Research Article

Effectiveness of Aqueous Versus Alcoholic Extracts of *Melia azedarach* in Amelioration of Lipid Profile, Liver Enzymes and Innate Inflammatory Indices for White New Zealand Rabbits

Marah Salim Hameed[1], Raad Mahmood Hussein Al Zubaidi[2], Ali Ibrahim Ali Al-Ezzy[3]*

[1]Department of Physiology, College of Veterinary Medicine, University of Diyala, Iraq; [2]Department of Medicine, College of Veterinary Medicine, University of Diyala, Iraq; [3]Department of Pathology, College of Veterinary Medicine, University of Diyala, Iraq.

Abstract | *M. azedarach* is widespread over most tropical and subtropical areas and is popular to tropical Asia. To determine the physiological effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in amelioration of lipid profile, liver enzymes and innate Inflammatory indices for white New Zealand rabbits. Thirty adult white New Zealand male rabbits were classified equally in to 3 groups, G1 were administrated 28mg/day of aqueous extract of *Melia azedarach* for 14 days, G2, were administrated 28mg/day of ethanolic extract of *Melia azedarach* for 14 day, G3 administrated pellets and green food for 14 days. Blood samples were taken at (0, 7, 14) days for separation of serum. Serum cholesterol, triglyceride, total serum protein, albumin and globulin, total serum cortisol, total serum bilirubin, liver enzymes (ALT, AST, and ALP) were determined. Significant variation was reported in serum cholesterol between ethanolic extract vs control. Significant variation in total Serum albumin was reported between aqueous extract vs ethanolic group. Significant variation was reported in total Serum bilirubin between groups (aqueous extract vs control). No significant variation was reported in total serum bilirubin between groups (ethanolic extract vs control, aqueous extract vs ethanolic group; aqueous extract, ethanolic vs control. No significant variation in serum ALT, AST, ALP were reported between groups. Ethanolic extract of *Melia azedarach* at 28mg/day dose has significant effects on serum cholesterol level and total serum albumin. Aqueous extract of *Melia azedarach* at 28mg/day dose has significant effects on total serum bilirubin. Ethanolic and aqueous extracts of *Melia azedarach* have equal effects on ALT, AST, ALP enzymes and innate inflammatory indices for white New Zealand rabbits.

Keywords | Inflammatory indices, Lipid profile, Liver enzymes, *Melia azedarach,* New Zealand rabbits, Physiological effect

Received | March 10, 2024; **Accepted** | April 07, 2024; **Published** | May 18, 2024
***Correspondence** | Ali Ibrahim Ali Al-Ezzy, Department of Pathology, College of Veterinary Medicine, University of Diyala, Iraq; **Email:** alizziibrahim@gmail.com, ali.ib@uodiyala.edu.iq
Citation | Hameed MS, Al-Zubaidi RMH, Al-Ezzy AIA (2024). Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in amelioration of lipid profile, liver enzymes and innate inflammatory indices for white New Zealand rabbits. Adv. Anim. Vet. Sci., 12(7):1256-1265.
DOI | https://dx.doi.org/10.17582/journal.aavs/2024/12.7.1256.1265
ISSN (Online) | 2307-8316

INTRODUCTION

Most indigenous medicines are primarily composed of medicinal plants, and many Western medical formulations contain one or more components that are derived from plants (Jamshidi-Kia *et al*., 2020; Ralte *et al*., 2024). The medicines that are used now are unquestionably not the same as those that were utilized in the past, whether it be ancient or more contemporary (Dias *et al*., 2012; Ullah *et al*., 2024). The kind, quality, presentation, and idea of a pharmaceutical treatment are always changing thanks to a variety of alterations, advancements, complexity, and new

discoveries (Mohs and Greig, 2017; Najmi *et al*., 2022). As human knowledge increased, scientists worked to separate various chemical components from plants, subject them to biological and pharmacological testing, and use this information to make contemporary medications (Salmerón-Manzano *et al*., 2020; Chaachouay and Zidane, 2024).

One of the most beneficial medicinal plants in India's ancient medical system is *Melia azedarach* (family: Meliaceae). *M. azedarach* is widespread over most tropical and subtropical areas and is popular to tropical Asia (Song *et al*., 2023). Different active ingredients were extracted from the fruit of Melia azedarach such as melianoninol (I), melianol (II), melianone (III), meliandiol (IV), vanillin (V), and vanillic acid have all been extracted from fruit (VI) (Ahmed *et al*., 2012; Song *et al*., 2023). The M. azedarach used for curing of leprosy, inflammations, and heart conditions (Gondwal and Rana, 2021). Its fruit extracts have larvicidal and ovicidal properties (Carpinella *et al*., 2007; Ahmed *et al*., 2012).

The majority of hepatoprotective investigations are conducted on Melia azedarach linn leaves, however other portions such as roots and fruits have also been used (Wajdy *et al*., 2021). Ahmed *et al*. (2012) and Sumathi (2013) conducted separate investigations on the hepatoprotective potential of *Melia azedarach*. They found that, AST, ALT, ALP, and serum bilirubin levels were evaluated. The study discovered that after treatment with *Melia azedarach* extracts, the high levels of the biochemical parameter were lowered and returned to normal levels, yielding a promising result (Ahmed *et al*., 2012; Rao *et al*., 2012; Sumathi, 2013).

Akacha *et al*. (2016) investigated the anti-inflammatory effects of an ethanolic extract of Melia azedarach leaves (Akacha *et al*., 2016). Carrageenan-induced paw edema was utilized to assess activity of *Melia azedarach*. The study found that *Melia azedarach* has strong anti-inflammatory action at a level of 150mg/kg (Wajdy *et al*., 2021).

Current study designed to determine the effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in amelioration of lipid profile, liver enzymes and innate inflammatory indices for white New Zealand rabbits.

MATERIALS AND METHODS

Procurement of white New Zealand rabbits

Thirty males of white New Zealand rabbits with a weight range of 1250-1500 g were procured from local market. Animals were housed under standard conditions with 12/12 hours' light–dark cycle. All animals had free access to water and a standard diet *ad libitum*.

Ethical consideration

Current randomized experimental procedures were performed in accordance with the guides for the care and use of laboratory animals and confirmed by the ethics committee at pathology department, college of veterinary medicine, University of Diyala Iraq. The approval No.CVM-UOD-25 /202.

Source of *Melia azedarach*

Melia azedarach fruit were collected from gardens of University of Diyala- Iraq at the summer. The fruits were identified at the department of Biology, Faculty of science, University of Diyala.

Preparation of ethanolic extract of *Melia azedarach* fruit

Melia azedarach fruit dried in shade. Five hundred grams of the dried fruit was soaked in 70% ethanolic solution for 72 hours. The extract filtered through a filter paper and then dried using a rotary evaporator. Finally, the dried extract was kept in a dark bottle (Afkhami-Ardekani *et al*., 2017). The final outcome of ethanolic extract was weighed and preserved at 4°C in airtight bottles until use. The required dose of dried ethanolic *Melia azedarach* fruit extract (28mg/kg) was prepared by dilution with dimethyl sulfoxide (DMSO) (Al-Khafaji *et al*., 2016).

