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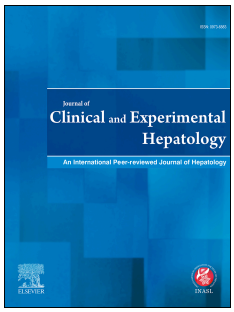

Stanozolol-induced liver injury: A distinctive cholestatic clinical and biochemical phenotype at presentation

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Stanozolol-induced liver injury: A distinctive biochemical pattern at presentation

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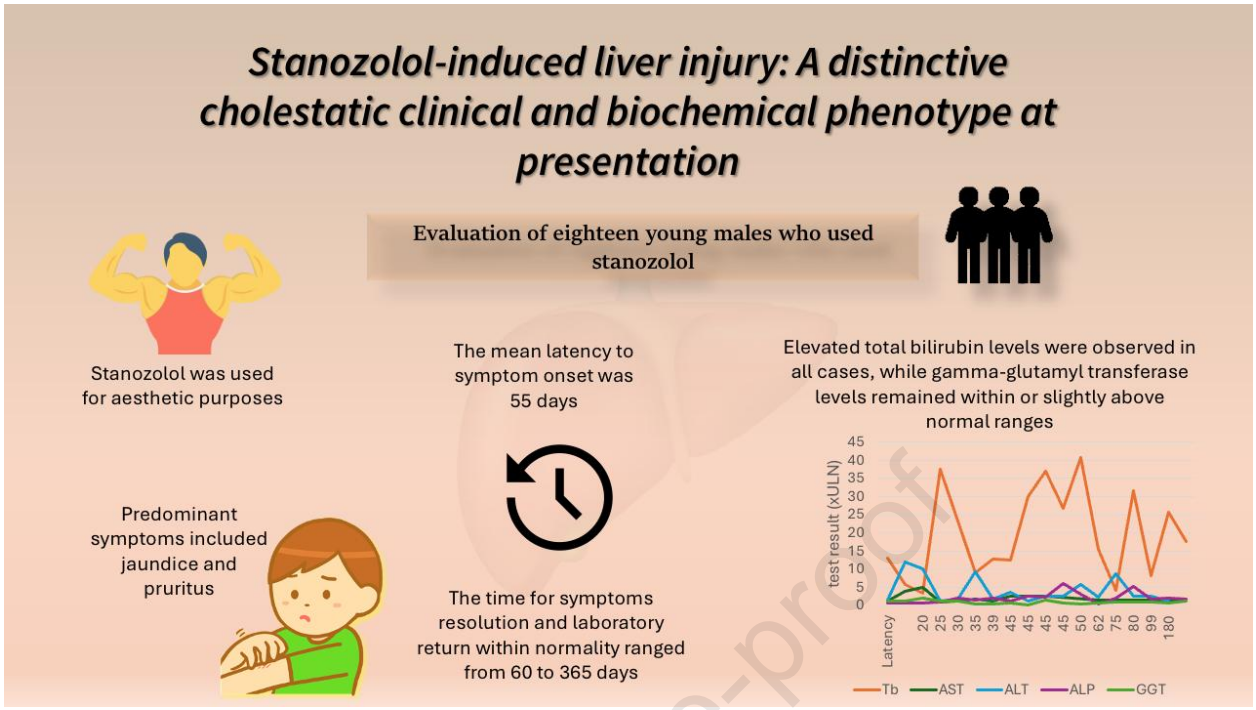
Electronic word count: 1902 **Tables:** 1 **Figure:** 1

Abbreviations:
AAS- Anabolic androgenic steroids
DILI- Drug induced liver injury
ANA- Anti-nuclear antibodies
AMA- Antimitochondrial antibody
SMA- Smooth muscle antibody
ANCA- Anti-neutrophil cytoplasm antibodies
SLATINDILI- Spanish Latin American DILI Registry
BCAA- Branched-Chain amino acids
TB- Total bilirrubina
GGT- Gamma-glutamyl transferase
ALP- Alkaline phosphatase
AST- Aspartate aminotransferase
ALT- Alanine aminotransferase
BSEP- Bile salt export pump

Conflicts of interest of all authors: none

Financial disclosure: Our group highlights the support received from Maria Emilia Foundation and IDOR to support the research line in neglected liver diseases.



