenolic phytonutrient known as anthocyanins has attracted considerable interest due to their potential health promoting effects highlighted as ant-inflammatory

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(Burgos-Edwards et al., 2019), ant-oxidant (Li et al., 2020), anti-cancer (Marczylo *et al*.,2009), immunomodulating (min *et al*., 2015), anti-microbial (Cisow Since their possible health benefits have been increasingly supported, the field of research studies dedicated to anthocyanins has revived in the 21st century as well (Wallace and Giusti, 2019)

This has seen anthocyanins enjoying a lot of attention by the food, medicine and treatment industries. They are used as traditionally used as food colourants to functional foods. They however have limited bioavailability in most cases owing to their unfavorable stability and solubility characteristics. In addition, the level of these bioactive phytonutrients may change dramatically because of numerous factors, including interspecies and intercultivar variations, seasonal and environmental factors, agricultural procedures, processing activities, and storage conditions (Tiwari and Cummins, 2013). Consequently, there exists different food processing techniques and approaches that are specifically used in an endeavor to preserve or even increase these bioactive compounds in an effort to retain their stability and bioactivity (Hocine *et al*., 2018).

The study aimed to examine the effect of two doses of the extract (12.5 mg/kg and 25 mg/kg) when administered orally during body weight, liver and kidney functions, and a possible histopathological impact, as well as the course of body weight change in three weeks. The safety study of the extract and an evaluation of possible toxicity were carried out by observing the critical biochemical markers, ALT, AST, bilirubin, creatinine, and urea, as well as weight and tissue morphology of the organs. The research is also a further attempt to study natural, vegetable colorants as safe alternatives that can be used in the food and pharmaceutical industries.

MATERIAL & METHODS

Collection and Preparation

These Pistacia lentiscus fruits were obtained in the middle od February in a number of locations around the town of Darna; al-Fattaih, Raas Al-Hilal and Lathronon. Fruits were hand-picked in their mature stage and healthy condition and dried under cool lab conditions in the air at normal room temperature. The fruits were then wiped dry after adequate washing with water, dried in a hot

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air oven at 50 C temperature after a time duration of 10 hours and grounded on a fitting milling apparatus to yield the resultant powder -18 C. The defuting process was completed using a soxhlet extraction process based on petroleum ether and in order to extract anthocyanins; a solvent containing 50% ethanol and 4% citric acid was used in a 30 ml/g ratio. This was done twice in the shaking of 40 C water bath at 30 minutes. It was filtered with the help of a B uchner funnel and further concentrated under low pressure in the form of a rotating evaporator at 40-50 C. The crude extract was frozen in -18 DEG C then freeze-dried to improve the stability of the pigments and maintain the chemical integrity of the heat sensitive constituents and the end product was stored in the same conditions.

HPLC Analysis of Anthocyanin Extract

Sample preparation: Dried anthocyanin extract was dissolved in acidified methanol, 1g of methanol containing 100 ml of 1g of 1% HCl; a standard procedure was followed to stabilize pigments and make them soluble. The solution was then filtered by a 0.45 um membrane filter and directly pushed to the HPLC system (Tsai *et al*., 2024).

Performance of HPLC analysis: an HPLC analysis of a Knauer Azura HPLC equipped with Clarity Chrom software and using a reversed-phase column (C18) of 100 mm size was carried out. Its mobile phase was the isocratic methanol: acetonitrile (50:50) at a flow-rate of 1.5 mL/min. The column was equilibrated in ambient temperature and samples (100 uL) inserted through it by using injection.

Anthocyanin pigments absorb light at a wavelength of 520 nm, and thus the eluent was subjected to diode-array detector (DAD) at 520 nm (Muti et al., 2025). The anthocyanins were detected characterized by the presence of characteristic retention time and UV-spectra; with no standards available, identification was defined by precedent in the literature. Related HPLC-DAD approaches are also comprised of using similar solvents and conditions of detection plant anthocyanins (Muti *et al*., 2025).

Experimental Work:

The Wistar albino rats were procured in the form of 15 healthy, female rats, weighing an average of about 164 g after purchase, and different, which were taken at random. The animals were maintained in the standard laboratory conditions comprising of

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proper ventilation, 12-hour light/dark schedule and free access to pure drinking water. Ad libitum standard pelleted diet possessed 21.27 percent protein, 2.83 percent fat and 2.46 percent fiber.