Preparation of aqueous extract of *Melia azedarach* fruit

Dried *Melia azedarach fruit* (500 g) was ground to a fine powder then it was poured with double distilled water, and left for 72 h at room temperature. The flask refluxed over hot water bath for 10 h and the mother liquor was filtered. The distilled water was refluxed and filtered by vacuum filtration through filter paper (Whatman no. 40). This process was repeated for four times. The filtrate was evaporated to complete dryness under reduced pressure on a water bath. Thus, the residue was aqueous *Melia azedarach* fruit extract (Jazzar and Hammad, 2003) that weighed and preserved at 4°C in airtight bottles until use. The required dose of dried aqueous plant extract (28mg/kg) was prepared by dilution with dimethyl sulfoxide (DMSO) (Al-Khafaji *et al*., 2016).

Study design

A total of 30 males of white New Zealand rabbits were enrolled as a typical ample size according to sample size calculator software (Tech, 2024).

Group 1: A ten adult white New Zealand male rabbits were administrated 28mg/kg/day of aqueous extract of *Melia azedarach* for 14 days.

Group 2: A ten adult white New Zealand male rabbits were administrated 28mg/kg /day of ethanolic extract of *Melia azedarach* for 14 days.

Group 3: A ten adult white New Zealand male rabbits

were administrated pellets and green food for 14 days.

Biochemical analysis

Blood samples were taken at (0, 7, 14) day for separation of serum as described by (Al-Ezzy, 2016; Al-Ezzy *et al*., 2020). Serum cholesterol and triglyceride, total serum protein, albumin, globulin, total serum cortisol, total serum bilirubin, liver enzymes (ALT, AST, Alkaline phosphatase) were determined by Cobas Integra 400 Plus according to (Al-Ezzy *et al*., 2016; Hameed *et al*., 2020).

Statistical analysis

Data were expressed as (Mean±SE) (Al-Ezzy *et al*., 2020). One–way analysis of variance (one–way ANOVA) of Vassar Stats online program was used (Al-Ezzy, 2015; Hameed and Al-Ezzy 2019). SPSS used for determination of between t test value with significant level (P<0.05) (Al-Ezzy, 2016).

RESULTS AND DISCUSSION

As shown in Table 1 and Figure 1, the mean serum cholesterol level was (52.6667±11.05039 mg/dL) in white rabbit administered aqueous extract of Melia azedarach; (70.7500±5.85057 mg/dL) in white rabbit administered ethanolic extract, (49.6667±6.8879 mg/dL) among control group. No significant variation was reported between groups (aqueous extract vs control, *P* value =0.41630; aqueous extract vs ethanolic extract, *P* value= 0.17819; aqueous extract, ethanolic vs control, (*P* value=0.17185). These results come in contrary with that reported by (Ilahi *et al*., 2014), stated a significant reduction in the mean serum cholesterol level after treatment with different fractions of *Melia azedarach* (ethyl acetate fraction, n-hexane fraction, chloroform fraction and aqueous fraction) at a dose 50 mg/kg for 20 days. The contrary of current study and that achieved by (Ilahi *et al*., 2014), regarding serum cholesterol, belongs to the using of different fractions by (Ilahi *et al*., 2014), which give a wide range for evaluation of anticholesterol activity of *Melia azedarach*, beside the use of higher dose for longtime which is one of limitation of current study.

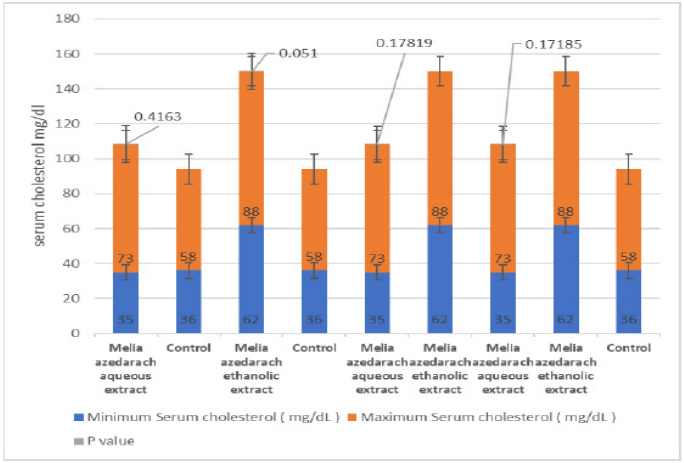

Figure 1: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in serum cholesterol.

Table 1: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in lipid profile.

Parameters	Experimental groups	Minimum serum cholesterol (mg/dL)	Maximum serum cholesterol (mg/dL)	Mean± SE serum cholesterol (mg/dL)	P value
Serum cholesterol	*Melia azedarach* aqueous extract	35	73	52.6667±11.0503	0.41630
	Control	36	58	49.6667±6.8879	
	Melia azedarach ethanolic extract	62	88	70.7500±5.8505	0.051
	Control	36	58	49.6667±6.8879	
	Melia azedarach aqueous extract	35	73	52.6667±11.0503	0.17819
	Melia azedarach ethanolic extract	62	88	70.7500±5.8505	
	Melia azedarach aqueous extract	35	73	52.6667±11.0503	0.17185
	Melia azedarach ethanolic extract	62	88	70.7500±5.8505	
	Control	36	58	49.6667±6.8879	
Serum triglyceride	*Melia azedarach* aqueous extract	48	66	54.6667±5.6960	0.18
	Control	30	60	43.6667±8.7622	
	Melia azedarach ethanolic extract	26	105	68.2500±16.8145	0.149
	Control	30	60	43.6667±8.7622	
	Melia azedarach aqueous extract	48	66	54.6667±5.6960	0.268
	Melia azedarach ethanolic extract	26	105	68.2500±16.8145	
	Melia azedarach aqueous extract	48	66	54.6667±5.6960	0.444
	Melia azedarach ethanolic extract	26	105	68.2500±16.8145	
	Control	30	60	43.6667±8.7622	

A shown in Table 1 and Figure 1, Significant variation in serum cholesterol was reported between (ethanolic extract vs control, p value =0.051). This result come in accordance with that reported by (Herlina *et al.* 2019), reported significant reduction in the level of cholesterol of high fat diet fed rat after treatment with *Melia azedarach* extracts. On the other hand, (Ilahi *et al.*, 2014) stated a significant reduction in the mean serum cholesterol level after treatment with butanol fraction of Melia azedarach at a dose 50 mg/kg for 20 days. The differences in the mean value of serum cholesterol may attributed to the difference in dose and duration as well as the solvent used for extraction of active ingredients. Current results come in line with (Sumathi, 2013), who reported a reduction of serum cholesterol after treatment with 100-200mg/kg ethyl acetate extract of leaves of Melia azedarach indicating a hepatoprotective effects. Current results come in line with (Kumar *et al.*, 2013) after treatment with 100-200mg/kg aqueous and methanolic extract of *Melia azedarach*.

