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HEPATOPROTECTIVE EFFECTS OF AQUEOUS AND METHANOLIC LEAVES EXTRACTS OF *AZADIRACTA INDICA* AGAINST CARBON TETRACHLORIDE (CCL_4) INDUCED HEPATOTOXICITY IN WISTAR RATS

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Abstract

The hepatoprotective effects of aqueous and methanolic leaf extracts of *Azadirachta indica* on carbon tetrachloride (CCl_4) induced liver damage was determined in this study. A total of thirty-five (35) wistar rats were divided into seven (7) groups, each containing five (5) rats. The modified procedure for inducing hepatotoxicity by carbon tetrachloride was used, all Wister rats in Group I (Control) were administered with 1 ml/kg body weight dosage of liquid paraffin daily from the first to the fifth day. However, from the second to the fifth day, all wister rats in Group II (Induced Control), Group III (Test group), Group IV (Test group), Group V (Test group), and Group VI (Test group) were administered with 1 ml/kg body weight of liquid paraffin containing 40% carbon tetrachloride, 1 ml/kg body weight of CCl_4 and a 50 mg/kg body weight daily oral dose of aqueous leaf extract, 1 ml/kg body weight of CCl_4 and 150 mg/kg body weight of aqueous leaf extract (orally), 1 ml/kg body weight of CCl_4 and a daily oral dose of 50 mg/kg body weight of methanol leaf extract, 1 ml/kg body weight of CCl_4 and 150 mg/kg body weight of methanolic leaf extract orally, 1 ml/kg body weight of CCl_4 and an oral dose of 100 mg/kg body weight of Silymarin respectively, with CCl_4 injected intraperitoneally. Hepatoprotective effects of aqueous and methanolic leaf extracts of *A. indica* were determined by evaluating the liver function enzymes namely; Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total bilirubin (T. bil) and Alkaline phosphatase (ALP). Results showed weight gain in groups (I, III, IV, V, VI, and VII) and weight loss in group II. Significant ($p < 0.05$) increase in levels of AST, ALT, ALP, and T.BIL in CCl_4-intoxicated rats were restored through normalization in aqueous and methanolic extracts' treated rats in a dose-dependent manner. The aqueous extracts (150mg/kg body weight), methanolic leaf extracts (150mg/kg body weight) and silymarin (100mg/kg) showed potential hepatoprotective effects with a significant decrease ($p < 0.05$). The study suggests that aqueous and methanolic leaf treatments of *A. indica* has shown to be a potentially good hepatoprotective medicinal plant at a higher dose of 150mg/kg body weight.

Keywords: *Azadiracta indica*, Hepatoprotective, Hepatotoxicity, CCL_4-induced, Leaves extracts

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1.0 INTRODUCTION

The liver is a key component of metabolism and excretion. Due to its special characteristics, the liver is a significant target for the toxicity of xenobiotics and oxidative stress (Huo *et al*., 2011). According to Alshawsh *et al.* (2011), the most frequent causes of liver injury in developed nations include excessive alcohol use, viral infections, environmental pollutants, liver viruses, parasite infections, and chemotherapy. Liver disease is one of the biggest health hazards in the world, characterized by the liver's impaired metabolic and secretory function in the form of cirrhosis, jaundice, liver cancer, hepatitis, and finally liver failure (Gayol *et al.,* 2012). According to Sharma and Sharma (2010), liver disorders cause about 20,000 fatalities annually. With more than 250,000 new cases each year, among the top 10 most prevalent cancers worldwide is hepatocellular carcinoma (Meganatha *et al.,* 2011).

Certain natural substances, like plant-based and conventional herbal remedies, can shield the liver from hepatotoxin-induced harm. Worldwide sales of hepatoprotective herbal medications for commercial usage have exceeded 600 (Girish and Pradhan, 2012). There have been reports of hepatoprotective action for over 170 phytoconstituents that were extracted from 110 plants across 55 families. Nonetheless, the safety and efficacy of a tiny portion of the hepatoprotective herbal formulations and remedies utilized in traditional medicine have been pharmacologically evaluated (Girish and Pradhan, 2012). The primary reasons of the pathophysiology of liver injury includeglutathione depletion, oxidative stress, DNA damage, lipid peroxidation, participation of a fatal agent, and bio-activation of free radicals that trigger an immune response (Bedi *et al.,* 2016).

