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SAFETY EVALUATION OF AQUEOUS EXTRACT OF *Solanum nigrum* LEAF IN ANASTROZOLE-INDUCED POLYCYSTIC OVARIAN SYNDROME IN WISTAR RATS

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder prevalent among women of reproductive age. *Solanum nigrum leaf* is used traditionally in the treatment of various gynecological disorders. The toxicological effects of aqueous extract of *Solanum nigrum* leaves (AESNL) at 200 mg/kg body weight on anastrozole-induced in polycystic ovarian syndrome was investigated in female Wistar rats. Sixteen female Wistar rats (190.56 ± 5.35g) were assigned into 4 groups (A - D) of four animals each: animals in group A received 0.5 ml of distilled water orally on daily basis for 14 days while the anastrozole-induced rats in groups B, C, and D also received orally 0.5 ml of distilled water, 7.14mg/kg of metformin (reference drug) and same volume of the extract corresponding to 200 mg/kg body weight of AESNL respectively. Parameters assayed for were liver function tests, kidney function tests and enzyme assays. The result obtained revealed that AESNL mitigated anastrozole-treatment related increases in albumin, uric acid, total protein parameters, total bilirubin, liver and serum aspartate aminotransferase, kidney alkaline phosphatase, liver gamma-glutamyl transferase activities, and aggravated the increases in urea, direct bilirubin, creatinine, liver and serum alkaline phosphatase, and serum gamma-glutamyl transferase activities. Therefore, the aqueous extract of *Solanum nigrum* leaves at the 200 mg/kg body weight might not be completely safe when used in the treatment of PCOS due to alterations in toxicological parameters evaluated in this study.

Keywords: Polycystic Ovarian Syndrome, *Solanum nigrum*, Toxicology, Anastrozole

INTRODUCTION

Phytomedicine is a treatment option which involves the use of plant-based products for medicinal purposes (Chien *et al*., 2021). Polycystic ovary syndrome (PCOS) is a complex hormonal disorder affecting women of reproductive age and characterized by multiple metabolic aberrations, including insulin resistance (IR), hyperinsulinemia, impaired glucose tolerance, visceral obesity, inflammation, endothelial dysfunction, hypertension, and dyslipidemia (Bargiota and Diamanti-Kandarakis, 2012). PCOS is the main cause of infertility in women of reproductive age due to anovulation (Jin and Xie, 2018). The treatments options for PCOS include lifestyle modifications and the use of medications such as metformin (Chien *et al*., 2021). However, emerging studies have explored the potential benefits of phytomedicine in managing PCOS (Chien *et al*., 2021).

Solanum nigrum, commonly known as 'black nightshade', is a plant that has been used in traditional medicine for various therapeutic purposes including gynecological disorders. The plant contains bioactive components such as flavonoids, saponins, alkaloids, and phytosterols (Chen *et al.,* 2022). Research has shown that *Solanum nigrum* has hepatoprotective effects and have been found to increase the activities of antioxidant enzymes and decrease oxidative stress markers in the liver (Elshater *et al.,* 2013; Ogunsuyi *et al.,* 2021). The high content of polyphenols, alkaloids, and saponins in Solanum nigrum contributes to its antioxidant activities (Elshater *et al.,* 2013). Another study proposed the possible role of *Solanum nigrum* in managing the symptoms of COVID-19 and its post-COVID complications, including kidney damage (Sharma *et al*., 2023). Sharma *et al* (2023) suggested that *Solanum nigrum* may reduce proinflammatory cytokines and protect various organs, including the kidneys, from multi-organ failure.

Herbal medicine has been explored as a potential treatment for PCOS and associated fertility disorders (Lakshmi *et al.,* 2023). Traditional Persian and Chinese medicine have a long history of using herbal medicine for gynecological problems and infertility, including PCOS (Moini *et al.,* 2019). The usage of *Solanum nigrum* leaf in as herbal interventions has been scientifically proven to restore female hormones imbalances, mitigate hyperhydrogenism, improve insulin resistance, and enhance lipid metabolism in PCOS (Kwon *et al.,* 2020).