The mechanism for lowering serum cholesterol by aqueous extract of *Melia azedarach* attributed to the effect of saponin which to help in eliminating bile acids and neutral fats, plasma lipids with intestinal content hence lowering cholesterol level (Nkosi *et al.*, 2005). Another mechanism attributed to the presence of other substances that are stimulated by the presence of glucose in the blood and work to inhibit the enzyme hydroxy methyl glutaryl-CoA reductase responsible for the formation of cholesterol (Oakenfull *et al.*, 1979). Further direct supportive evidence for antihyperlipidemic effect of *Melia azedarach* comes from (Davies *et al.*, 2003) reported that a flavonoid fraction of *Melia azedarach* has antioxidant activity which delay lipid oxidation, reducing LDL and total cholesterol levels. Further supporting evidence come from (Agustina 2009), found that alkaloid substances of *Melia azedarach* impair lipase enzyme function, resulting in reduced fat absorption. As reported by (Herlina *et al.*, 2019) flavonoids of *Melia azedarach* reduce the activity of the 3-hydroxy-3-methyl-glutaril-CoA enzyme, lowering cholesterol production. Further evidence come s from a study of (Herlina *et al.*, 2019) reported that the tannin components in the ethanol extract of *Melia azedarach* reduce LDL levels by reducing the activity of the enzyme HMG-CoA reductase, which is involved in the production of cholesterol.

As shown in Table 1 and Figure 2, the mean serum triglyceride level was (54.6667±5.69600mg/dL) in white rabbit administered aqueous extract of *Melia azedarach* while it was (68.2500±16.81455 mg/dL) in white rabbit administered ethanolic extract of Melia azedarach, (43.6667±8.7622 mg/dL for control). No significant variation was reported between groups (aqueous extract vs control, *P* value =0.18; ethanolic extract vs control, p value= 0.149; aqueous extract vs ethanolic extract, p value= 0.268; aqueous extract, ethanolic vs control, (*P* value =0.444). These results come in contrary with that reported by (Ilahi *et al.*, 2014), stated a significant reduction in the mean serum triglyceride level after treatment with ethyl acetate fraction, n-hexane fraction with minimum reduction with aqueous fraction for 20 days at a dose 50 mg/kg. Current result come in line with (Kumar *et al.*, 2013) who reported no significant variation between aqueous and methanolic extract of *Melia azedarach* in lowering triglyceride. The differences in the mean value of serum triglyceride may attributed to the difference in dose and duration as well as the use of different solvent for extraction of wide range of active ingredients of *Melia azedarach*.

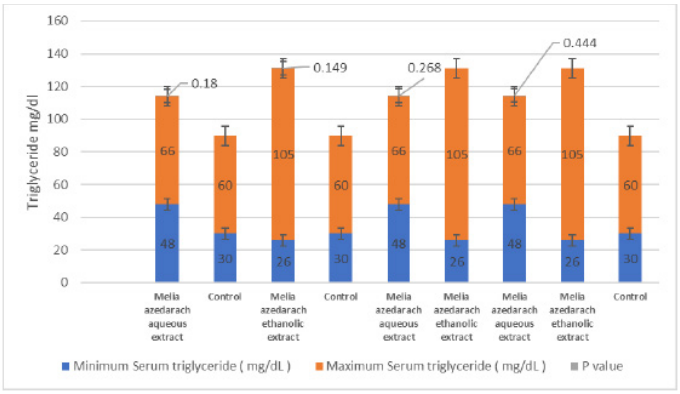

Figure 2: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in serum triglyceride.

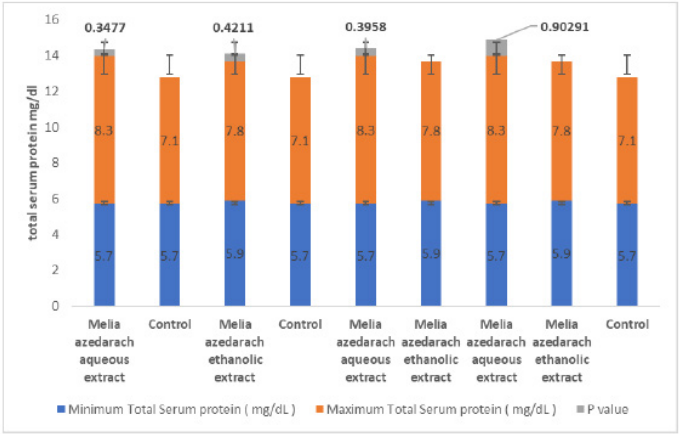

Figure 3: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in total serum protein.

As shown in Table 2 and Figure 3, the mean of total serum protein was (6.8333±0.76884 mg/dL) in white rabbit administered aqueous extract of Melia azedarach; (6.6000±0.45277 mg/dL) in white rabbit administered ethanolic extract, (6.4667±0.40961mg/dL) among control group. Both aqueous and ethanolic extracts of *Melia azedarach* have equal activity in maintaining the total serum protein close to its normal level with no significant variation between aqueous extract vs control, (p value= 0.3477); ethanolic extract vs control, p value= 0.4211); aqueous extract vs ethanolic extract, p value=

0.3958; aqueous extract, ethanolic vs control, (p value= 0.90291). These results come in contrary with (Soni *et al*., 2014), stated that a decrease in total protein content was reported in rat after treatment with 300-500mg/kg *Melia azedarach*. On the other hand, current results come in agreement with (Ahmed *et al*., 2011; Sumathi, 2013), indicating the hepatoprotective role for *Melia azedarach* by maintaining the serum protein level and metabolic activity of hepatocytes at normal physiological level. These discrepancy in reported results of total protein levels may attributed to the wide range of dose under investigation 28mg/kg in current study versus 300-500mg/kg in a study of (Soni *et al*., 2014).

As shown in Table 2 and Figure 4, the mean of total serum albumin was (5.5667±.06667 mg/dL) in white rabbit administered aqueous extract of *Melia azedarach*; (6.6000±.45277 mg/dL) in white rabbit administered ethanolic extract, (5.5333±0.17638 mg/dL) among control group. No significant variation was reported between groups (aqueous extract vs control, *P* value= 0.434137); ethanolic extract vs control, *P* value = 0.181243). Significant variation was reported between aqueous extract vs ethanolic group (*P* value = 0.030392). These results come in contrary with that reported by (Rao *et al*., 2012; Soni *et al*., 2014) stated no significant changes in total serum proteins which include serum albumin for rabbit treated with 300mg/kg -500mg/kg body weight *Melia azedarach* for 14 day. This effect might be due to the plant's ability to interfere with protein metabolism or its hepatic function.