In the assessment and treatment of individuals with hepatic dysfunction, a number of biochemical tests are helpful (Shivaraj *et al*., 2009). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), serum bilirubin, albumin and total protein are routinely measured while assessing liver function. The elevation pattern can help in the differential diagnosis, and these examinations can help determine liver disease (Lala *et al.,* 2021).

Azadirachta indica generally known as India neem or margosa tree has long been used widely in homoeopathic, unani, and Ayurvedic treatment. "Nimba" (Sitasiwi *et al*., 2018) is the Sanskrit term for "good health." Over time, this term evolved into "Neem," and the tree is also known as "Sarvaroganivarini," which means "cure all ailments". Further, neem also possesses compound s that act as immunomodulatory, anti-inflammatory, antidiabetic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antimutagenic as well as anticancer (Saleem *et al.,* 2018).

According to Agada *et al.* (2020), liver involvements in addition to other health challenges are the major causes of mortality worldwide, ranking as the second top cause of mortality, accounting for over 2 million deaths annually. Chronic illnesses claim the lives of almost 35 million people annually as WHO estimates that chronic liver disorders account for 46% of all global diseases and 59% of all deaths worldwide (Bose *et al*., 2021).

The absence of precise treatment plans for liver ailments has resulted to a deteriorating situation, long-term use of Silymarin is hindered by systemic toxicity and current liver disease treatments are ineffective. Since the toxicity factor is minimal, medicinal plants have been utilized in traditional medicine for treating liver problems for centuries (Ghosh *et al*., 2011). There are numerous medicinal plants that can be utilized to treat liver disorders as

there are no reliable liver protective drugs available currently (Sanmugapriya and Venkataraman, 2006).
Therefore, *A. indica* has been chosen as a candidate plant in this study to evaluate the aqueous extract as well as methanolic leaf extract of the medicinal plant for the management of liver damage induced by CCL_4.

2.0 MATERIALS AND METHODS

Experimental Animals

Thirty-five (35) wistar rats (Wister strains) weighing between 120 and 250g were acquired from the College of Agriculture and Animal Science in Mando, Kaduna State. Throughout the two-week acclimatization period, rats were accommodated in a cage made of wire mesh, placed in a well-ventilated room with free access to water source as well as food supply. Rats were fed regular animal feed (Chikun feeds), which were produced by Crown Flour Mill in Kaduna State, as well as unlimited access to clean tap water. Ethics for the examination of experimental pain in conscious animals were followed during the experiment's execution (Zimmerman, 1983). Oral delivery of the extracts was accomplished using a normal rat feeding cannula.

Preparation of Plant Extracts

The collected *Azadirachta indica* leaves were cleaned, washed twice with tap water and air-dried under shade for a week. The dried leaves were pulverized into power form using mortar and pestle, followed by an electrical blender (Fadar plus FD-998).

Aqueous extract was prepared by measuring two hundred and fifty grams (250 g) of the powdered leaves using an electronic scale (G&G T1000), this was placed in a clean container and soaked in two (2l) liters of distilled water. Similarly, methanolic extract was also prepared by measuring two hundred and fifty grams (250 g) of the powdered leaves which was soaked in two (2l) liters of methanol. Both containers with the contents were shaken for 20 minutes, sealed and stored for 72 hours at room temperature. Whatman filter paper (No.1 Bibby RE 200, Sterilin Ltd, UK) was used to filter the mixtures and then the filtrates were concentrated using rotary evaporator (RE-52A PEC Medical USA) at 45ºC and water bath (HH-S6 PEC Medical USA) at 40ºC (Olaniyan *et al.,* 2016).

The yield of the extract was calculated as follows:

Percentage (%) yield = $\frac{(W_2 - W_1)\ X\ 100}{W_0}$

Where W_2 represents the weight of the extract and container, W_1 represents the weight of the empty container and W_0 represents the weight of the initial dried leaves (Anokwuru *et al.*, 2011).