However, *Solanum nigrum* has been used in the treatment of PCOS as an herbal option but there are no studies or report in open scientific literature that has addressed the toxicity of the aqueous extract of *S. nigrum* leaves in anastrozole-induced PCOS female rats. Therefore, this study was aimed at evaluating the toxicological effect of aqueous extract of *S. nigrum* leaf on anastrozole-induced PCOS in female Wistar rats.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Solanum nigrum* were collected in the month of March, 2023, from Mountain Top University permanent site, Ogun State, Nigeria. The plant authentication was carried out at the Department of Plant Biology, University of Lagos, Nigeria. A voucher specimen number LUH 10037 was prepared and deposited at the herbarium of the department.

Animals

Sixteen, healthy female Wistar rats (190.56±5.35g) were obtained from the animal holding unit of the Mountain Top

University, Ogun State, Nigeria. The animals were kept in a well-ventilated house condition (temperature: 22±3ºC; photoperiod: 12h/12h light/dark cycle; humidity: 45-50 %) and fed with rat pellets (New Hope, Grand Cereals, Lagos, Nigeria) and water *ad libitum*

Drugs, Assay Kits, and Chemicals

Albumin, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), urea, uric acid, creatinine and protein assay kits were products of Randox Laboratory, Co-Atrim, United Kingdom. Anastrozole was a product of Ani Pharmaceuticals, Minnesota, USA. All other reagents used were from Sigma Chemicals, St. Louis, USA.

Preparation of Extract

A known weight (2 kg) of *Solanum nigrum* leaves was washed, air-dried and pulverized in a warring electric blender. The powdered material (715 g) was extracted in distilled water using the ratio $(1:4)^3$ for 48 hours and filtered with Whatman No. 1 filter paper. The filtrate was lyophilized to give a yield of 17.4 g which corresponded to 2.43%. The resulting powder was reconstituted in distilled water to obtain the dose, 200 mg/kg body weight used in this study

Animal grouping and extract administration for pharmacological study

A total of 16 female rats were acclimatized for 2 weeks and completely randomized into 4 groups of four (4) animals each. PCOS was then induced in twelve female Wistar rats in groups designated B - D with 1 mg of anastrozole described by Yakubu and Ibiyo (2013) for a period of 21 days. The extract administration was done according to the groups was carried out as follows:

Group A (non PCOS induced control) received 0.5ml of distilled water

Group B (untreated PCOS) received 0.5ml of distilled water

Group C (PCOS induced) received 7.14mg/kg body weight of metformin and 2mg/kg

body weight clomiphene citrate (Reference drug)

Group D (PCOS induced) received 200mg/kg body weight of *Solanium nigrum* leaves extract.

The extract, distilled water and reference drugs were once daily administered for fourteen days. Twenty-four hours after the last administration (end of the experimental period), the rats were anesthetized using diethyl ether and sacrificed by jugular puncture. The blood samples were collected using the procedures described by Yakubu and Ibiyo (2013). Thereafter, the serum and tissue supernatants were used to carry out further biochemical assays.

Preparation of Tissue and Serum Supernatants

The rats were weighed individually and thereafter anaesthetized in a jar containing cotton wool soaked in diethyl ether. The neck area was cleared of fur and skin to expose the jugular veins. The jugular veins were displaced slightly from the neck region and thereafter cut with a sharp sterile blade. The animals were held head downwards, allowed to bleed into clean, dry sample tubes and the collected blood was preserved. The blood samples were centrifuged at 4000rpm for 10 minutes to obtain the supernatant from the stock using Thermo Scientific Centrifuge (Heraeus Megafuge 8). The sera were thereafter aspirated using microflux pipette into clean, dry, sample bottles and were then stored frozen (-4ºC) overnight. The animals were quickly dissected, the liver and kidney were excised, cleaned of fatty layers, weighed and transferred into ice cold 0.25M sucrose solution and homogenized separately in ice cold 0.25M sucrose solution (1:4 w/v) based on their different dilution factors used such as kidney (x60) and liver (x30). The homogenates obtained were centrifuged at 4000 rpm for 10 minutes to obtain the supernatants which were gently collected into sample bottles, stored frozen (-4 ºC) overnight before used for the various biochemical assays (Aufrere *et al.*, 1977; Abraham *et al.*, 1981).