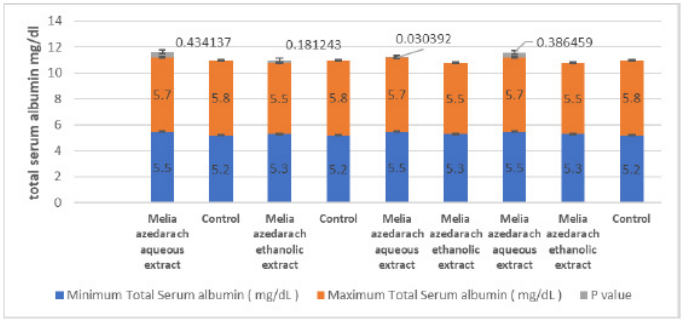

Figure 4: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in total serum albumin.

Table 2: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in erum protein indices.

Parameter	Experimental groups	Minimum total serum protein (mg/dL)	Maximum total Serum protein (mg/dL)	Mean± SE Total serum protein (mg/dL)	P value
Total serum protein (mg/dL)	*Melia azedarach* aqueous extract	5.70	8.30	6.8333±0.76884	0.3477
	Control	5.70	7.10	6.4667±0.40961	
	Melia azedarach ethanolic extract	5.90	7.80	6.6000±.45277	0.4211
	Control	5.70	7.10	6.4667±0.40961	
	Melia azedarach aqueous extract	5.70	8.30	6.8333±.76884	0.3958
	Melia azedarach ethanolic extract	5.90	7.80	6.6000±.45277	
	Melia azedarach aqueous extract	5.70	8.30	6.8333±.76884	0.90291
	Melia azedarach ethanolic extract	5.90	7.80	6.6000±.45277	
	Control	5.70	7.10	6.4667±0.40961	
Total serum albumin	*Melia azedarach* aqueous extract	5.50	5.70	5.5667±.06667	0.434137
	Control	5.20	5.80	5.5333±.17638	
	Melia azedarach ethanolic extract	5.30	5.50	5.3750±.04787	0.181243
	Control	5.20	5.80	5.5333±.17638	
	Melia azedarach aqueous extract	5.50	5.70	5.5667±.06667	0.030392
	Melia azedarach ethanolic extract	5.30	5.50	5.3750±.04787	
	Melia azedarach aqueous extract	5.50	5.70	5.5667±.06667	0.386459
	Melia azedarach ethanolic extract	5.30	5.50	5.3750±.04787	
	Control	5.20	5.80	5.5333±.17638	
Total serum globulin	*Melia azedarach* aqueous extract	0.20	2.80	1.2667±.78599	0.363519
	Control	0.10	1.40	0.9333±0.41767	
	Melia azedarach ethanolic extract	0.50	2.30	1.2250±.42303	0.452534
	Control	0.10	1.40	0.9333±0.41767	
	Melia azedarach aqueous extract	0.20	2.80	1.2667±.78599	0.3308
	Melia azedarach ethanolic extract	0.50	2.30	1.2250±.42303	
	Melia azedarach aqueous extract	0.20	2.80	1.2667±.78599	0.8153512
	Melia azedarach ethanolic extract	0.50	2.30	1.2250±.42303	
	Control	0.10	1.40	0.9333±0.41767	

Table 3: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in Total Serum cortisol.

Parameter	Experimental groups	Minimum total serum cortisol (mg/dL)	Maximum total serum cortisol (mg/dL)	Mean± SE total serum cortisol (mg/dL)	P value
Total serum cortisol (mg/dL)	*Melia azedarach* aqueous extract	76	98	85.3333±6.56591	0.4596
	Control	66	100	86.6667±10.47749	
	Melia azedarach ethanolic extract	39	103	73.25±13.20590	0.24383
	Control	66	100	86.6667±10.47749	
	Melia azedarach aqueous extract	76	98	85.3333±6.56591	0.24919
	Melia azedarach ethanolic extract	39	103	73.25±13.20590	
	Melia azedarach aqueous extract	76	98	85.3333±6.56591	0.65095
	Melia azedarach ethanolic extract	39	103	73.25±13.20590	
	Control	66	100	86.6667±10.47749	

As shown in Table 2 and Figure 5, the mean of total serum globulin was (1.2667±.78599 mg/dL) in white rabbit administered aqueous extract of *Melia azedarach*; (0.9333±0.41767mg/dL) in white rabbit administered ethanolic extract, (0.9333±0.41767 mg/dL) among control group. No significant variation was reported between groups (aqueous extract vs control, *P* value =0.363519); ethanolic extract vs control, p value =0.452534), aqueous extract vs ethanolic group (*P* value = 0.3308); aqueous extract, ethanolic vs control, (*P* value =0.8153512). These results come in agreement with (Shekhar *et al*., 2018), stated that lesser reduction in total protein, albumin, globulin and albumin: globulin ratio was observed in broilers treated with methanolic extract of *Melia azedarach*. The reduction in previous parameters was attributed to the increase in cortisol level which enhance the catabolism of protein (Shekhar *et al*., 2018).

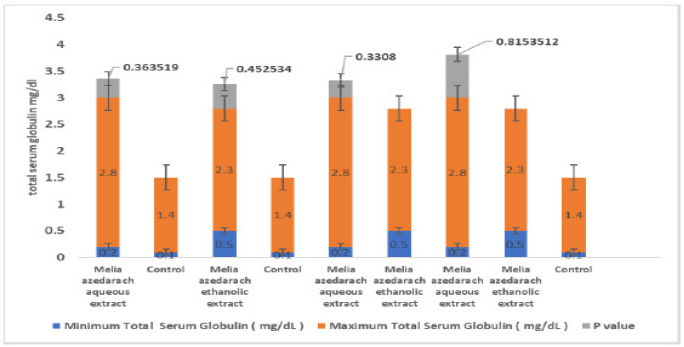

Figure 5: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in total serum globulin.

As shown in Table 3 and Figure 6, the mean of total serum cortisol was (85.3333±6.56591 mg/dL) in white rabbit administered aqueous extract of Melia azedarach; (73.25±13.20590 mg/dL) in white rabbit administered ethanolic extract, (86.6667±10.47749 mg/dL) among control group. No significant variation was reported between groups (aqueous extract vs control, p value =0.4596); ethanolic extract vs control, *P* value = 0.24383), aqueous extract vs ethanolic group (*P* value = 0.24919); aqueous extract, ethanolic vs control, (*P* value = 0.65095). However, there is some evidence that *Melia azedarach* have anti-inflammatory and analgesic properties by activation of central mechanisms also through peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in pain which could potentially affect cortisol levels due to the activity of flavonoids, glycosides, steroids and tannins in extract (Bibi *et al*., 2010; Vekariya *et al*., 2016).

