

Effects of Methanol Leaf Extract of *Datura stramonium* on Some Liver Biomarkers of Levonorgestrel-Induced Albino Rats

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Abstract

Levonorgestrel is a synthetic hormone orally taken to prevent pregnancy. It is absorbed rapidly after oral administration. Metabolism is handled by the liver, as metabolites are excreted through the kidneys. The current study investigates the Effects of Methanol Leaf Extract of *Datura stramonium* on Some Liver Biomarkers of Levonorgestrel-induced Albino Rats. Levonorgestrel was the drug administered. Hepatogenic parameters were assayed to determine the toxic effects of the drug on the liver, and the Plant extract was administered for its ameliorating effects. Twenty-five (25) rats grouped into five (n=5) were administered levonorgestrel and the methanol leaf extract as indicated in the experimental design. The animals were sacrificed after the experimental periods of 21 days. Blood was collected for assay of biochemical parameters by cardiac puncture. The concentrations of ALP ($222.06 \pm 148.99b$), GGT ($260.55 \pm 174.09ab$), and TP ($2.48 \pm 1.65ab$) were significantly ($p < 0.05$) reduced in groups treated with levonorgestrel as compared with the normal control. However, treatment with plant extract significantly ($p < 0.05$) elevated ALP ($308.69 \pm 22.16ab$), GGT ($316.71 \pm 6.02ab$), and TP ($3.44 \pm 0.10ab$) when 100mg/kg extract was administered. Slide elevation in ALP ($283.99 \pm 8.51ab$), GGT ($283.99 \pm 8.51ab$), and TP ($3.37 \pm 0.06ab$) was observed as 300mg/kg dose of the extract was administered. The extract showed a dose-dependent response. The result showed that frequent use of levonorgestrel suppresses ALP, GGT activity, and TP concentration. It can be concluded that the extract demonstrated a restorative potential on the harmful effect of levonorgestrel on the biliary obstructing and protein synthetic effects of the liver.

Keywords: Levonorgestrel, Biomarkers, Albino Rats, *Datura stramonium*, Liver.

Introduction

Levonorgestrel is a synthetic hormone orally taken to prevent pregnancy by destabilizing ovulation leading to failed fertilization (Endler *et al.*, 2022). It is a second-generation synthetic progesterone. It is the active component of the racemic mixture of norgestrel. Levonorgestrel is absorbed rapidly following oral administration and undergo metabolism through hydroxylation, conjugation and reduction in the liver. The metabolites are later excreted through the urine (Preeti & Tusher, 2025). Bulk of drugs administered orally go into the gastrointestinal tract (GIT), get absorb into the bloodstream through the alveoli to the liver where it gets metabolize before reaching the target site. More than 900 drugs are

known to cause liver damage, which most time are not noticed early, making it the leading causes of liver failures (Simran *et al.*, 2024). According to Simran *et al.* (2024), "A biomarker is a measurable and quantifiable attribute used to evaluate the efficacy of a treatment or to diagnose a disease". These biomarkers can be cellular or molecular (e.g., DNA, RNA, Proteins or Metabolites) and body fluids like the Blood, Urine, Saliva, serum, cerebrospinal fluid etc., are used to determine them (Srinitha, 2021). Biomarkers are increasingly being used as a primary warning in a perceived pathological condition. They are produced in organs, tissues and cells that are diseased, in response to various pathological states or as an outcome of an individual's reaction to drug taken. It reveals the presence of systemic or tissue diseased condition (Varahalarao & Sandeep, 2024). Biomarkers play an important role in clinical diagnosis because its early identification reduces damage to the body (Bodaghi *et al.*, 2023). Several methods and technologies have been developed and applied in tumor biomarker discovery and detection. These technologies include genomics, transcriptomics, proteomics, metabolomics, the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) gene-editing technology and high-throughput sequencing make it possible to obtain large-scale information on the diversity of tumor biomarkers (Zhou *et al.*, 2024). Medicinal plants elucidate their pharmaceutical properties on biomarkers by reducing or lowering the effect of an inducing compound or pathological conditions. Some demonstrate immunomodulatory benefits as such could possess potential lead compound for the development and design of immunomodulatory drugs (Joshua *et al.*, 2022). Many medicinal plants like *Datura stramonium* manifest a beneficial effect by restoring altered drug and pathological hepatotoxicity effects on the liver, restoring biochemical in chemotherapeutic condition (Ester *et al.*, 2023). The multiple and frequent use of Levonorgestrel as contraceptive is on the increase by females both unmarried and the married as reported by Abdulrahman *et al.* (2024) who examined the effect of long term usage of this contraceptives on the lipid profile in albino rats. Since this drug is metabolize in the liver, it would important to also investigate its effects on the liver. Therefore, it is imperative to investigate the effects of this drug on the liver using animal models like the albino rats. The aim of this research is to investigate the effect of Methanol Leaf Extract of *Datura stramonium* on liver biomarkers of rat induced with Levonorgestrel.