Stanozolol-induced liver injury: A distinctive cholestatic clinical and biochemical phenotype at presentation

Electronic word count: 1848 **Tables:** 1 **Figure:** 2

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Abstract:

Background & Aims: The misuse of Anabolic Androgenic Steroids (AAS), including Stanozolol, for performance enhancement has emerged as a significant cause of liver damage. This study aims to elucidate the distinctive hepatotoxicity profiles induced by Stanozolol.
Methods: Eighteen individuals were prospectively evaluated by the Latin American DILI Registry from 2013 to 2023. Data regarding Stanozolol administration, symptoms onset, and clinical manifestations were collected. Comprehensive assessments including serologies, abdominal imaging, and in some cases, liver biopsies were performed to identify Stanozolol-induced liver injury and exclude other etiologies.
Results: All patients were young males aged between 19 to 48, utilizing Stanozolol for aesthetic purposes. The mean latency to symptom onset was 55 days. Predominant symptoms included jaundice and pruritus. Elevated total bilirubin levels were observed in all cases, while gamma-glutamyl transferase levels remained within or slightly above

normal ranges. Concurrent use of other substances was reported in ten cases, showcasing a trend of poly-substance abuse.
Conclusions: The study identified a specific biochemical profile of Stanozolol-induced liver injury in young men using it for aesthetic purposes. The characteristic liver injury profile has marked elevation of bilirubin, mild rise in transaminases and near normal GGT

Keywords: Anabolic Androgenic Steroids, Drug Induced Liver Injury, Cholestasis, bland cholestasis

Lay Summary: The increasing use of anabolic steroids for aesthetic purposes has become a worrying trend, with liver damage as one of its consequences.
Stanazolol toxicity presents itself in a peculiar way: significant elevation of bilirubin with normal or slightly elevated GGT levels.
Knowing the laboratory manifestation as well as the prognosis of liver injury is important to provide optimal medical support and differentiate from toxicities caused by other substances commonly used in association

Introduction

Anabolic androgenic steroids (AAS) are synthetic compounds derived from testosterone and are primarily used to treat various medical conditions such as hypogonadism, breast cancer, hereditary angioedema, osteoporosis, and even cirrhotic sarcopenia.[1,2,3] However, their misuse for performance enhancement and aesthetic purposes has become a worrying trend.[2]

The use of AAS, herbal supplements, and dietary supplements has increased significantly in recent years, highlighting the emergence of these substances as a major cause of liver damage.[4] Liver effects induced by AAS include acute hepatitis, intrahepatic cholestasis, adenomas, hepatocellular carcinoma, hepatic peliosis, and hepatic rupture, among others.[4,5]

Stanozolol, a 17α-alkylated synthetic anabolic steroid derived from dihydrotestosterone, is widely available online and is predominantly administered intramuscularly, with a dose-dependent effect.[5] It poses a high risk of liver injury due to delayed metabolism and prolonged hepatic exposure.[6] Furthermore, 17α-alkylated steroids have been shown to induce oxidative stress in the rat livers, despite increased compensatory antioxidant mechanisms.[7]

The use of stanozolol is often associated with the concomitant use of other supplements or AAS. It is therefore important to fully understand the clinical and laboratory manifestations of hepatotoxicity associated with each drug. In addition, factors such as latency period and prognosis should be considered to provide optimal support for each individual case. In this case series of 18 individuals, we aim to highlight the distinctive phenotypic characteristics of idiosyncratic drug-induced liver injury (DILI) caused by stanozolol.