The three experimental groups (n = 5 corresponding groups) of rats were randomly assigned, as follows:

1.Control group (C): was only on standard diet.

2.Low-dose group (L): they received 12.5 mg/kg of the pigments of anthocyanin origin extracted by Pistacia lentiscus fruits orally once a day, and normal diet.

3.High-dose group (H): received 25 mg/kg of anthocyanin pigments extracted of Pistacia lentiscus fruits orally once per day as well as standard diet.

The measurements of body weights were taken after two days until the end of the experimental process (21 days). Each group was used to determine the percentage of cumulative weight gain; this is to estimate the effects of the treatment.

Anesthetic killing was done to all the animals at the end of experiment. Biochemical examination was done by cardiac puncture of blood. Kidneys and liver were removed, weighed and stored to conduct further assessments on hepatic and renal products.

Liver and kidney tissues were also taken and prepared to be subjected to histopathological analysis in order to determine, using a microscope, any structural changes that could have been caused by the effect of treatments.

Biochemical investigation:

The levels of plasma aspartate aminotransferase (AST) and alanine transaminase (ALT) were assessed in strict compliance with the peculiarities of the BIOLABO Assay Kit Manual (Cat# 80027).

The amount of plasma bilirubin was measured using the AGAPPE Assay Kit Manual (Cat# 51003005) instructions. The level of creatinine in plasma was calculated based on the instructions of Biomaghreb Assay Kit Manual (Cat# 25043). Biochemistry analysis was carried out by the procedure described in the BIOLABO Assay Kit Manual (Cat# 92032), to assay the plasma urea levels

Histological investigations

After collecting tissues of the liver region of rats, the samples were removed and underwent histological examination after fixation in 10% phosphate-buffered formalin (pH 7.4) and histological investigation. Sectioning was done in serial 5 microns thick and

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prepared using hematoxylin and eosin stain(Carleton *et al.*, 1967) which was examined under the bright field microscope.

Statistical Analysis:

Data were presented as mean p SE. One- way analysis of variance (ANOVA) was employed to calculate statistical significance followed by the multiple comparisons which were done using the post-hoc tests. The entire statistical test was performed by use of SPSS 23 software. The significance of the difference was set at P 0.05.

RESULTS

Identification of Anthocyanins by HPLC-DAD Analysis

The HPLC chromatogram (monitored at 520 nm) of the P. lentiscus fruit extract showed 11 distinct peaks (Figure 1, Table 1) eluting between 0.573 and 9.880 minutes. The major peaks were observed at retention times 4.317 min (peak 6) and 7.060 min (peak 9), with integrated areas of 24.4% and 22.6% of the total anthocyanin signal, respectively. Other significant peaks occurred at 3.993 min (6.6% area, peak 5), 6.810 min (13.4%, peak 8), 9.520 min (12.6%, peak 10), and 9.880 min (11.6%, peak 11). The remaining peaks (at 0.573, 1.407, 1.650, 3.660, and 5.903 min; peaks 1–4,7) each contributed only 0.3–2.6% of total area, indicating minor components. Peak heights and half-widths (W05 ≈0.08–0.16 min) reflect well-resolved, sharp peaks for most compounds.

Table 1 summarizes the retention times, peak areas, heights, and width at 5% height (W05) for each detected compound.

Signal number	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.573	8.758	3.188	0.3	0.9	0.02
2	1.407	39.489	3.235	1.4	0.9	0.16
3	1.650	65.396	10.186	2.3	2.8	0.08
4	3.660	63.390	8.596	2.2	2.4	0.12
5	3.993	185.250	22.061	6.6	6.1	0.13
6	4.317	688.956	79.918	24.4	21.9	0.14
7	5.903	74.342	8.352	2.6	2.3	0.13
8	6.810	378.298	51.203	13.4	14.1	0.12
9	7.060	639.259	75.943	22.6	20.9	0.13
10	9.520	355.890	51.614	12.6	14.2	0.11
11	9.880	329.110	49.906	11.6	13.7	0.11