Hepatoprotective Activity

A total of thirty-five (35) wistar rats were divided into seven (7) groups, each containing five (5) rats, at random. The procedure used to induce hepatotoxicity by carbon tetrachloride was modified from the methods described by Guntupalli (2006).

Group I Control: From the first to the fifth day, a daily oral dose of liquid paraffin (1 ml/kg body weight) was given.

Group II Induced Control: From the second to the fifth day, an intraperitoneal injection of liquid paraffin containing 40% carbon tetrachloride (1 ml/kg body weight) was administered.

Group III Test group: from the second to the fifth day, an intraperitoneal injection of CCl_4 (1 ml/kg body weight) and a daily oral dose of aqueous leaf extract in the form of aqueous suspension (50 mg/kg body weight) were administered.

Group IV Test group received an intraperitoneal injection of CCl_4 (1 ml/kg body weight) and 150 mg/kg body weight of aqueous leaf extract (orally) every day from the second to the fifth day in the form of an aqueous solution.

Group V Test group: From the second to the fifth day, CCl_4 (1 ml/kg body weight, intraperitoneal injection) and a daily dose of methanol leaf extract (50 mg/kg body weight, orally) in the form of an aqueous suspension were given.

Group VI Test group received an intraperitoneal injection of CCl_4 (1 ml/kg body weight) and 150 mg/kg body weight of methanolic leaf extract orally every day in the form of an aqueous suspension from the second to the fifth day.
Group VII: From the second to the fifth day, an oral dose of Silymarin (100 mg/kg), a known hepatoprotective drug was administered along with an intraperitoneal injection of CCl_4 (1 ml/kg body weight).

Following a 14-day course of therapy, all the animals were weighed and, 48 hours following the final injection, they were killed under chloroform anesthesia. Samples of blood were taken by heart puncture into plain sample tubes, allowed to clot for half an hour, and then spun using a bench centrifuge for five minutes at 3000 revolutions per minute at 37°C. Before being used for biochemical analyses of liver enzymes, including total bilirubin, alanine aminotransferases (ALT), aspartate aminotransferases (AST), and alkaline phosphatases (ALP), the supernatant (serum) was kept at -20 °C in a refrigerator (Ali *et al.,* 2013). Serum level of total bilirubin, ALT, AST, and ALP were determined using diagnostic kits (Human Diagnostic, Germanity), following the user manual for diliution, the samples were analyzed in a Biochemistry Analyzer (Humalyzer 3000, USA) (Ali *et al.,* 2013).

Data Analysis
The statistical software for social sciences (version 23.0, SPSS Inc., Chicago, USA) was utilized for the analysis of results. ANOVA, or one-way analysis of variance, was employed. The findings were presented as Mean ± SEM with a 95% confidence limit.

3.0 RESULTS

The result of the weight of wistar rats before the induction, after the induction and administration of extracts, and the hepatoprotective effects of *A. indica* leaves extract on CCL_4 treated wistar rats are shown in Tables 1 and 2 respectively.

The body weight of wistar rats in all groups were measured before induction and after treatments. The wistar rats induced with carbon tetrachloride (CCl_4) without treatment (group 2) showed a decrease in body weight after 14 days of induction. However, rats induced with CCl_4 and treated with 50mg/kg, 150mg/kg methanol extract, 50mg/kg, 150mg/kg aqueous extract and 100mg/kg of silymarin (groups 3,4,5,6 and 7) revealed increased body weight after the 14 days of treatment. After receiving treatments for 14 days, the group of Wistar rats thatwere not induced or treated (group 1) gained the most body weight (Table 1).