Methods

Cases were included after prospective evaluation by specialists from the Latin American DILI Registry between 2013 and 2023. Information such as latency, route of administration, dose, purpose of use, duration of symptoms, and concomitant use of other substances was collected. Serologies for viral hepatitis (IgM antibody to hepatitis A virus, hepatitis B surface antigen, IgM anti-HBc, anti-hepatitis C virus, IgM anti-cytomegalovirus, and IgM anti-Epstein-Barr virus) and autoimmune markers (ANA, AMA, SMA, Anti-LKM1) were performed to exclude other etiologies of liver damage. All cases had liver biochemistry available during follow-up, including ALT, AST, GGT, ALP, bilirubin, albumin, and prothrombin time.

Abdominal ultrasonography or magnetic resonance imaging of the biliary tract revealed no biliary obstruction. Liver biopsies were performed in only 3 cases. All included patients had no history of excessive alcohol consumption, defined as an intake of more than 20 g of alcohol per day.

DILI was diagnosed using the Roussel Uclaf Causality Assessment Method (RUCAM), with cases classified as probable or highly probable. The pattern of liver injury was defined according to the R value, with hepatocellular injury defined as R >5, mixed injury as R between 2 and 5, and cholestatic injury as R <2.

Twelve cases followed a standardized report form, that was meticulously completed by the responsible clinician. Subsequently, a detailed case description was sent to the coordinating physician within each participating country. The description underwent initial scrutiny and evaluation before being forwarded to the coordinating center situated at the University of Malaga in Spain. There, it underwent a thorough reevaluation process conducted by a panel consisting of three experts specializing in DILI. Only after this rigorous assessment was the case deemed eligible for inclusion in the comprehensive database. The operational framework of this network, including data recording and case ascertainment procedures, has been comprehensively described in a previous publication. The remaining six cases were not included in the SLATINDILI database as they were monitored in Brazilian centers that were not part of this collaborative network. However, all these cases were supervised by experienced hepatologists at prominent university-affiliated institutions, and the case details were reviewed by hepatologists who were active participants in the network. All necessary information was readily available and appropriately presented to validate these cases.
After DILI diagnosis, patient management varied according to specialist preference and included the use of medications such as ursodeoxycholic acid, rifampicin, sertraline, cholestyramine, and antihistamines to alleviate symptoms.

Exclusion criteria included previous liver disease or use of other medications or supplements with potential hepatotoxic effects where causality assessments indicated a higher probability of liver damage due to causes other than stanozolol. Patients with inaccessible data were also excluded. Written informed consent was obtained from all patients. The study adhered to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional review board of the Federal University of Bahia, Brazil.

Descriptive analyses were conducted using R software

Results

Between 2013 and 2023, specialists from the Latin America DILI Registry prospectively evaluated 18 reports of liver damage induced by stanozolol. Twelve of these cases occurred in Brazil, two in Argentina, two in Uruguay, and two in Spain.

The cohort comprised 18 young men (19–48 years) without comorbidities, using stanozolol intramuscularly for muscle hypertrophy. Exact dosages, reported in seven cases, ranged from 50–200 mg daily or three times weekly for up to 8 weeks. The mean latency to symptoms onset was 55 days of use (Table 1).

The Roussel Uclaf Causality Assessment Method (RUCAM) was applied, classifying causality as probable or highly probable in all patients.

All patients presented with jaundice and pruritus. Other common symptoms were nausea, weight loss and fatigue. The time for symptoms resolution and laboratory return to normal ranged from 60 to 365 days, with a median value of 153 days, with one patient remains under monitoring, without complete resolution to date. In addition, ten patients reported concurrent use of other drugs, such as testosterone cypionate, nandrolone, BCAA, whey protein, drostanolone, testosterone enanthate, trenbolone, and durateston. No deaths or liver transplants were reported.