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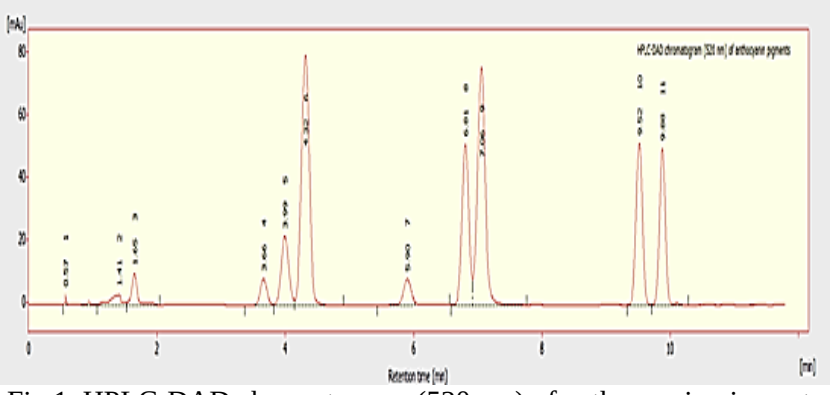

Fig 1. HPLC-DAD chromatogram (520 nm) of anthocyanin pigments extracted from Pistacia lentiscus fruits.

Body weight:

The summary of Table (2) and Figure (2) gives the percentage change of body weight of the three groups of female rats given orally 12.5 mg/kg (L group) and 25 mg/kg (H group) of the anthocyanin pigments extracted in Pistacia lentiscus fruits after three weeks of three consecutive times versus the control group (C).At the start of experiment the body weights of three groups, were not significantly distinguished ($F = 0.52$; $P>0.05$), which implies a homogenous beginning. During the treatment period: Week 1: During week 1, there was moderate gain in body weight in all three groups without any statistically significant variance experienced ($F = 0.59$; $P > 0.05$). Week 2: The weight gain was observed in all the three groups, comparable values showing 19.30 (2.07)% in the control, 19.26 (1.31)% in the low-dose and 19.51 (3.75)% in the high-dose categories. Nor were there found any significant differences (F 0.003; $P > 0.05$). Week 3: It was noted that there was a significant difference in weight gain. The highest GE increase was demonstrated by the low-dose group (29.38 1.81%), the second-highest increase was presented by the control group (27.64 2.09%). On the contrary the gain was significantly (18.83 + 4.97 %) low in the high-dose group distributions and the group difference was also significant ($F = 2.96$; $P < 0.05$). This indicates that the lower doses of the final powder could increase the weight gain of the bodies but there is a suppressive or side effect to the higher dose which might indicate toxicity or even interruption of positive or normal body growth with regard to the high amounts of the substance.

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Table. 2. Percent increase in body weight of female rats administered 12.5 mg/kg and 25 mg/kg anthocyanin pigments extracted from Pistacia lentiscus fruits three weeks

	C	L	H	F test
Starting body weight	146.4±11.59	145.2±8.18	160±13.58	**0.52**
1 week	10.62 ±2.03	9.5 ± 1.97	12.52 ± 1.94	**0.59**
2 week	19.30 ±2.07	19.26 ± 1.31	19.51 ± 3.75	**0.003**
3 week	27.64 ±2.09	29.38 ± 1.81	18.83 ± 4.97*	**2.96**

Each result represent the mean ± SE. (n=5). * means significant at P<0.05. Abbreviations; C , Control; L , Low-dose group ; H , High-dose group

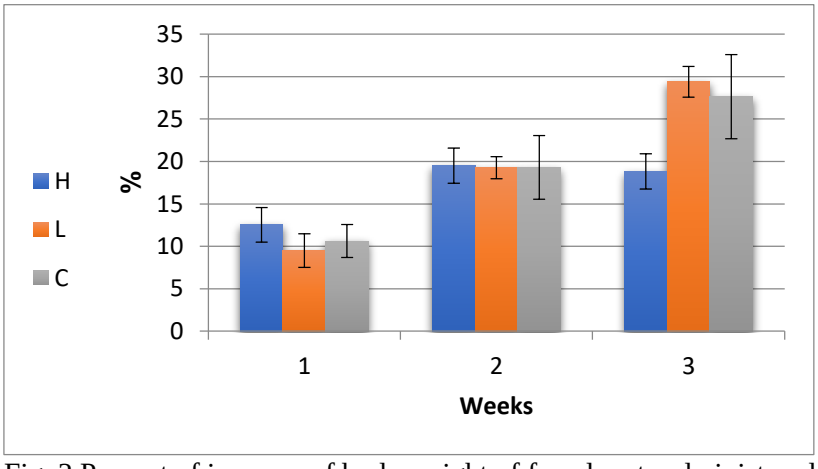

Fig. 2 Percent of increase of body weight of female rats administered 12.5 mg/kg and 25 mg/kg of anthocyanin pigments extracted from Pistacia lentiscus fruits for three weeks. Each result represent the mean ± SE. (n=5). * means significant at P<0.05. Abbreviations; C , Control; L , Low-dose group ; H , High-dose group