Justification and significance of the Study

Drug-induced liver is the leading cause of death in many countries in sub-Saharan African countries. The consumption of levonogestrel is rampant, being carried away by the immediate remedy it offers, neglecting its effects on the liver. The drug leaflets from the manufacturers do not carry useful information in many cases, on the effects of its long-time usage on the liver.

Methodology

Chemicals and reagents

Chemicals and reagents of Standard-grade were purchased from reputable vendors for use in the experiment.

Biological materials

Fresh leaves of *Datura stramonium* and albino rats (Wistar) are the biological materials employed in this research. Mature leaf of *Datura stramonium* was harvested at Anguwan NEPA and Anguwan Tiv in October 2024 within Bali metropolis. The plant was identified and certified with accession number MAU/PLS/0079, in the department of plant science of Modibbo Adama University, Yola.

Animal care

Wistar albino rats numbering 25 (mixed sex) within standard weight were procured from the Department of Animal Science, Modibbo Adama University Yola. The rats were kept for two weeks to get acclimatized to the new environment in the Animal House, located in the biological garden, behind the SLT department of Federal Polytechnic Bali. Animals were kept in clean cages, under standard laboratory condition (Temperature $25\pm 5^{\circ}\text{C}$, Relative humidity 50-60% and a 12/12 hours light and dark cycle) and were fed standard diet and water *ad-libitum*.

Sample preparation

Following certification, leaves were washed under running water and dried under shed for 30 days before grinding into smooth powder and storing in an air-tight bottle. Cool maceration method by Khadayat *et al.* (2020) was adopted in extract preparation. One hundred and fifty gram (150g) of powdered leaf was soaked in 850mls of methanol placed in a tight container with constant agitation for 72hours. Followed by evaporation of the solvent in a water bath.

Experimental design

After two weeks of adaptation, the 25 albino rats were grouped into 5 groups of 5 rats each.

Group 1: Normal control (given feed and water only)

Group 2: 0.75mg of levonorgestrel (inducer only)

Group 3: 0.75mg levonorgestrel+ 100 mg/kg b.w extract

Group 4: 0.75mg levonorgestrel+ 200 mg/kg b.w extract

Group 5: 0.75mg levonorgestrel+ 300 mg/kg b.w extract

All administration was done orally.

Sample collection from experimental animals

After 21 days of extract administration, rats were starved all night and Whole blood was collected into plan sample bottles by cardio puncture according to IACUC (2022) guidelines for further analysis.

Assay of Hepatological Indices

Serum concentration of Albumin and total protein, the activity of liver enzymes such as ALT, AST, ALP and GGT were determined using standard methods.

Aspartate aminotransferase (AST): Amador and Wacker (1962) was adopted in the determination of AST activity.

Alanine aminotransferase (ALT): Amador and Wacker (1962) method was used in ALT determination.

Alkaline Phosphatase (ALP): Serum activity of alkaline phosphatase (ALP) was determined by the method described by Haussament, (1977).

Gamma glutamyl transferase (GGT): serum activity of GGT was determined by method describe by Szewczuk & Orlowski (1960).

Total protein: It was determined colometrically as described by Fine (1935).

Albumin Level: Doumas *et al.* (1971) Method was adopted.

Statistical Analysis

All data obtained were expressed as mean $\pm$ SD. One way ANOVA was used to test for difference among groups using spss version 27. Post Hoc test was used to test for significant differences among group mean. A p- value of < 0.05 was considered statistically significant.

Results and Discussion

Results

The result of liver biomarkers of levonogestrel intoxication revealed the suppressing effects of levonogestrel or inducer on ALP (222.06 ± 148.99^b), GGT (260.55 ± 174.09^{ab}) and TP (2.48 ± 1.65^{ab}) when compared with the normal control as indicated in Table 1. There was a significant ($p<0.05$) elevation in the activity of ALP (308.69 ± 22.16^{ab}) and GGT (316.71 ± 6.02^{ab}) as well as TP (3.44 ± 0.10^{ab}) concentration following 100mg/kg extract treatment across the groups. Increased dose of the extract to 300mg/kg mildly elevated the activity of ALP (283.99 ± 8.51^{ab}), GGT (283.99 ± 8.51^{ab}) and TP (3.37 ± 0.06^{ab}) concentration when compared to drug control group. Dose of 200mg/kg did show no effect possibly because it was not properly treated. While the activity of ALT and AST as well as ALB concentration were not significant ($p<0.05$) among the groups, relatively small elevation were observed, due to the effects of the plant leaf extract on the liver enzymes.

Table 1: Effects of Methanol Leaf Extract of *Datura stramonium* on Some Liver Biomarkers of Levonorgestrel-induced Albino Rats.