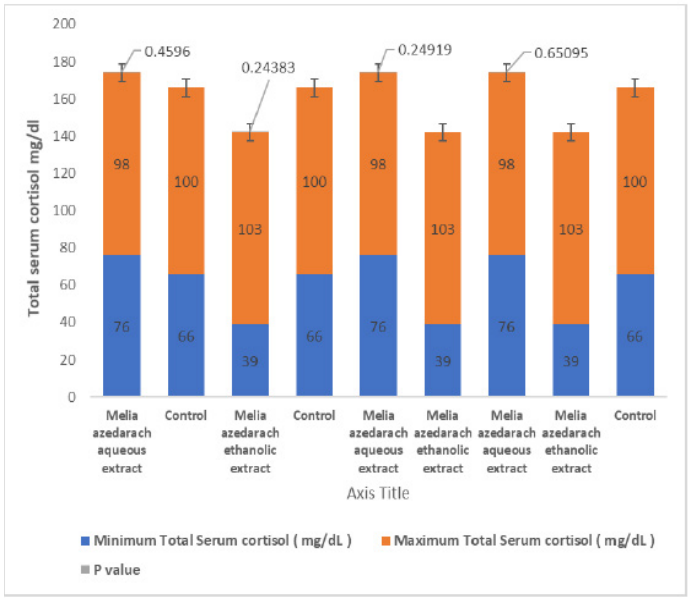

Figure 6: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in total serum cortisol.

As shown in Table 4 and Figure 7, the aqueous extract has superior activity than ethanolic in maintaining of total serum bilirubin level. The mean serum total Serum Bilirubin was (0.6000±0.05774 mg/dL) in white rabbit administered aqueous extract of *Melia azedarach*; (0.5750±10308 mg/dL) in white rabbit administered ethanolic extract, (0.400±.05774 mg/dL) among control group. Significant variation was reported between groups (aqueous extract

vs control (p value=0.0352). No significant variation was reported between groups (ethanolic extract vs control, *P* value =0.11986), aqueous extract vs ethanolic group (*P* value = 0.428177); aqueous extract, ethanolic vs control, (*P* value =0.279893). These results come in line with that reported by (Rajeswary *et al*., 2011; Rao *et al*., 2012; Soni *et al*. 2014; Al-Khafaji *et al*., 2019), which indicate the hepatoprotective effect for aqueous extract of the *Melia azedarach* at a dose 300-500 mg /day. Hepatoprotective effect of *Melia azedarach* may attributed in part to the effects of phenolic compounds which have antioxidant effects that inactivate lipid free radicals or prevent the decomposition of hydro peroxides into free radicals (Rao *et al*., 2012; Mendonça *et al*., 2022). Current results come in line with (Sumathi, 2013), who reported a reduction of serum bilirubin after treatment with 100-200mg/kg ethyl acetate extract of leaves of *Melia azedarach* indicating a hepatoprotective effects

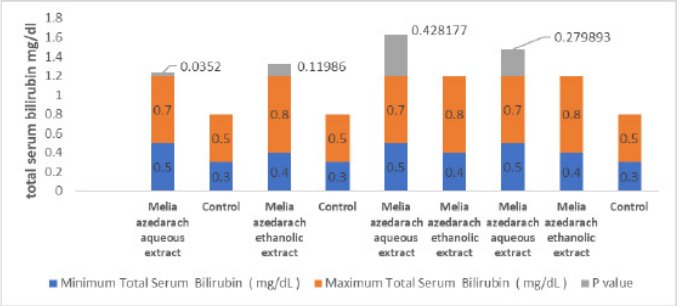

Figure 7: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in total serum Bilirubin

As shown in Table 5 and Figure 8, the mean of serum ALT was (52±2.51661mg/dL) in white rabbit administered aqueous extract of *Melia azedarach*; (52.33±8.11035mg/dL) in white rabbit administered ethanolic extract, (52.33±8.11035mg/dL) among control group. No significant variation was reported between groups (aqueous extract vs control, p value= 0.48528; ethanolic extract vs control, P value =0.3458092), aqueous extract vs ethanolic group (P value = 0.3192); aqueous extract, ethanolic vs control, (P value =0.8394).

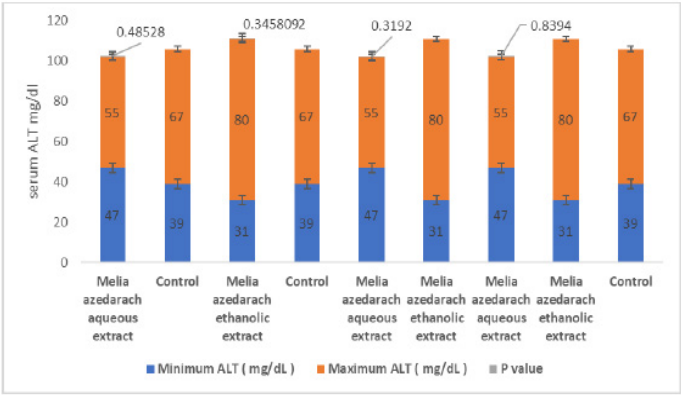

Figure 8: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in serum ALT Level.

As shown in Table 5 and Figure 9, The mean of serum AST was (41.3333±8.19214 mg/dL) in white rabbit administered aqueous extract of Melia azedarach; (40 ±6.79461 mg/dL) in white rabbit administered ethanolic extract, (37±10.39765 mg/dL) among control group. No significant variation was reported between groups (aqueous extract vs control, *P* value = 0.388787; ethanolic extract vs control, *P* value =0.41539), aqueous extract vs ethanolic group (p value= 0.452242); aqueous extract, ethanolic vs control, (*P* value =0.947215). As shown in Table 5 and Figure 10, the mean of serum ALP was (59±14.73092mg/dL) in white rabbit administered aqueous extract of *Melia azedarach*; (64.2500±5.40640mg/dL) in white rabbit administered ethanolic extract, (102±25.79406mg/dL) among control group. No significant variation was reported between groups (aqueous extract vs control, *P* value = 0.110; ethanolic extract vs control, *P* value =0.077183), aqueous extract vs ethanolic group (*P* value = 0.36044); aqueous extract, ethanolic vs control, (*P* value =0.19149197).

Table 4: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in Total Serum Bilirubin.

Parameter	Experimental groups	Minimum total serum bilirubin (mg/dL)	Maximum total serum bilirubin (mg/dL)	Mean± SE total serum bilirubin (mg/dL)	P value
Total serum bilirubin (mg/dL)	*Melia azedarach* aqueous extract	0.50	0.70	0.6000±0.05774	0.0352
	Control	0.3	0.5	0.400±.05774	
	Melia azedarach ethanolic extract	0.40	0.80	0.5750±10308	0.11986
	Control	0.3	0.5	0.400±.05774	
	Melia azedarach aqueous extract	0.50	0.70	0.6000±0.05774	0.428177
	Melia azedarach ethanolic extract	0.40	0.80	0.5750±10308	
	Melia azedarach aqueous extract	0.50	0.70	0.6000±0.05774	0.279893
	Melia azedarach ethanolic extract	0.40	0.80	0.5750±10308	
	Control	0.3	0.5	0.400±.05774	

Table 5: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in liver enzymes.