Absolute and relative liver weight:

In Table (3) and Figure (3) it is observed that when the natural plant derived colorant (anthocyanin pigments extracted in the fruits of Pistacia lentiscus) was administered to female rats orally (doses of 12.5 mg/kg and 25 mg/kg), with a treatment period of three weeks there were different responses on the liver weight of the treated animals when compared with that of the control group as indicated in the table and figure(s). The final body weights were relatively similar in every group, the means of which were 186.20

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+/-12.51 g in the control group, 188.20 +/-12.16 g in the low-dose group, and, 187.60 +/-9.10 g in the high dose group (F = 0.008, P > 0.05), which revealed no considerable effect on overall body mass. The absolute liver weight however gave a statistically significant result between the low dose group (8.47 + 0.42) and the control group (6.46 + 0.40) with the high dose group having a half way value of (7.30 + 0.72) (F = 3.48, P < 0.05). In a similar manner, the relatively weight of liver was increased in the low-dose group (4.54+0.26) as well as the high-dose group (3.92+0.43) than that in control, (3.54+0.36) with statistical significance noted (F 1.95, P< 0.05). These findings indicate that the use of this natural food colorant could be used to affect liver mass, especially as low doses, which could be reflective of adaptive hepatic processes.

Table 3 Body weight ,Absolute liver and relative liver weight of female rats administered 12.5 mg/kg and 25 mg/kg of anthocyanin pigments extracted from Pistacia lentiscus fruits for three weeks

	C	L	H	F test
Body weight (g)	168	166	200	
	155	153	166	
	208	206	196	
	178	216	166	
	222	200	210	
	±12.5186.20	**188.20±12.16**	**187.60±9.10**	**0.008**
Absolute weight of liver(g)	7.84	7	7.36	
	6.46	8.4	5.2	
	5.91	8.34	9	
	5.44	9.25	8.75	
	6.68	9.4	6.2	
	6.46±0.40	**±0.42*8.47**	**7.30±0.72**	**3.48**
Relative weight of liver(%)	4.66	4.21	3.68	
	4.16	5.49	3.13	
	2.84	4.04	4.59	
	3.05	4.28	5.25	
	3	4.7	2.95	
	3.54±0.36	**4.54±0.26**	**3.92±0.43**	**1.95**

Each result represent the mean ± SE. (n=5). * means significant at P<0.05. Abbreviations; C , Control; L , Low-dose group ; H , High-dose group

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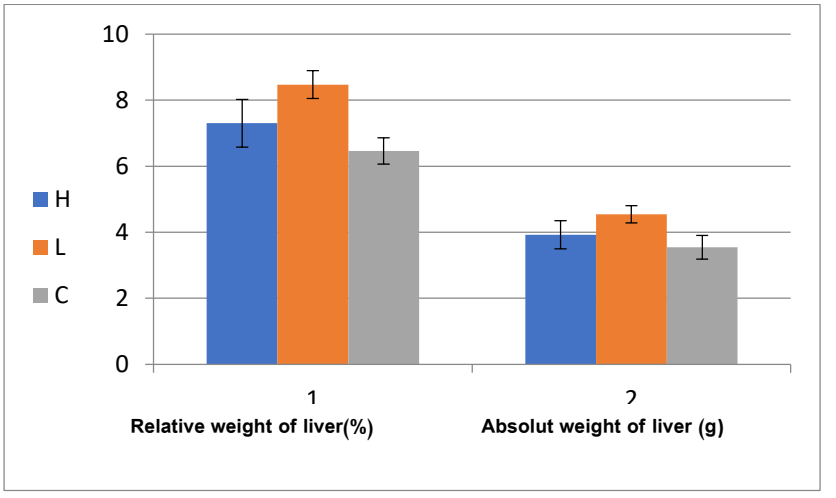