Determination of Biochemical Parameters

The concentrations of albumin, bilirubin, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), alkaline phosphate (ALP), urea, uric acid, creatinine and protein were determined by adopting standard procedures as described in the assay kits manufacturers procedure.

Statistical Analysis

Data were expressed as the mean ± standard error mean of four determinations and data were analyzed for statistical significant at $P < 0.05$ using One Way Analysis of Variance and Duncan Multiple Range Test performed with Statistical Package for Social Sciences, version 21.0 (IBM Inc., Chicago, USA).

RESULTS AND DISCUSSION

The result obtained in this study showed that, anastrozole administration significantly increased ($p<0.05$) the serum total bilirubin and direct bilirubin concentration (Table 1). However, the serum albumin and creatinine concentration significantly decreased ($p<0.05$) when compared to the control group (Table1 & 2). Administration of anastrozole significantly increased ($p<0.05$) the serum uric acid, and significantly decreased ($p<0.05$) the serum urea levels compared to the control group (Table 2) while 200 mg/kg of AESNL significantly increased ($p<0.05$) the serum direct bilirubin, creatinine, and total bilirubin (Table 2). The 200 mg/kg of AESNL administration significantly increased ($p<0.05$) the serum uric acid and urea levels, when compared to the control group (Table 2). Compared to the reference drug, the extract significantly increased ($p<0.05$) the serum albumin concentration in the liver (Table 1), significantly decreased ($p<0.05$) the serum total bilirubin, and serum (Table 1), and compared favorably ($p>0.05$) with the direct bilirubin levels (Table 1). The administration of the reference drug significantly decreased ($p<0.05$) the urea levels, significantly increased ($p<0.05$) the creatinine, and compared favorably ($p>0.05$) with the uric acid, compared to the control group (Table 2).

Administration of anastrozole significantly decreased ($p<0.05$) the activity of ALT in the liver, but significantly increased ($p<0.05$) in the serum, compared to the control group (Figure 1). The administration of the reference drug significantly increased ($p<0.05$) the activity of ALT in the serum, but significantly decreased ($p<0.05$) the activity in the liver, compared to the control group (Figure 1). The extract administration significantly increased ($p<0.05$) the activity of ALT in the serum, whereas significantly decreased ($p<0.05$) the activity in the liver (Figure 1).

Administration of anastrozole significantly increased ($p<0.05$) the ALP activity in the serum and kidneys while the ALP activity significantly decreased ($p<0.05$) in the liver, when compared to the control group (Figure 2). Treatment with the reference drug significantly decreased ($p<0.05$) the ALP activity in the serum, liver and kidneys compared to the control group (Figure B). Administration of the extract significantly decreased ($p<0.05$) the ALP activity in the

serum, liver and kidneys compared to the control group (Figure 2). Compared to the reference drug, the ALP activity significantly increased ($p<0.05$) in the liver and serum, but compared favorably ($p>0.05$) in the kidneys (Figure 2).

Anastrozole administration significantly increased ($p<0.05$) the activity of AST in the serum, whereas significantly decreased ($p<0.05$) the activity in the liver, compared to the control group (Figure 3). Treatment with the reference drug significantly decreased ($p<0.05$) AST activity in the serum and liver when compared with the control group. Administration of the extract significantly increased ($p<0.05$) the activity of AST in the serum, but significantly decreased ($p<0.05$) the activity in the liver (Figure 3). Compared to the reference drug, the AST activity significantly increased ($p<0.05$) in the serum, whereas significantly decreased ($p<0.05$) in the liver (Figure 3).

Administration of anastrozole significantly increased ($p<0.05$) GGT activity in the serum, however it was significantly decreased ($p<0.05$) in the liver compared to the control group (Figure 4). Treatment with the reference drug significantly decreased ($p<0.05$) GGT activity in the liver, but significantly increased ($p<0.05$) in the serum compared to the control group (Figure 4). Extract administration significantly increased ($p<0.05$) the GGT activity in the serum, but was significantly decreased ($p<0.05$) in the liver (Figure 4). Compared to the reference drug, the extract administration significantly decreased ($p<0.05$) GGT activity in both the liver and serum (Figure 4).