Parameter	Experimental groups	Minimum ALT (mg/dL)	Maximum ALT (mg/dL)	Mean± SE ALT (mg/dL)	P value
ALT (mg/dL)	*Melia azedarach* aqueous extract	47	55	52±2.51661	0.48528
	Control	39	67	52.33±8.11035	
	Melia azedarach ethanolic extract	31	80	58.25±10.41933	0.3458092
	Control	39	67	52.33±8.11035	
	Melia azedarach aqueous extract	47	55	52±2.51661	0.3192
	Melia azedarach ethanolic extract	31	80	58.25±10.41933	
	Melia azedarach aqueous extract	47	55	52±2.51661	0.8394
	Melia azedarach ethanolic extract	31	80	58.25±10.41933	
	Control	39	67	52.33±8.11035	
AST (mg/dL)	**Experimental groups**	**Minimum AST (mg/dL)**	**Maximum AST (mg/dL)**	**Mean± SE AST (mg/dL)**	**P value**
	Melia azedarach aqueous extract	26	54	41.3333±8.19214	0.388787
	Control	25	58	37±10.39765	
	Melia azedarach ethanolic extract	25	58	40 ±6.79461	0.41539
	Control	25	58	37±10.39765	
	Melia azedarach aqueous extract	26	54	41.3333±8.19214	0.452242
	Melia azedarach ethanolic extract	25	58	40 ±6.79461	
	Melia azedarach aqueous extract	26	54	41.3333±8.19214	0.947215
	Melia azedarach ethanolic extract	25	58	40 ±6.79461	
	Control	25	58	37±10.39765	
ALP (mg/dL)	**Experimental groups**	**Minimum ALP (mg/dL)**	**Maximum ALP (mg/dL)**	**Mean± SE ALP (mg/dL)**	**P value**
	Melia azedarach aqueous extract	34	85	59±14.73092	0.110
	Control	66	152	102±25.79406	
	Melia azedarach ethanolic extract	53	78	64.2500±5.40640	0.077183
	Control	66	152	102±25.79406	
	Melia azedarach aqueous extract	34	85	59±14.73092	0.36044
	Melia azedarach ethanolic extract	53	78	64.2500±5.40640	
	Melia azedarach aqueous extract	34	85	59±14.73092	0.19149197
	Melia azedarach ethanolic extract	53	78	64.2500±5.40640	
	Control	66	152	102±25.79406	

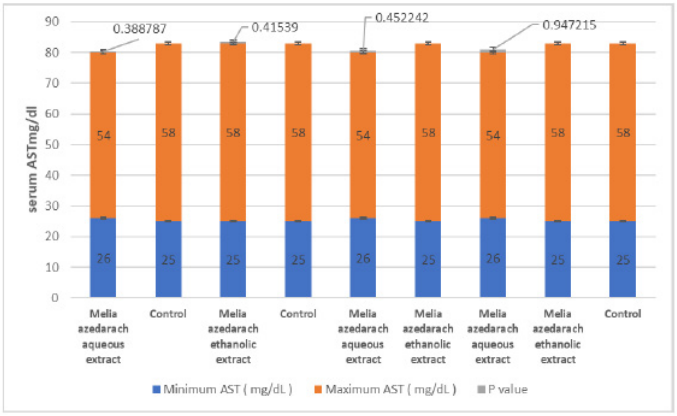

Figure 9: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in serum AST Level.

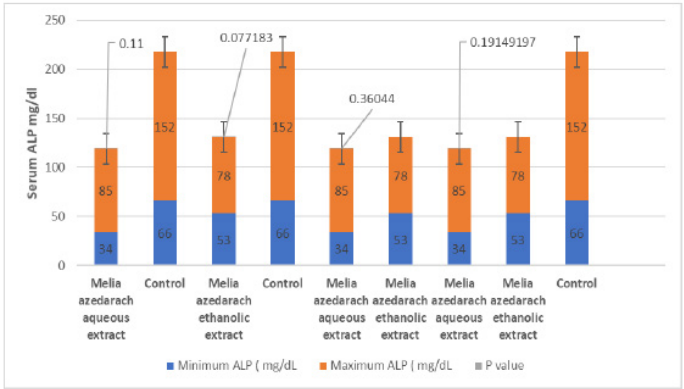

Figure 10: Effectiveness of aqueous versus alcoholic extracts of *Melia azedarach* in serum ALP level.

In current study, both aqueous extract and ethanolic extract of Melia azedarach have equal activity in keeping of the serum level of ALT, AST, ALP, close to normal value. In general, no significant variation was reported in ALT between groups (aqueous extract vs control; ethanolic extract vs control, aqueous extract vs ethanolic group; aqueous extract, ethanolic vs control. These results come in line with that reported by (Ke and Roma, 2005; Rajeswary *et al*., 2011; Rao *et al*., 2012; Al-Khafaji *et al*., 2019), which indicate the equivalent hepatoprotective effect for ethanolic and aqueous extract of the *Melia azedarach* as the level of enzymes in extra hepatic environment was reduced (Sen and Batra, 2012) which was confirmed in PhD thesis project by (Kale, 2013). Moreover, demonstrating their hepatoprotective action, *Melia azedarach* ethanolic extracts (300 mg/kg and 500 mg/kg, P.O.) inhibit the histological alterations of the liver. The protective effect may attributed to the activity of Catechins which are one of the phytochemical content of *Melia azedarach* may be responsible for lowering the levels of (ALT and AST) in treated animals (Nkosi *et al*., 2005). The antioxidant properties of phenolic compounds may be responsible for the protective benefits (Rao *et al*., 2012). Similar results were reported by (Soni *et al*., 2014), stated that ALT, AST, ALP remain at normal level after treatment with *Melia azedarach* at a dose (300-500mg/kg). Current results come in line with (Sumathi, 2013), who reported a reduction of serum ALT, AST, ALP to normal levels after treatment with 100-200mg/kg ethyl acetate extract of leaves of Melia azedarach indicating a hepatoprotective effects. Usually, the difference in antioxidant activity attributed to the total concentration of phenolic compounds which in turn depends on the type of solvent used for extraction of phenolic compounds (Sen and Batra, 2012; Shi *et al*., 2022). In current study, it is appeared to be that both ethanol and aqueous extraction methods have the same efficacy in extraction of phenolic compounds from *Melia azedarach*.

CONCLUSIONS AND RECOMMENDATIONS

Ethanolic extract of *Melia azedarach* at 28mg/day dose has significant effects on serum cholesterol level, total serum albumin. Aqueous extract of *Melia azedarach* at 28mg/day dose has significant effects on total serum bilirubin. Ethanolic and aqueous extracts of *Melia azedarach* have equal effects on ALT, AST, ALP enzymes and innate inflammatory indices for white New Zealand rabbits.