Fig.3 Absolute and relative liver weight of female rats administered 12.5 mg/kg and 25 mg/kg of anthocyanin pigments extracted from Pistacia lentiscus fruits for three weeks. Each result represent the mean ± SE. (n=5). * means significant at P<0.05. Abbreviations; C , Control; L , Low-dose group ; H , High-dose group

Biochemical Observations

The biological properties of the plant-based pigment (anthocyanin pigments obtained by extracting Pistacia lentiscus fruit) tested on the activity of liver enzymes were performed by determining the concentration of aphenylalanine-transaminase (ALT) and aspartate aminotransferase (AST) in the plasma of female rats following three weeks of administration by mouth (Table 4.4). The average values of ALT were 22.47 +/- 0.51 U/L, 29.17 +/- 7.33 U/L, in the low and high dose groups respectively. The control, low-dose and high-dose received AST levels of 24.88 +/- 2.60 U/L, 22.39 +/- 5.08 U/L as well as 27.89 +/- 3.35 U/L individually. The significant difference was not observed between the groups according to the level of liver enzymes ALT ($F = 0.56$) or AST ($F = 0.51$) based on the results of statistical analysis (one-way ANOVA (F-test); $P > 0.05$). The outcomes are indicative of the fact that the administrated doses of the colorant caused no significant hepatic damage or defragmentation of enzymes into the bloodstream throughout the course of the treatment. And (Table 4) and (Fig 4).

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Table. 4 Plasma transaminases levels of female rats administered 12.5 mg/kg and 25 mg/kg of anthocyanin pigments extracted from Pistacia lentiscus fruits for three weeks

	AST (U/L)	ALT (U/L)
C	24.88± 2.60	22.47 ± 0.51
L	22.39 ± 5.08	29.17 ± 7.33
H	27.89 ± 3.35	26.50 ± 0.91
F test	0.51	0.56

Each result represent the mean ± SE. (n=5). * means significant at P<0.05. Abbreviations; C , Control; L , Low-dose group ; H , High-dose group;. AST, Aspartate aminotransferase; ALT, Alanine transaminase.

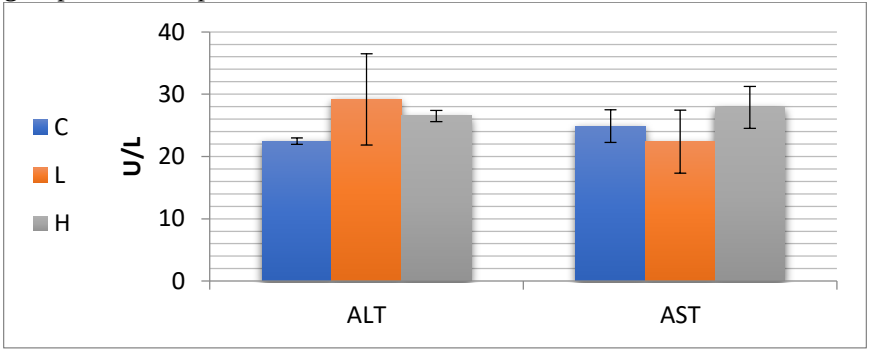

Fig 4 Plasma transaminases levels of female rats administered 12.5 mg/kg and 25 mg/kg of anthocyanin pigments extracted from Pistacia lentiscus fruits for three weeks. Each result represent the mean ± SE. (n=5). * means significant at P<0.05. Abbreviations; C, Control; L, Low-dose group; H , High-dose group;. AST, Aspartate aminotransferase; ALT, Alanine transaminase

Based on Table (5) and Figure (5), plasma bilirubin concentration was employed to measure the probability of hepatic malfunctioning after exposing the animal to oral doses of the natural plant-based colorant at the doses of 12.5 mg/kg and 25 mg/kg within three weeks. The average level of plasma bilirubin in the control population was 0.75 +/- 0.01 g/dl, in the low-dose group (L) and the high-dose group (H) the results were slightly different 0.68 +/- 0.03 g/dl and 0.69 +/- 0.04 g/dl respectively (Table 5). The results obtained by statistical analyzing with F test (F = 1.05) showed that there was no significant difference between the groups (P>0.05). These results imply that the applied amount of the final powder did not implicate a serious impact on the

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bilirubin metabolism or mark liver dysfunction in the conditions of the present.