Table 1: Effect of aqueous extract of *Solanum nigrum* leaf on liver function indices in anastrozole-induced PCOS rats

Groups / Parameters	Albumin (g/dl)	Globulin (g/dl)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
Control	200.30 ± 0.40 ^{a}	64.06 ± 1.34^{a}	55.98± 0.11^{a}	10.01 ± 0.01 ^{a}
PCOS +Distilled water	186.95 ± 0.30^{d}	57.11± 2.34^{b}	62.02 ± 0.09^{c}	11.02 ± 0.01 ^{b}
PCOS+MET+CC	145.13 ± 0.30^{b}	131.32±0.90^{c}	78.12 ± 0.08^{b}	11.99 ± 0.00 ^{b}
PCOS + 200 mg/kg b.wt. of AESNL	162.53 ± 0.79^{c}	70.02 ± 4.09^{d}	59.07 ± 0.11^{d}	12.03 ± 0.02 ^{c}

Data are means of four determinations ± SEM. Values with different superscripts in each column are significantly different (P<0.05). Metformin- MET, clomiphene citrate- CC

Table 2: Effect of aqueous extract of *Solanum nigrum* leaf on kidney function indices in anastrozole-induced PCOS rats

Groups / Parameters	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Control	75.98 ± 0.02^{a}	8199.8 ± 0.27^{a}	0.4325 ± 0.01^{a}
PCOS +Distilled water	75.76 ± 0.25^{a}	6798.75 ± 0.75^{c}	0.65 ±0.00^{c}
PCOS+MET+CC	78.01 ± 0.01^{b}	7198.75 ± 0.75^{d}	0.435 ± 0.01^{a}
PCOS +200 mg/kg b.wt. of AESNL	78.99 ± 0.00^{c}	8598.75 ± 0.75^{b}	0.4725 ± 0.00^{b}

Data are means of four determinations ± SEM. Values with different superscripts in each column are significantly different (P<0.05). Metformin- MET, clomiphene citrate- CC

1

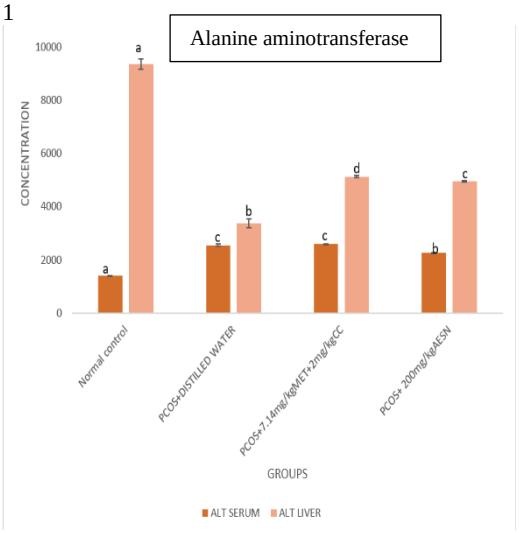

Figure 1: Alanine aminotransferase activity in the liver and serum concentration of anastrozole -induced PCOS rats administered 200mg/kg body weight of AESNL

2

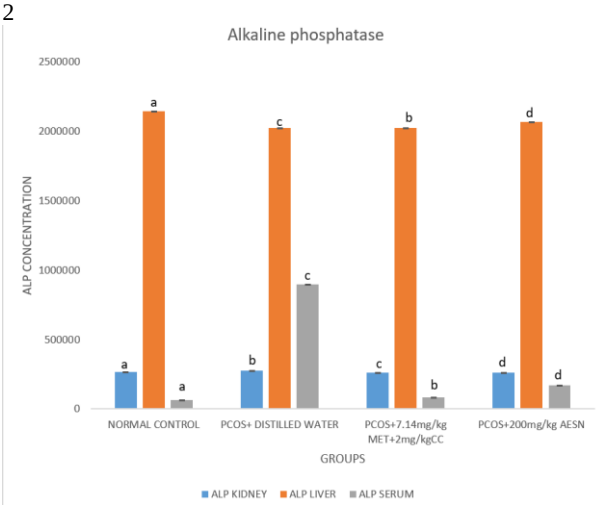

Figure 2: Alkaline phosphatase activity in the liver, kidney and serum concentration of anastrozole -induced PCOS rats administered 200mg/kg body weight of AESNL