Figure 1 illustrates a characteristic enzymatic pattern observed in these cases over time, while Figure 2 presents the pattern of enzymatic changes in DILI caused by stanozolol. Total bilirubin (TB) levels were elevated in all cases, with a mean of 23.3 mg/dl, with levels exceeding 30 mg/dL in 38.8% of patients, while gamma-glutamyl transferase (GGT) remained within the normal range or slightly elevated (mean value of 53.7 IU/L and normal value below 60 IU/L). Although GGT levels were below 90 IU/L in all cases, the majority of cases (77.7%) had elevated alkaline phosphatase (ALP) levels, with a mean of 264.8 IU/L, which is equivalent to 2 times the upper limit of normality. Prothrombin time was within the normal range in all patients, with a minimum value of 85%.

Aminotransferases were slightly elevated, with mean values of 80 U/L for aspartate aminotransferase (AST) and 165.4 U/L for alanine aminotransferase (ALT), approximately four times the upper limit of normal. The mean R value was 3.7, with 55.5% of patients presenting a cholestatic injury pattern, 22.2% presenting a mixed pattern, and 16.6% presenting a hepatocellular injury pattern.

In the few patients who underwent liver biopsy, the findings were consistent with bland cholestasis, predominantly in zone 3, with minimal inflammatory cell infiltration.

Discussion

All cases involved young males aged between 19 and 48, presenting with jaundice and pruritus after using stanozolol for aesthetic purposes. Elevated total bilirubin levels and alkaline phosphatase were noted in all cases, while gamma-glutamyl transferase levels remained within or slightly above normal limits.

The use of anabolic steroids has been a part of clinical practice since the 1950s, primarily for the treatment of hypogonadism.[3] Although their aesthetic use has been recognized since the drug's inception, the pattern of use has evolved. Once restricted to bodybuilders or specific niches, AAS use has become increasingly popular among non-athletes, with a significant rise in consumption, often without medical supervision. This trend now affects approximately 3% of the American population and 70% of individuals attending fitness centers, predominantly young men, as evidenced by our case series.[8,9]

Stanozolol is a synthetic steroid that was introduced to the market in 1962. Major side effects include cardiovascular, psychiatric, and reproductive disorders.[10] Regarding hepatotoxicity, the characteristic presentation of AAS typically involves cholestatic injury with elevated bilirubin levels,[11] accompanied by minimal inflammation, a pattern known as “bland cholestasis.”[12] Additionally, there is a higher likelihood of associated renal injury.[4] Isolating stanozolol cases revealed that despite cholestatic injury and elevated bilirubin levels, outcomes were favorable following discontinuation, irrespective of treatment, though resolution could be prolonged.

Another notable feature was the finding that, despite hyperbilirubinemia, aminotransferases averaged below 165 U/L, and alkaline phosphatase was elevated to more than twice the normal range in all cases, while GGT remained consistently below 80 U/L, close to normal levels. This resembles what occurs in familial intrahepatic cholestasis.[13] This concept is supported by other cases described in the literature, including immunohistochemical staining of BSEP (bile salt export pump) in liver biopsies from patients with stanozolol-induced drug-induced liver injury (DILI), which showed an absence of BSEP expression, suggesting mutations in the ABCB11 gene.[5,7] This observation may indicate a dependence on bile salt exporters in stanozolol models.

The strengths of our article lie in gathering rare cases of DILI evaluated by experts in the field, from multiple centers, secondary to a single drug, and establishing a unique pattern, facilitating its recognition, early diagnosis, and prognosis. However, we acknowledge certain limitations. The data were based on a limited number of cases, albeit rare ones. Only severe cases tend to seek specialized care, creating a selection bias in which mild or subclinical cases are not reported. Additionally, serological testing for hepatitis E and liver biopsy were conducted in only a limited number of subjects.

Conclusions

Liver injury induced by stanozolol has been predominantly observed in a population of young men using it for aesthetic purposes. These patients exhibited high total bilirubin levels, modest aminotransferase elevations, and GGT levels close to the normal range. This lays the foundation for further investigations into stanozolol’s hepatotoxic effects,

thereby contributing to improved clinical management and awareness regarding AAS misuse-associated hepatotoxicity.