Table 5 Plasma bilirubin levels of female rats administered 12.5 mg/kg and 25 mg/kg of anthocyanin pigments extracted from Pistacia lentiscus fruits for three weeks

	Plasma bilirubin (gm/dl)
C	0.75 ±0.01
L	0.68± 0.03
H	0.69± 0.04
F test	1.05

Each result represent the mean ± SE. (n=5). * means significant at P<0.05. Abbreviations; Abbreviations; C , Control; L , Low-dose group ; H , High-dose group

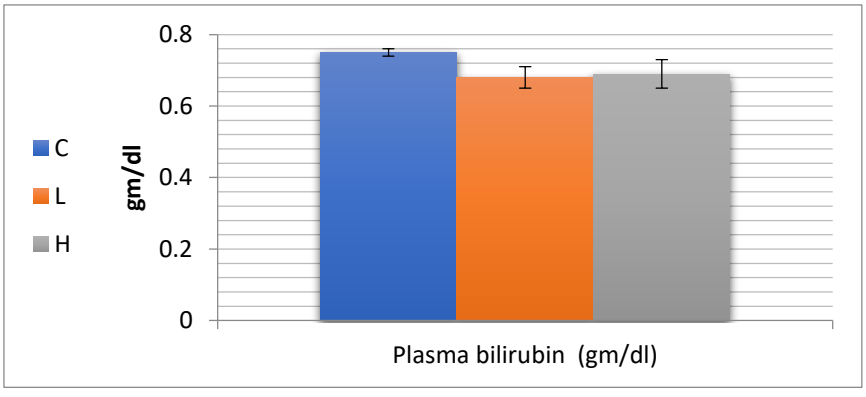

Fig.5. Plasma bilirubin levels of female rats administered 12.5 mg/kg and 25 mg/kg of anthocyanin pigments extracted from Pistacia lentiscus fruits for three weeks. Each result represent the mean ± SE. (n=5). * means significant at P<0.05. Abbreviations; C , Control; L , Low-dose group ; H , High-dose group

The renal parameters were estimated in terms of plasma urea and creatinine levels in response to administration of anthocyanin pigment of Pistacia lentiscus fruit in dose of 12.5 mg/kg and 25 mg/kg orally at day 0 and continued till 21 days.
The control group had a plasma urea level of 50.84 +/- 0.87 g/dl whereas the low dose (L) and high dose (H) group showed a slightly lower urea level (49.03 +/- 2.65 g/dl and 49.03 +/- 8.10 g/dl respectively as indicated in Table 6). There was no statistically significant difference in the levels of urea as per the

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groups (F = 0.78, P > 0.05) and thus the treatment was not able to influence significantly the urea levels.

Plasma creatinine levels, on the contrary, varied more significantly. The average creatinine in the control group was 0.84 G/dl and 0.57 G / dl in the low- dose group, and it showed a significant drop. The group with a higher dose of drugs also reduced to 0.75 +/- 0.05 g/dl. The value of F test was 4.89, meaning that there is significant difference (P < 0.05) in the level of creatinine across groups. The results could imply that the powder displays a dose-dependent influence on the parameters of the renal functions, but none of the parameters went beyond a physiologically acceptable range.

Table. 6 Plasma urea and creatinine plasma levels of female rats administered 12.5 mg/kg and 25 mg/kg of anthocyanin pigments extracted from Pistacia lentiscus fruits for three weeks

	Creatinine Plasma (gm/dl)	Plasma urea (gm/dl)
C	**0.84 ±0.05**	**50.84±0.87**
L	**0.57± 0.07**	**49.03±2.65**
H	**0.75± 0.05**	**49.03±8.10**
F test	**4.89**	**0.78**

Each result represent the mean ± SE. (n=5). * means significant at P<0.05. Abbreviations; C , Control; L , Low-dose group ; H , High-dose group.