Total bilirubin (T.Bil), alkaline phosphatase (ALP), aspartate aminotransferases (AST), and alanine aminotransferases (ALT) were the liver enzymes used to assess the hepatoprotective effects of aqueous and methanolic extracts. When compared to the enzyme activities of normal control groups, wistar rats fed with 40% (v/v) CCl_4 in liquid paraffin demonstrated significant ($p < 0.05$) increases in the levelsof ALT, AST, ALP, and T.bil. (Table 2). When compared to CCl_4-intoxicated groups, oral administration of *A. indica* aqueous extract at doses of 50 mg/kg and 150 mg/kg resulted in a significant ($p < 0.05$) drop in serum enzyme activity levels. The serum enzyme level is

likewise reduced by *A. indica* methanolic extract at the same dose of 50 mg/kg and 150 mg/kg during CCl_4 intoxication. Sulymarin, a known hepatoprotective drug at 100 mg/kg dosage demonstrated a significant ($p<0.05$) decrease in serum enzyme levels of ALT, AST, ALP, and T.BIL. However, from the results obtained, oral administration of methanol and aqueous extracts of *A. indica* at 150mg/kg dosage and 100mg/kg dosage of silymarin showed more hepatoprotective effects in reducingthe level of liver damage induced by carbon tetrachloride when compared to normal control animals.

Table 1 Mean Body weight of Wistar Rats Before and After Induction and Administration of Extracts

Groups	Induction Treatment	Mean ± SEM of Initial Weight	Mean ± SEM of Final Weight	P-Value
I	Control: (1ml/Kg b.w) Paraffin	141.3± 4.78	181.0±9.10	39.6±4.35*
II	40% CCL_4 in liquid paraffin	166.0±2.47	130.0 ±2.77	- 36.0±1.83*
III	ALE(50 mg/kg b.w) + CCl_4	181.6± 1.33	216.0±8.26	34.4±7.01*
IV	ALE(150 mg/kg b.w) + CCl_4	207.6 ± 3.41	224.8±11.74	17.2±8.53
V	MLE(50 mg/kg b.w) + CCl_4	173.6±1.20	209.0 ±2.47	35.4±1.28*
VI	MLE(150 mg/kg b.w) + CCl_4	197.8±1.74	235.6±15.89	37.8±14.32*
VII	Silymarin (100 mg/kg b.w) + CCl_4	247.2 ±8.49	264.2±12.42	17.0±5.06

Data are expressed as Mean±S.E.M (n = 5).
* Significantly different ($P<0.05$) from Initial weight.
ALE: Aqueous leaf extract, MLE: Methanolic leaf extract, b.w: Body weight

Table 2 Effects of Aqueous and Methanolic Leaf Extracts of *Azadirachta Indica* (neem) on CCL_4 Induced Alterationin Liver Function of Wistar rats.

Groups	Treatment	ALT(U/L)	AST(U/L)	ALP(U/L)	T.BIL(mg/dL)
I	Control (1mg/Kg b.w) Paraffin	16.0±4.78	63.8±11.52	258.0±86.02	13.3±3.82
II	40% CCL_4 (Induced)	24.6±3.79*	99.2±5.91*	438.6±46.19*	38.0±2.70*
III	ALE (50 mg/kg b.w) + CCl_4	18.4±0.68**	85.2±3.97	391.8±45.47	13.2±1.24*
IV	ALE(150 mg/kg b.w) + CCl_4	19.0±1.87	70.2±3.58**	308.8±50.92	7.20±0.86**
V	MLE (50 mg/kg b.w) + CCl_4	17.8±3.398**	78.4±3.92*	229.6±59.56**	13.0±2.07*
VI	MLE(150 mg/kg b.w) + CCl_4	17.2±1.83**	60.4±7.3**	221.4±33.67**	7.20±1.46**
VII	Silymarin (100 mg/kg b.w) + CCl_4	18.2±1.16**	73.6±6.23**	262.0±23.99**	8.60±0.68**

Data are expressed as Mean±S.E.M (n = 5).
* Significantly different ($P<0.05$) from Control group I.
** Significantly different ($P<0.05$) from CCl_4 group II for Duncan's post hoc test.
AST: Aspartate transaminase, ALP: Alkaline phosphatase, ALT: Alanine transaminase, T.BIL: Total bilirubin, ALE: Aqueous leave extract, MLE: Methanolic leave extract, b.w: Body weight.