Group	Normal Control	Drug Control	Drug+100mg/kg extract	Drug+200mg/kg extract	Drug+300mg/kg extract
AST(I/U)	65.17±1.522 ^a	46.99±31.33 ^a	61.43±0.35 ^a	30.89±35.67 ^a	60.81±034 ^a
ALT(I/U)	30.72±0.77 ^a	22.00±14.67 ^a	28.76±0.27 ^a	14.52±16.17 ^a	28.48±0.22 ^a
ALP(I/U)	302.50±39.60 ^b	222.06±148.99 ^b	308.69±22.16 ^{ab}	136.13±1.57 ^a	283.99±8.51 ^{ab}
GGT(I/U)	356.09±31.02 ^b	260.55±174.09 ^{ab}	316.71±6.02 ^{ab}	153.44±177.19 ^a	283.99±8.51 ^{ab}
ALB(mg/dl)	2.95±0.04 ^a	2.20±1.47 ^a	2.91±0.06 ^a	1.56±1.80 ^a	3.05±0.07 ^a
TP(mg/dl)	3.61±0.09 ^b	2.48±1.65 ^{ab}	3.44±0.10 ^{ab}	1.64±1.90 ^a	3.37±0.06 ^{ab}

Results are expressed as mean $\pm$ SD (n=5). Significant at $p < 0.05$ compared with control using analysis of Variance.

Groups with same superscript in the row are not significantly different

Group with different superscripts in the same row are significantly different

Discussion

Plant parts have been used for medicinal purposes since ancient times. The reliance on herbs for curative and preventive purposes has doubled by 80% worldwide. In developing countries, most people still relies heavily on herbal medicine to meet their health needs. The poorest countries of the world use herbal medicine because of its availability and affordability (Chavban *et al.*, 2018). *Datura stramonium* is one of such plants. It is used traditionally to relieve pain, fevers, liver disorders, and anti-inflammatory properties etc., owed to the presence of alkaloids, saponins, tannins, steroids, polyphenols and glycosides (Bakht *et al.*, 2022).

The liver, being the largest and one of the vital organ of the body play immense roles in life processes. Some important function of the liver includes metabolic functions, excretory functions, protective/detoxification functions, hematological functions and storage functions (Ozougwu, 2017). Most clinically used drugs get metabolized in the liver through oxidation, reduction and hydrolysis (phase -1 reaction). Cytochrome P₄₅₀ which primarily catalyzes oxidation reaction play major role in the metabolism of about 50% of drugs used clinically (Tatsuki *et al.*, 2022). Defective liver function could lead to instant death. Damages to liver are caused by alcohols, viruses, Drugs, toxins, hepatocellular carcinoma among others. Plant leaf extract of *Datura stramonium* does not cause harm to the liver (Alum *et al.*, 2022). Levonorgestrel suppresses the activity ALP, GGT and TP but the plant extract elevated their activities. Levonorgestrel also increases cardiac, hepatic and kidney total cholesterol (Abdulrahman *et al.*, 2024). Bakht *et al.*, (2022) reports that *Datura stramonium* exhibited anti- inflammatory action in CCL₄-induced hepatic injury.

Liver enzymes are located in specific areas within the hepatocytes and cholangiocytes. Cholangiocytes are injured in biliary tract disease such as cholangitis, biliary obstruction. Alkaline phosphatase (ALP) is produced in the cholangiocytes. It is also produced in the kidney, bone, placenta, intestine and gut. Gamma Glutamyl transpeptidase (GGT) is a

microsomal enzyme and raised in its level indicates liver injury. Decreased in the activity of ALP and GGT also indicates liver injury (Isah *et al.*, 2017). There was an elevation in the activity of ALP, GGT and TP concentration when extract was administered as compared to that of drug control which altered the enzyme activity (Table 1). Decreased in the activity of GGT, ALT and TP in the drug control as compared to the normal control signified the effect of the drug on the microsomal enzymes or cholestasis liver injury.

Total protein measures the amount of proteins, primarily albumin and globulins, found in serum and plasma and decrease albumin indicates liver disease or malnutrition. Serum albumin, synthesize solely in the liver has the half-life of 20-25 days. Low albumin is commonly observe in patients with severe liver damage. The drug administered lowered total protein concentration as compared to the normal control but the extract reversed the protein concentration (Table 1). Low protein concentration (albumin+globulins) in the serum is implicated to hepatic abnormality (Chukwudoruo *et al.*, 2021). This study showed that levonogestrel interferes with protein synthesizing function of the liver but the methanol leaf extract of *Datura stramonium* reverses the harm caused by the drug. Alkaline phosphatase and Gamma-Glutamyl Transpeptidase activities were disrupted by levonogestrel administration, but the leaf extract restored the activities of the enzymes. It therefore showed that the plant leaf extract of *Datura stramonium* has the ability to improve free biliary transport and synthetic functions of the liver (Atamgba, 2018). It also implies that levonogestrel could be implicated in biliary obstruction.

Conclusion

The leaf extract of *Datura stramonium* reverses the harm caused by levonogestrel intoxication on the liver cells of albino rats. Therefore, the leaf extract of *Datura stramonium* may be useful in alleviating liver abnormality as observed from the result.

Conflicts of interest

No conflict of interest declared by the authors.

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