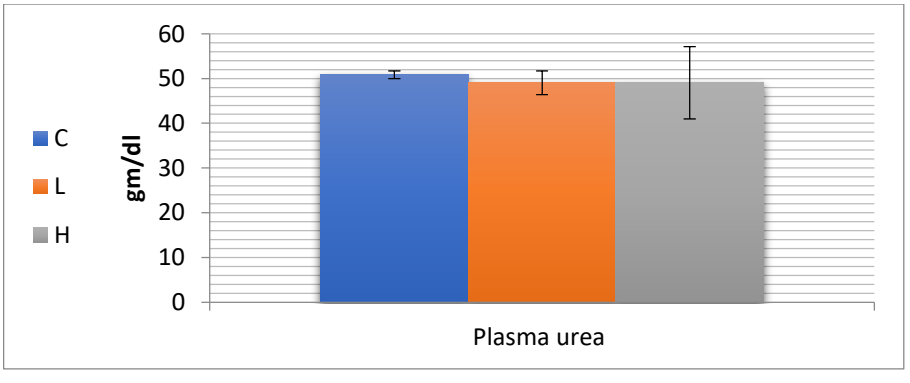

Fig 6 Plasma urea levels of female rats administered 12.5 mg/kg and 25 mg/kg of anthocyanin pigments extracted from Pistacia lentiscus fruits for three weeks. Each result represent the mean ± SE. (n=5). * means significant at P<0.05. Abbreviations; C , Control; L , Low-dose group ; H , High-dose group

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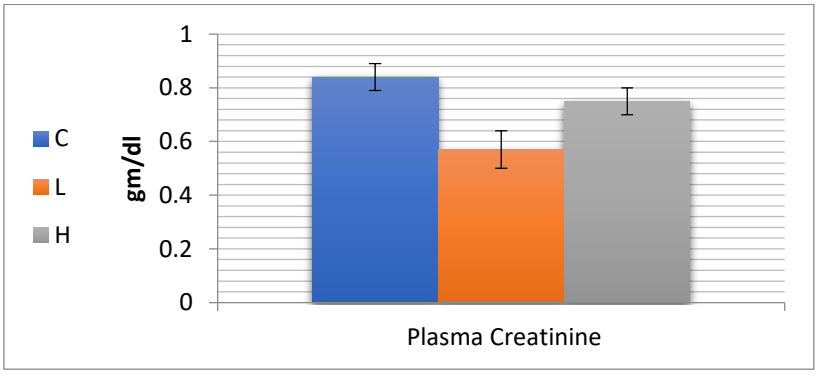

Fig 7 Plasma Creatinine levels of female rats administered 12.5 mg/kg and 25 mg/kg of anthocyanin pigments extracted from Pistacia lentiscus fruits for three weeks. Each result represent the mean ± SE. (n=5). * means significant at P<0.05. Abbreviations; C , Control; L , Low-dose group ; H , High-dose group

Histopathological examination of liver section:

The liver histological observation of the control group (A and B) has an intact hepatic architecture and intact cellular arrangement. The hepatocytes are radiating, polygonal in shape and the hepatic cords surround the central hepatocyte with a well-marked central vein. The cells have central round nuclei with equal staining that characterizes a healthy cell. The hepatic sinusoids are distributed regularly between the hepatocyte cords and seem moderately dilated to permit the adequate blood circulation. No histologic evidence is seen of cell degeneration, tissue/cell necrosis, and congestion or inflammatory infiltrates. The portal triads lack prominence in these sections which is characteristic in central-lobular view. All in all, liver histological sections depict normal histological appearance of healthy liver tissue in untreated female Wistar rats.

Photomicrograph C and D are liver tissue sections of female rats which were exposed to low dose (12.5 mg/kg) of the anthocyanin pigments which were extracted out of the fruits of Pistacia lentiscus after 3 weeks. The general liver structure is fairly retained with the hepatic cords radiating around the central vein. There is however, the early histopathological changes such as mild dilation of the hepatic sinusoids and mild vascular congestion. All these characteristics indicate development of hepatocellular stress or emerging responses of inflammation to the injected drug.

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On the contrary, the liver tissue photomicrographs E and F that represent the photomicrographs of rats that received a higher dose (25 mg/kg) of the anthocyanin pigments extracted in Pistacia lentiscus fruits show more evident histological changes. These are evident overcrowding of central veins, severe dilatation of sinusoids, and to some degree, the distortion of the normal hepatic cord structure. Hepatocytes are swollen and even some ones have got some abnormalities in their nuclei and some of them have got early changes in degenerating. The detachment of a lobular structure in some regions further the argument on having moderate to severe hepatic injury.