3

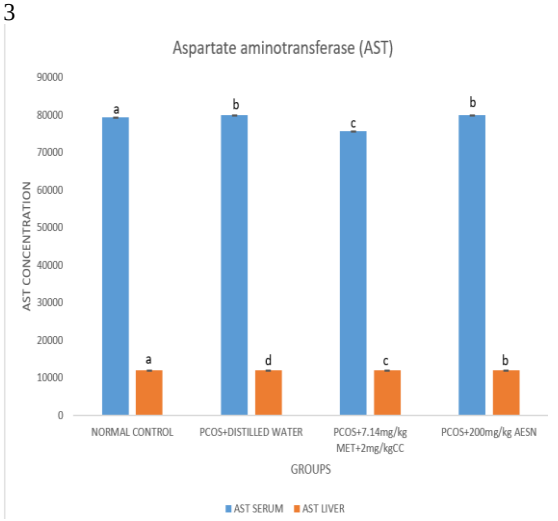

Figure 3: Aspartate aminotransferase activity in the liver and serum concentration of anastrozole -induced PCOS rats administered 200mg/kg body weight of AESNL

4

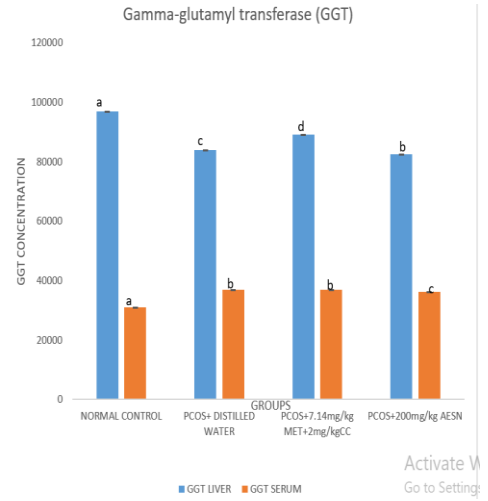

Figure 4: Gamma-glutamyl transferese activity in the liver and serum concentration of anastrozole -induced PCOS rats administered 200mg/kg body weight of AESNL

Data are mean of four determinations ± SEM; Values with different superscripts in each column are significantly different (P<0.05).

Discussion

The toxicological evaluation of aqueous extract of *Solanum nigrum* leaves on anastrozole-induced polycystic ovarian syndrome in female Wistar rats has provided additional information on its effects on the liver and kidney of the animals.

Bilirubin is a product of hemoglobin breakdown and is typically conjugated in the liver before excretion (McDonagh, 2009). Elevated levels of bilirubin can indicate impaired liver function, potentially affecting the rats' ability to detoxify substances and metabolize drugs (Hamoud *et al.*, 2018). The observed significant increase in total bilirubin, direct bilirubin, and total protein concentration in the liver and kidneys following anastrozole administration is suggestive of potential liver and kidney dysfunction.

Albumin is synthesized by the liver and functions to maintain oncotic pressure and transport various molecules in the blood (Quinlan and Martin, 2005). A decrease in albumin levels may indicate liver dysfunction or impaired liver function, as these proteins are synthesized by the liver. The decreased serum albumin and creatinine levels in the anastrozole group may suggest impaired renal function (Kaysen *et al.*, 2004). Low serum albumin can lead to decreased oncotic pressure and impaired transportation of essential molecules in the bloodstream. Creatinine is a waste product of muscle metabolism excreted by the kidneys, and elevated serum creatinine levels suggest decreased renal filtration (Gounden *et al.*, 2023).

The significant increase in serum uric acid and decrease in serum urea levels in the anastrozole group (Table 2) may further reflect renal dysfunction. Uric acid is primarily excreted by the kidneys, and elevated levels can be indicative of impaired renal clearance or suggest altered purine metabolism (Seki *et al.*, 2010). Elevated uric acid levels can potentially lead to conditions like gout. Reduced serum urea levels may imply decreased renal filtration or altered protein metabolism.