Limitation of current study

In current experimental study several limitations that could play a role in discrepancy of obtaining results such as, using of only two fractions (crude ethanolic fraction, aqueous fraction of *Melia azedarach*), shortening of time for clinical investigation, 14 days, using of low dose of 28mg/kg compared with high doses that used in another studies.

Recommendations

To overcome limitation of current study ,and to eliminate any source of bias and for comprehensive evaluation for the clinical usefulness of *Melia azedarach* it is recommended to use different doses, different exposure time, different solvents (n-hexane, chloroform, ethyl acetate, butanol) for further fractionation of ethanolic extract of *Melia azedarach* and using of these fractions for evaluation the anti-hyperlipidemic ,hepatoprotective and renal protective effects of *Melia azedarach*. Future studies to evaluate the effect of different fractions of *Melia azedarach* on cardiac and pulmonary physiological functions.

ACKNOWLEDGMENT

Authors express an appreciated acknowledgement to the department of pathology, college of veterinary medicine, university of Diyala for support.

NOVELTY STATEMENT

The novelty of current study is determination of the effectiveness of aqueous versus alcoholic extracts of Melia azedarach in amelioration of lipid profile, liver enzymes and innate inflammatory indices for white New Zealand rabbits.

AUTHOR'S CONTRIBUTION

All authors are equally contributed in planning, writing a draft and final manuscript, experimental design and laboratory work, statistical analysis.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Afkhami-Ardekani O, Esmailidehaj M, Afkhami-Ardekani M, Esmaeili H, Afkhami-Ardekani A (2017). *Melia azedarach* L. fruit extract effect on plasma lipid profile and cardiac and hepatic functions of diabetic rats. Iran. J. Diabetes Obesity, 9(4): 155-163.

Agustina R (2009). Efek pemberian ekstrak etanol 70% daging buah jambu biji (*Psidium guajava* L.) bagian dalam terhadap kadar kolesterol dalam serum darah tikus putih jantan wistar ratus norvegicus, universitas muhammadiyah surakarta.

Ahmed MF, Rao AS, Thayyil H, Ahemad SR, Ibrahim M (2011). Role of *Melia azedarach* leaf extract in Paracetamol induced Hepatic damage in rats. Pharmacogn. J., 3(21): 60-64.

https://doi.org/10.5530/pj.2011.21.10

Ahmed MF, Rao AS, Ahemad SR, Ibrahim M (2012). Phytochemical studies and antioxidant activity of *Melia azedarach* Linn leaves by DPPH scavenging assay. Int. J. Pharm. Appl., 3(1): 271-276.

Akacha M., Lahbib K, Remadi MD (2016). Antibacterial, antifungal and anti-inflammatory activities of *Melia azedarach* ethanolic leaf extract. Bangladesh J. Pharmacol., 11(3): 666-674. https://doi.org/10.3329/bjp.v11i3.27000

Al-Ezzy AIA (2015). Evaluation of clinicopathological and risk factors for nonmalignant H. pylori associated gastroduodenal disorders in Iraqi patients. Open Access Macedon. J. Med. Sci., 3: 645. https://doi.org/10.3889/oamjms.2015.113

Al-Ezzy AIA (2016). In situ nick end labeling as a molecular immunopathological indicator for the severity of DNA fragmentationand gastroduodenal tissue damage among H. Pylori Cag A positive patients. Indian J. Sci. Technol., 9. https://doi.org/10.17485/ijst/2016/v9i2/78512

Al-Ezzy AIA (2016). The accuracy of elisa versus latex agglutination tests in diagnosis of rotavirus acute gastroenteritis and the clinical usefulness of C-reactive protein in Iraqi children. South East Eur. J. Immunol., 2016: 1-5. https://doi.org/10.3889/seejim.2016.20008

Al-Ezzy AIA, Al-Khalidi AAH, Hameed MS (2020). Evaluation of C-reactive protein in Iraqi children presented with acute enteropathogenic *Escherichia coli* associated diarrhea with special emphasis to age and gender. Gazi Med. J., 31: 143-148. https://doi.org/10.12996/gmj.2020.38

Al-Ezzy AIA, Hameed MS, Jalil WI (2016). Pathophysiological effects of vitamin C and E- selenium combination on lipid profile and serum glucose of experimentally induced sodium nitrate intoxication in mice. Res. J. Pharm. Biol. Chem. Sci., 7(2).

Al-Khafaji MN, Shlash EY, Nayef AI, Shaker NS (2019). Protective effect of aqueous–methanol extract of melia azedarach against paracetamol–induced hepatitis in rabbits. Diyala J. For. Pure Sci., 15(04). https://doi.org/10.24237/djps.15.04.500D

Al-Khafaji NJ, Al-Zubaedi RM, Al-Azawi SJ (2016). Evaluation of antibacterial effects of Melia azedarach fruit extracts against some isolated pathogenic bacteria. Vet. Sci. Dev., 6(1). https://doi.org/10.4081/vsd.2015.6080

Bibi Y, Nisa S, Waheed A, Zia M, Sarwar S, Ahmed S, Chaudhary MF (2010). Evaluation of viburnum foetens for anticancer and antibacterial potential and phytochemical analysis. Afr. J. Biotechnol., 9(34): 5611.

Carpinella MC, Miranda M, Almirón WR, Ferrayoli CG, Almeida FL, Palacios SM (2007). *In vitro* pediculicidal and ovicidal activity of an extract and oil from fruits of *Melia azedarach* L. J. Am. Acad. Dermatol., 56(2): 250-256. https://doi.org/10.1016/j.jaad.2006.10.027

Chaachouay N, Zidane L (2024). Plant-derived natural products: A source for drug discovery and development. Drugs Drug Candidates, 3(1): 184-207. https://doi.org/10.3390/ddc3010011

Davies MJ, Judd JT, Baer DJ, Clevidence BA, Paul DR, Edwards AJ, Wiseman SA, Muesing RA, Chen SC (2003). Black tea consumption reduces total and LDL cholesterol in mildly hypercholesterolemic adults. J. Nutr., 133(10): 3298S-3302S. https://doi.org/10.1093/jn/133.10.3298S

Dias DA, Urban S, Roessner U (2012). A historical overview of natural products in drug discovery. Metabolites, 2(2): 303-336. https://doi.org/10.3390/metabo2020303

Gondwal S, Rana A (2021). Ethnobotanical uses, pharmacological activities and phytochemistry of *Melia azedarach* Linn: A review. World J. Pharma. Res., 10(2): 571-584.

Hameed MS, Al-Ezzy AIA (2019). Evaluation of possible stress factors affecting physiological level of gamma interferon during first six months of life in healthy calves. Adv. Anim. Vet. Sci., 7(5): 370-377. https://doi.org/10.17582/journal.aavs/2019/7.5.370.377

Hameed MS, Al-Ezzy AIA, Jalil WI, Al-Khalidi AAH (2020). Physiological protective effects of ascorbic acid versus Dl-A-tocopheryl acetate–sodium selenite combination in mice under experimental sodium nitrate intoxication. Biochem. Cell. Arch., 20(1): 2593-2601.