4.0 DISCUSSION

The body weight changes of wistar rats before and after treatment appeared to summarize the overall effect of extracts and drug on all treated wister rats. The significant loss of weight in CCl_4-induced wistar rats could be attributed to the mechanism of action of trichloromethyl free radicals (CCl_3) and trichloromethyl peroxide radical (CCl_3OO^*), while the groups with

significant weight gain observed in wistar rats treated with aqueous and methanol extract of *A. indica* or silymarin reflects the inhibitory effect of *A. indica* leaves on oxidative stress. This is in agreement with Dineshkumar *et al.* (2013) that reported significant weight loss in CCl_4 treated rats. Report from Nwobodo *et al*. (2018) also revealed weight loss in paracetamol treated wistar rats. The fall in weight of body could be due to reduced water intake and a loss of appetite after intraperitoneal administration of CCl_4. This agrees with the work of Dikwa *et al.* (2021) that loss in body weight recorded in infected but not treated and infected but treated mice compared to normal control mice could be due to a resultant decrease in food intake resulting from loss of appetite, increased metabolic rate, and feed conversion efficiency. Also, Hassan *et al.* (2010) reported that weight loss may result from a decrease in thirst and appetite following the extract's consumption.

In comparison to the CCl_4-induced group, wistar rats' hepatic parameters; AST, ALT, ALP, and T.Bil, were found to be less affected by aqueous and methanolic extracts at dose levels of 150 mg/kg body weight and silymarin at 100 mg/kg. The free radical CCl_3, a metabolite of CCl_4 alkalyzes proteins and other macromolecules while simultaneously attacking polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxide, which damages the liver (Sanmugapriya and Venkataraman, 2006).

As a result of the liver's release of enzymes into the bloodstream, hepatocellular necrosis causes the serum marker enzymes to rise (Ashok *et al*, 2002). Increased AST, ALT, ALP, and T.Bil levels are recognized indicators of liver damage (Achiliya *et al*., 2004). This is in accordance with the previously published research on the hepatoprotective qualities of *A. indica* leaf, and is consistent with the work of iivani *et al*. (2009).

The elevation of the liver marker enzymes, AST, ALP, T.Bil and ALT, that was seen in CCl_4 induced but not treated rats (Group 2), is congruent with the results of Guntupali *et al.* (2006) and Dineshkumar *et al.* (2013) on heptotoxic effects of CCL_4. Oral administration of aqueous and methanolic extracts at a high dose of 150mg/kg showed effective hepatoprotection which was nearly identical to that obtained from the silymarin therapy (100mg/kg). This agrees with the work of Kalaivani *et al.* (2009) that observed maximum hepatoprotective effects at a higher dose of 500mg/kg.

5.0 CONCLUSION

The CCl_4 was found to cause injury to the liver in wistar rats at a 40% concentration in liquid paraffin. Such chronic liver damage was observed in groups of wistar rats administered with CCl_4 without treatments compared to groups of wistar rats administered with aqueous extract, methanolic extract, and silymarin.

Changes in the weight of wistar rats of various treatment groups were observed after induction and administration of extracts. Weight loss was observed in wistar rats induced with 40% CCl_4 without treatment compared to induced and treated wistar rats, which showed an increase in body weight.

The liver function enzymes, such as ALT, AST, ALP, and T.biI, were found to be elevated in wistar rats administered with CCl_4. When compared to a normal control, treatment with 150 mg/kg of *Azadirachta indica* leaf extract in aqueous and methanolic forms and 100 mg/kg of silymarin had a greater hepatoprotective impact in lowering the levels of liver function enzymes in wistar rats.

5.1 RECOMMENDATION

There is need to further purify and study the mechanism of action of *Azadirachta indica* leaf extract as a promising hepatoprotective medicinal plant.

5.2 CONFLICTS OF INTEREST

No conflicts of interest.

5.3 ACKNOWLEDGMENTS

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5.4 AUTHORS CONTRIBUTION

DKB, SGA, AAI, VPA and NCT conceptualized and designed the study, SGA participated in fieldwork and data collection, performed the data analysis and interpreted the data. DKB, SGA, AAI, VPA and NCT participated in the preparation of the first draft of the manuscript, DKB, SGA, AAI, VPA, NCT and MM reviewed it. All authors contributed to the development of the final manuscript and approved its submission.