Alkaline phosphatase is an ectoenzyme of the plasma membrane (Benjawatanapon *et al.*, 1982) and is used to assess the integrity or damage to the plasma membrane. Increased serum ALP activity (Figure B) could be indicative of cholestatic liver injury or bone pathology, as ALP is found in both the liver and bone (Balbaied and Moore, 2019). Decreased liver ALP activity may reflect impaired liver function (Yakubu and Nurudeen, 2014).

Opposite effects were observed for ALT and AST activities in the liver and serum following anastrozole administration suggestive of a pattern of liver injury. ALT is primarily found in the liver, and it plays a key role in amino acid metabolism by converting the amino acid alanine into pyruvate. It is considered a specific marker of liver damage, while AST is found in various tissues, including the liver and heart. It is involved in the conversion of aspartate to oxaloacetate. Increased serum ALT and AST activities may indicate hepatocellular injury, while decreased liver ALT activity may reflect liver dysfunction (Isah *et al.*, 2023; Xu *et al.*, 2019).

GGT is a liver enzyme involved in detoxification and is found in bile ducts. The anastrozole administration caused an increase in serum GGT activity but decrease in liver GGT. Elevated serum GGT can be associated with liver or biliary disease (Itoh and Nakajima, 1986), whereas decreased liver GGT activity may indicate liver damage, releasing the enzymes into the bloodstream.

CONCLUSION

The aqueous extract of *Solanum nigrum* leaf may contain various phytochemicals that could have toxic effects on the liver and kidneys when used in the treatment of PCOS. These effects could result from the accumulation of toxic compounds or interference with normal metabolic processes, liver injury and kidney damage. This study therefore suggests that aqueous extract of *Solanum nigrum* leaves might not be completely safe when used in the treatment of PCOS due to alterations in toxicological parameters.

REFERENCES

Abraham, S., Lin, C. Y., Klein, H. P., Volkmann, C., Tigranyan, R. A., & Vetrova, E. G. (1981). Studies of specific hepatic enzymes involved in the conversion of carbohydrates to lipids in rats exposed to prolonged spaceflight aboard COSMOS-1129. In Gravitational Physiology (pp. 71-77). Pergamon.

Aufrere, M. B., Hoener, B. A., & Vore, M. E. (1977). High-performance liquid-chromatographic assay for nitrofurantoin in plasma and urine. Clinical chemistry, 23(12), 2207-2212.

Balbaied, T. and Moore, E. (2019). Overview of optical and electrochemical alkaline phosphatase (alp) biosensors: recent approaches in cells culture techniques. Biosensors, 9(3), 102

Bargiota, A., & Diamanti-Kandarakis, E. (2012). The effects of old, new and emerging medicines on metabolic aberrations in PCOS. Therapeutic advances in endocrinology and metabolism, 3(1), 27-47.

Benjawatanapon, C., Bell, L., & Williams, L. (1982). Ultrastructural localisation of alkaline phosphatase in adult human large intestine. Gut, 23, 134 - 136.

Chen X., Dai X., Liu Y., Yang Y., Yuan L., He X., and Gong G. (2022). *Solanum nigrum* Linn.: An Insight into Current Research on Traditional Uses, Phytochemistry, and Pharmacology. *Front Pharmacol.* 13:918071. doi: 10.3389/fphar.2022.918071. PMID: 36052142; PMCID: PMC9424827.

Chien, Y. J., Chang, C. Y., Wu, M. Y., Chen, C. H., Horng, Y. S., & Wu, H. C. (2021). Effects of curcumin on glycemic control and lipid profile in polycystic ovary syndrome: systematic review with meta-analysis and trial sequential analysis. Nutrients, 13(2), 684.

Elshater, A. E. A., Salman, M. M. A., & Mohamed, S. A. (2013). The hepato-ameliorating effect of Solanum nigrum against CCL4 induced liver toxicity in Albino rats. Egyptian academic journal of biological sciences. C, physiology and molecular biology, 5(1), 59-66.

Gounden V., Bhatt .H and Jialal I. (2023). Renal Function Tests. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK507821/

Hamoud, A., Weaver, L., Stec, D. E., & Hinds, T. D. (2018). Bilirubin in the liver–gut signaling axis. Trends in Endocrinology &Amp; Metabolism, 29(3), 140-150.