Herlina H, Untari B, Solihah I, Santia M (2019). Antihyperlipidemic activity of ethanol extract mindi's leaves (*Melia azedarach* Linn.) in male wistar rats induced propiltiouracil. Sci. Technol. Indonesia, 4(1): 24-30. https://doi.org/10.26554/sti.2019.4.1.24-30

Ilahi I, Qureshi IZ, Ahmad I (2014). Effects of fractions of *Melia azedarach* (L.) fruit extracts on some biochemical parameters in rabbits. Arch. Biol. Sci., 66(4): 1311-1319. https://doi.org/10.2298/ABS1404311I

Jamshidi-Kia F, Wibowo JP, Elachouri M, Masumi R, Salehifard-Jouneghani A, Abolhasanzadeh Z, Lorigooini Z (2020). Battle between plants as antioxidants with free radicals in human body. J. Herbmed Pharmacol., 9(3): 191-199. https://doi.org/10.34172/jhp.2020.25

Jazzar C, Hammad EAF (2003). The efficacy of enhanced aqueous extracts of Melia azedarach leaves and fruits integrated with the *Camptotylus reuteri* releases against the sweetpotato whitefly nymphs. Bull. Insectol., 56: 269-276.

Kale BP (2013). Comparison of antioxidant activity of azadirachta indica, ricinus commnius, eclipta alba, ascorbic acid (vitamin C) and Liv-52 in rabbits, an animal experimental study. Doctor of phylosophy (Medical Pharmacology), Datta Meghe Institute of Medical Sciences.

Ke R, Roma M (2005). Ricinus commnius pharmacognosy of indigenous drugs, Central council in Ayurvada and Siddha dehl: pp. 228-335.

Kumar SV, Sanghai DB, Rao CM, Shreedhara C (2013). Antioxidant and antihyperlipidemic activity of *Melia azedarach* Linn. extracts in rats. Res. J. Pharma. Technol., 6(11): 1195-1199.

Mendonça JDS, Guimarães RDCA, Zorgetto-Pinheiro VA, Fernandes CDP, Marcelino G, Bogo D, Freitas KDC, Hiane PA, de Pádua Melo ES, Vilela MLB (2022). Natural antioxidant evaluation: A review of detection methods. Molecules, 27(11): 3563. https://doi.org/10.3390/molecules27113563

Mohs RC, Greig NH (2017). Drug discovery and development: Role of basic biological research. Alzheimer's Dementia Transl. Res. Clin. Intervent., 3(4): 651-657. https://doi.org/10.1016/j.trci.2017.10.005

Najmi A, Javed SA, Al-Bratty M, Alhazmi HA (2022). Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. Molecules, 27(2): 349.

Nkosi C, Opoku A, Terblanche S (2005). Effect of pumpkin seed (*Cucurbita pepo*) protein isolate on the activity levels of certain plasma enzymes in CCl4-induced liver injury in low-protein fed rats. Phytother. Res. Int. J. Devoted Pharmacol. Toxicol. Eval. Natl. Prod. Derivat., 19(4): 341-345. https://doi.org/10.1002/ptr.1685

Oakenfull D, Fenwick DE, Hood R, Topping D, Illman R, Storer G (1979). Effects of saponins on bile acids and plasma lipids in the rat. Br. J. Nutr., 42(2): 209-216. https://doi.org/10.1079/BJN19790108

Rajeswary H, Vasuki R, Samudram P, Geetha A (2011). Hepatoprotective action of ethanolic extracts of *Melia azedarach* Linn. and *Piper longum* Linn and their combination on CCl 4 induced hepatotoxicity in rats.

Ralte L, Sailo H, Singh YT (2024). Ethnobotanical study of medicinal plants used by the indigenous community of the western region of Mizoram, India. J. Ethnobiol. Ethnomed., 20(1): 2. https://doi.org/10.1186/s13002-023-00642-z

Rao AS, Ahmed MF, Ibrahim M (2012). Hepatoprotective activity of *Melia azedarach* leaf extract against simvastatin induced Hepatotoxicity in rats. J. Appl. Pharma. Sci., 2(7): 144-148. https://doi.org/10.7324/JAPS.2012.2721

Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F (2020). Worldwide research trends on medicinal plants. Int. J. Environ. Res. Publ. Health, 17(10): 3376. https://doi.org/10.3390/ijerph17103376

Sen A, Batra A (2012). *In vivo* and *in vitro* comparative study of total phenol content and antioxidant activity of *Melia azedarach* L. J. Pharm. Res., 5(1): 47-50.

Shekhar S, Shula S, Bhatt P, Kumar M, Bisth D (2018). Comparative efficacy of melia azedarach extracts with amprolium against experimentally induced coccidiosis in broiler. Int. J. Curr. Microb. Appl. Sci., 7: 2656-2663. https://doi.org/10.20546/ijcmas.2018.704.303

Shi L, Zhao W, Yang Z, Subbiah V, Suleria HAR (2022). Extraction and characterization of phenolic compounds and their potential antioxidant activities. Environ. Sci. Pollut. Res., 29(54): 81112-81129. https://doi.org/10.1007/s11356-022-23337-6

Song M, Luo HJ, Li ZW, Qiu L, Zhao YX, He CW, Zhang XQ, Ye WC, Lin LG, Zhang QW (2023). Limonoids from the roots of *Melia azedarach* and their anti-inflammatory activity. Phytochemistry, 216: 113869. https://doi.org/10.1016/j.phytochem.2023.113869

Soni RK, Dixit V, Irchhaiya R, Alok S (2014). Potential herbal hepatoprotective plants: An overview. Int. J. Pharma. Life Sci., 5(3).

Sumathi A (2013). Investigation of hepatoprotective activity of *Melia azedarach* L. leaves. Pharma. Glob., 4(4): 1.

Tech M (2024). International LLC (2024), Sample size calculator.

Ullah N, Ullah B, Kaplan A, Dossou-Yovo HO, Wahab S, Iqbal M (2024). Quantitative ethnobotanical study of medicinal plants used by local communities in Chamla Valley, Buner District, Pakistan. Ethnobot. Res. Appl., 28: 1-22. https://doi.org/10.32859/era.28.38.1-22

Vekariya S, Nishteswar K, Patel BR, Nariya M (2016). Evaluation of analgesic and antipyretic activities of Mahanimba (*Melia azedarach* Linn.) leaf and root powder. Ayu 37: 140. https://doi.org/10.4103/ayu.AYU_69_15

Wajdy J, Tayseer A, Dheyaa K (2021). Constituents, pharmacological and toxicological effects of Meliaazadirachta. A review, SMJ.