REFERENCES

Achiliya, G. S., Wadodkar, S.O. and Dorle, A. K. (2004). Evaluation of Hepatoprotective Effect of *Amakadi Ghrita* Against Carbon Tetrachloride Induced Hepatic Damage in Rats, *Journal of Ethnopharmacology* 90:229-232.

Agada, S. A., Odama, R. I., Kenechukwu, C. O., Okang, S. O. and Ezeh, C. O. (2020). Epidemiology of Chronic Liver Disease in Nigeria: A Review. *Asian Journal of Advances in Medical Science* ,2(1): 1-6. .

Ali, M., Qadir, M.I., Saleem, M., Janbaz, K.H., Gul, H., Hussain, L. and Ahmad, B. (2013). Hepatoprotective Potential of *Convolvulus Arvensis* Against Paracentamol-Induced Hepatotoxicity. *Bangladesh Journal of Pharmacology;* 8: 4-300.

Alshawsh, M.A., Abdulla, M.A., Ismail, S. and Amin, Z.A. (2011). Hepatoprotective effect of *Orthosiphon stamineus* extract on thioacetamide-induced liver cirrhosis in rats. *Evidence Based Complementary and Alternative Medecine*.doi:10.1155/2011/103039.

Anokwuru, C.P., Anyasor, G.N., Ajibaye, O., Fakoya, O., Okebugwu, P. (2011). Effect of Extraction Solvents on Phenolic, Flavoinoid, and Antioxidant Activities of Three Nigerian Medicinal Plants. *Nature and Science*, 9(7), 53-61.

Ashok, S. K., Somayaji, S.N., and Bairy, K.L. (2002). Evaluation of Hepatoprotective Activity of *Gingo biloba* in Rats*, Indian Journal of Pharmacology*. 46(2), 167-174.

Bedi, O., Bijjem, K.R.V., Kumar, P. and Gauttam, V. (2016). Herbal Induced Hepatoprotection and Hepatotoxicity: A Critical Review. *Indian Journal of Physiology and Pharmacology*. 60: 6-21.

Bose, S., Yashoda, R. and Puranik, M. P. (2021). Association Between Oral Health and Alcoholic Liver Disease-A Cross-Sectional Analytical Study. *Journal of Dentistry Defence Section*, 15(1), 5.

Dikwa, K. B., Dibal, D.M., Muazu, M. and Obaje, G. (2021). Antiplasmodial Activity of Methanolic Leaf Extract of *Daniella oliveri* in Albino Mice Infected with *Plasmodium berghei* Nk65. *Nigerian. Journal of Parasitology;* 42(2); 411-421.

Dineshkumar, G., Rajakumar, I. R., Mani, P. and Johnbastin, T.M.M. (2013). Hepatoprotective Activity of Leaves Extract of *Eichhornia crassipes* Against CCl_4 Induced Hepatotoxicity Albino Rats. *International Journal of Pure and Applied Zoology*; 1 (3): 209-212.

Gayol, R., Ravishankar, B., Shukla, V.J. and Singh, M. (2012). Hepatoprotective Activity of *Rohitakaghrita*Against Paracetamol Induced Liver Injury in Rat. *Pharmacologia*, 3: 227-232.

Ghosh, N., Ghosh, R., Mandal, V. and Mandal, S. C. (2011). Recent Advances in Herbal Medicine for Treatment of Liver Diseases. *Pharmaceutical Biology*, 49(9), 970-988.

Girish, C. and Pradhan, S.C. (2012). Indian Herbal Medicines in the Treatment of Liver Diseases: Problems and Promises. *Fundamental and Clinical Pharmacology*, 26(2) 180-89.

Guntupalli, M. M. R., Chandana, V. R., Palpu P. and Annie S. (2006). Hepatoprotective Effect of Rubiadin, a Major Constitute of *Rubia cardifolia*Linn*: Journal Ethanopharmacology*. 103: 484-490.