These data overall suggest dose-dependent hepatotoxicity of anthocyanin pigments derived by the Pistacia lentiscus fruit, which causes more severe structural liver damage with increasing concentration of the extract. It is an indication of the importance of care towards the dosing and full toxicological assessment of this compound before any use that is therapeutic or experimental of this compound (Fig.8).

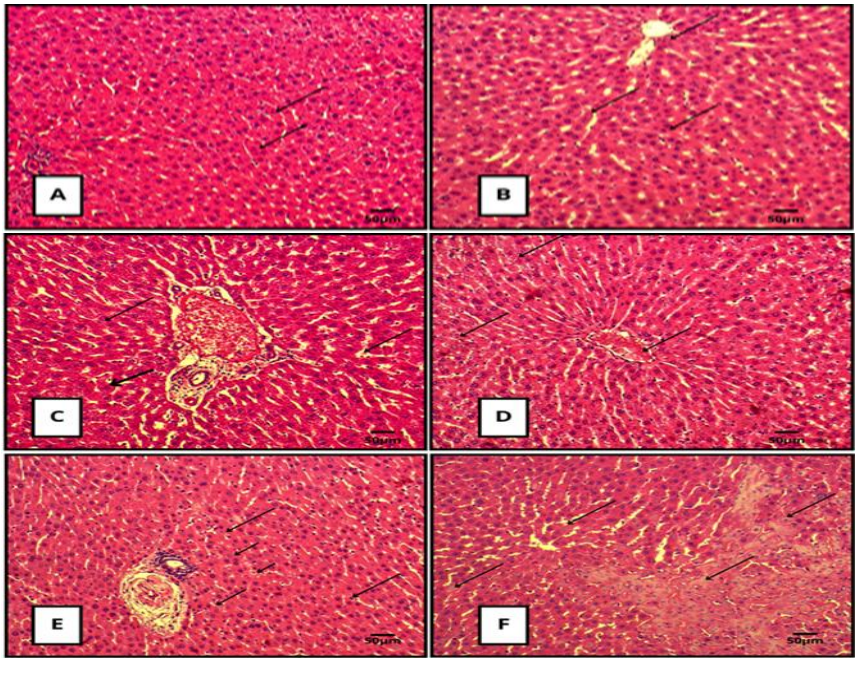

Fig. 8 Photomicrographs of vertical histological sections of liver tissue of female rats Control and administered 12.5 mg/kg and 25 mg/kg of anthocyanin pigments extracted from Pistacia lentiscus fruits for three weeks

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DISCUSSION

The anthocyanin pattern of the fruit extract of Pistacia lentiscus showed the prevalence of cyanidin-3-O-glucoside and delphinidin-3-O-glucoside peak, as was observed in prior research (Sehaki *et al.*, 2023; Milia *et al.*, 2021). Aissat *et al.*, (2022) and Muti *et al.*, (2025) also confirmed these findings by suggesting people found similar anthocyanin composition in lentisk extracts such as sugar-conjugated forms of cyanidin and delphinidin.

Physiologically, there was a significant increase in body weight and a significant liver weight of the low-dose group (12.5 mg/kg) with no adverse alteration on liver enzymes and kidney markers. It implies that this may have a positive metabolic effect at moderate doses, as indicated by Santamarina *et al.*, (2023) and Herrera-Balandrano *et al.*, (2021). On the contrary, the high dose group (25 mg/kg) in addition to showing mild changes in the liver tissues, including dilated sinusoids and congestion of the vascular tissue as well, had no abnormalities in the biochemical parameters. The same dose-related liver effects were demonstrated in the researches of Hocine *et al.*, (2018) and Sangsefidi *et al.*, (2021).

The low-dose group had a significant lowering of renal markers particularly creatinine, which implied its nephroprotective effect, which in line with the finding of Wang et al. (2024). Histology analysis also revealed that the high dose provoked an early hepatic stress, as revealed by Francavilla & Joye (2020) and Suresh & Vellapandian (2024a).

Overview, the extract seems to be safe and physically favorable in lower dose but in higher concentration it can cause mild deviations in liver. Such findings point up the fact that attention must be paid to dosage when wondering the nutritional or pharmaceutical use of anthocyanin-rich extracts.

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