Isah A. O., Idu M., & Amaechina F. C. (2023). Toxicity Profiles of Methanol Extract of *Crossopteryx febrifuga* Leaf (Afzel. Ex G. Don) Benth. *FUDMA JOURNAL OF SCIENCES*, *7*(2), 100 - 109. https://doi.org/10.33003/fjs-2023-0702-1391

Itoh, S., & Nakajima, M. (1986). Liver γ-Glutamyltransferase Activity in Viral Liver Disease. Digestion, 33, 121-125

Jin, P., & Xie, Y. (2018). Treatment strategies for women with polycystic ovary syndrome. Gynecological Endocrinology, 34(4), 272-277.

Kaysen GA, Dubin JA, Müller HG, Rosales LM, Levin NW, Mitch WE. Inflammation and reduced albumin synthesis associated with stable decline in serum albumin in hemodialysis patients. Kidney Int. 2004;65(4):1408-1415.

Kwon, C. Y., Cho, I. H., & Park, K. S. (2020). Therapeutic effects and mechanisms of herbal medicines for treating polycystic ovary syndrome: A review. Frontiers in Pharmacology, 11, 1192

Lakshmi J. N., Babu A. N., Kiran S. S. M., Nori L. P., Hassan N., Ashames A., Bhandare R. R., and Shaik A. B. (2023). Herbs as a Source for the Treatment of Polycystic Ovarian Syndrome: A Systematic Review. *BioTech (Basel)*, 3;12(1):4. doi: 10.3390/biotech12010004. PMID: 36648830; PMCID: PMC9844343.

McDonagh AF. The bilirubins: chemistry, metabolism, and clinical relevance. Adv Clin Chem. 2009;48:263-312. doi: 10.1016/S0065-2423(09)48007-8

Moini Jazani, A., Nasimi Doost Azgomi, H., Nasimi Doost Azgomi, A., & Nasimi Doost Azgomi, R. (2019). A comprehensive review of clinical studies with herbal medicine on polycystic ovary syndrome (PCOS). DARU Journal of Pharmaceutical Sciences, 27, 863-877.

Ogunsuyi, O. B., Oboh, G., Özek, G., & Göger, F. (2021). Solanum vegetable-based diets improve impairments in memory, redox imbalance, and altered critical enzyme activities in Drosophila melanogaster model of neurodegeneration. Journal of food biochemistry, 45(3), e13150

Quinlan, G. J., & Martin, G. S. (2005). Albumin: biochemical properties and therapeutic potential. Hepatology, 41(6), 1211-1219.

Seki, S., Tsutsui, K., Fujii, T., Yamazaki, K., Anzawa, R., & Yoshimura, M. (2010). Association of Uric Acid with Risk Factors for Chronic Kidney Disease and Metabolic Syndrome in Patients with Essential Hypertension. Clinical and Experimental Hypertension, 32, 270 - 277.

Sharma, D., Joshi, M., Apparsundaram, S., Goyal, R. K., Patel, B., & Dhobi, M. (2023). Solanum nigrum L. in COVID-19 and post-COVID complications: a propitious candidate. Molecular and Cellular Biochemistry, 1-20.

Xu, J., Xia, Y., Li, S., Cheng, X., Hu, S., Gao, Y., Zhou, X., Wang, G., & Zheng, Q. (2019). A retrospective pilot study to examine the potential of aspartate aminotransferase to alanine aminotransferase ratio as a predictor of postoperative acute kidney injury in patients with hepatocellular carcinoma. Annals of Clinical Biochemistry, 56, 357 - 366

Yakubu, M. T. and Nurudeen, Q. O. Toxicological effects of aqueous extract of Cnestis ferruginea (Vahl ex DC) root in paroxetine-induced sexually impaired male rats. J. Investig. Biochem. 2014; 3(5):39-45

Yakubu, M. T., & Ibiyo, B. O. (2013). Effects of Aqueous Extract of *Cnestis ferruginea* (Vahl Ex DC) Root on the Biochemical and Clinical Parameters of Anastrozole-Induced Polycystic Ovarian Syndrome Rat Model. *Journal of Endocrinology and Reproduction*, *17*(2), 99–112.