Hassan, S.W., Salawu K., Ladan, M. J., Hassan, L.G., Umar, R. A., and Fatihu, M.Y. (2010). Hepatoprotective, Antioxidant and Phytochemical Properties of Leaf Extracts of New bouldia Laevies.

International Journal of PharmTech Research, 2 (1) 573-584.

Huo, H. Z., Wang, B., Liang, Y. K., Bao, Y. Y. and Gu, Y. (2011). Hepatoprotective and Antioxidant Effects of *Licorice* Extract Against CCl_4-induced Oxidative Damage in Rat. *International Journal of Molecular Science*. 10: 6529-6543.

Kalaivani, T., Meignanam, E., Premkumar, N., Siva1, R., Vijayakumar, V., Rajasekaran, S., Ramya C. and Jayakumararaj, R. (2009). Studies on Hepatoprotective Properties of Leaf Extracts of *Azadirachta indica* A. Juss (Meliaceae). *Ethnobotanical Leaflets 13:* 70-165.

Lala, V., Goyal, A. and Minter, D. A., (2021). Liver Function Tests. In StatPearls [Internet].*StatPearlsPublishing*, 2021.

Meganathan, M., Madhana, M. G., Sasikala, P., Mohan, J., Gowdhaman, N. and Balamurugan, K. (2011). Evaluation of Antioxidant Effect of Omega 3-Fatty Acid Against Paracetamol Induced Liver Injury in Albino Rats. *Global Journal Pharmacology*. 1: 50-53.

Nwobodo, E. I., Nwosu, C. D., Ogbodo, O. S., Ugwuene, O. F., Ihim, C. A., Onuabuchi, A. N., Nnodim, K. J. and Okwudili, A. (2018). Effects of *Azadirachta indica* Leaf Aqueous Extract on the Antioxidant Enzymes in Paracetamol-Induced Hepatotoxicity in Wistar Rats. *International Journal Biological and Chemical Science.* 12(1): 1-10.

Olaniyan, J. M., Muhammad, H. L., Makun, H. A., Busari, M. B., and Abdullah, A. S. (2016). Acute and Sub-acute Toxicity Studies of Aqueous and Methanol Extracts of *Nelsonia campestris* in Rats. *Journal of Acute Disease*, 5(1), 62-70.

Saleem, S., Muhammad, G., Hussain, M.A. and Bukhari, S. N. A. (2018). A Comprehensive Review on Phytochemical Profile, Bioactive for Pharmaceuticals, and Pharmacological Attribute of *Azadirachta indica. Phytotherapy Research* 32(7), 1241-1272.

Sanmgapriya, K. M. and Venkataranganna, M. V. (2006). Protective Effect of HD-03, A Herbal Formulation Against Various Hepatotoxic Agents in Rats. *Journal of Ethanopharmacology*. 63(3):181-186.

Sanmugapriya, E. and Venkataraman, S. (2006). Studies on Hepatoprotective and Antioxidant Actions of *Strychnos Potatorum* Linn. Seeds on CCl_4-induced Acute Hepatic Injury in Experimental Rats, *Journal of Ethnopharmacology,* 105(1-2): 154-160.

Sharma, B. and Sharma, U. K. (2010). Hepatoprotective Activity of Some Indigious Plant. *International Journal of pharmaceutical Technology*.2 (1): 568-572.

Shivaraj, G., Prakash, B. D., Vinayak, V. H., Avinash, A. K. M., Sona, N. V. and Shruthi, S. K. (2009). A Review on Laboratory Liver Function Tests. *Pan African Medical Journal*; 3:17.

Sitasiwi, A. J., Isdadiyanto, S., and Mardiati, S. M. (2018). Effect of Ethanolic Neem (*Azadirachta indica*) Leaf Extract as an Herb Contraceptive on Hepato-somatic Index of the Male Mice (Mus muculus). *Journal of Physics Conference Series*, 1025.

Zimmerman, M. (1983). Ethical Guidelines for Investigation of Experimental Pain in Conscious Animal. *International Journal of Biological1ogical Chemistry*. 16 (2): 109-110.