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Patient	Age (years)	Latency (days)	TB Mg/DL	AST U/L	ALT U/L	ALP U/L	GGT U/L	R
1	21	45	15.2	52	72	258	17	0.9
2	31	45	15	99	147	146	36	3.2
3	26	180	9.7	60	103	217	53	1.5
4	29	15	15.7	53	61	100	75	2.0
5	29	30	45	38	50	139	71	1.1
6	25	-	31	47	49	250	45	0.6
7	22	-	21	48	-	241	80	-
8	29	45	36	-	46	319	13	0.4
9	37	45	44.3	105	98	281	89	1.1
10	31	50	32	-	98	775	42	0.4
11	37	80	5.1	-	355	253	51	4.6
12	36	99	38	-	104	689	52	0.4
13	26	62	49	-	228	387	22	1.9
14	40	25	3.9	195	399	101	117	12.9
15	34	75	18.7	62	88	66	39	4.4
16	36	35	-	50	67	276	72	0.7
17	19	39	10.7	75	374	195	17	6.2
18	48	20	6.81	156	474	74	77	21
Mean	**30.8**	**55.6**	**23.3**	**80**	**165.4**	**264.8**	**53.7**	**3.7**

Table 1: Clinical profile and main laboratory findings
TB: total bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl-transferase

Figure 1. The trend of liver biochemistry over time in patients with DILI caused by stanozolol

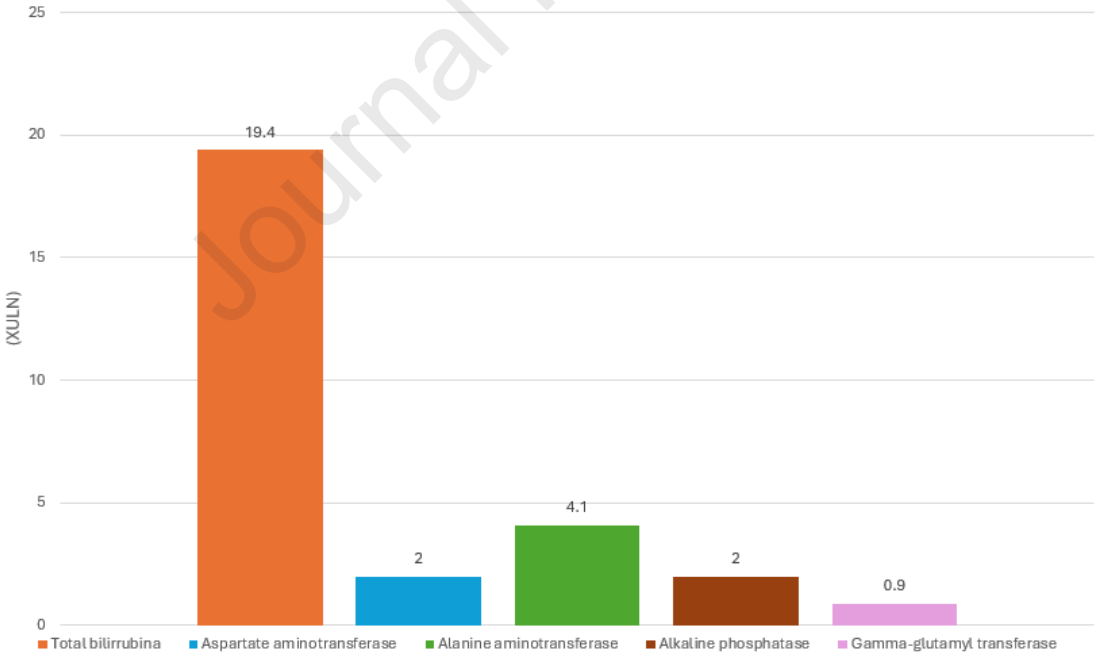
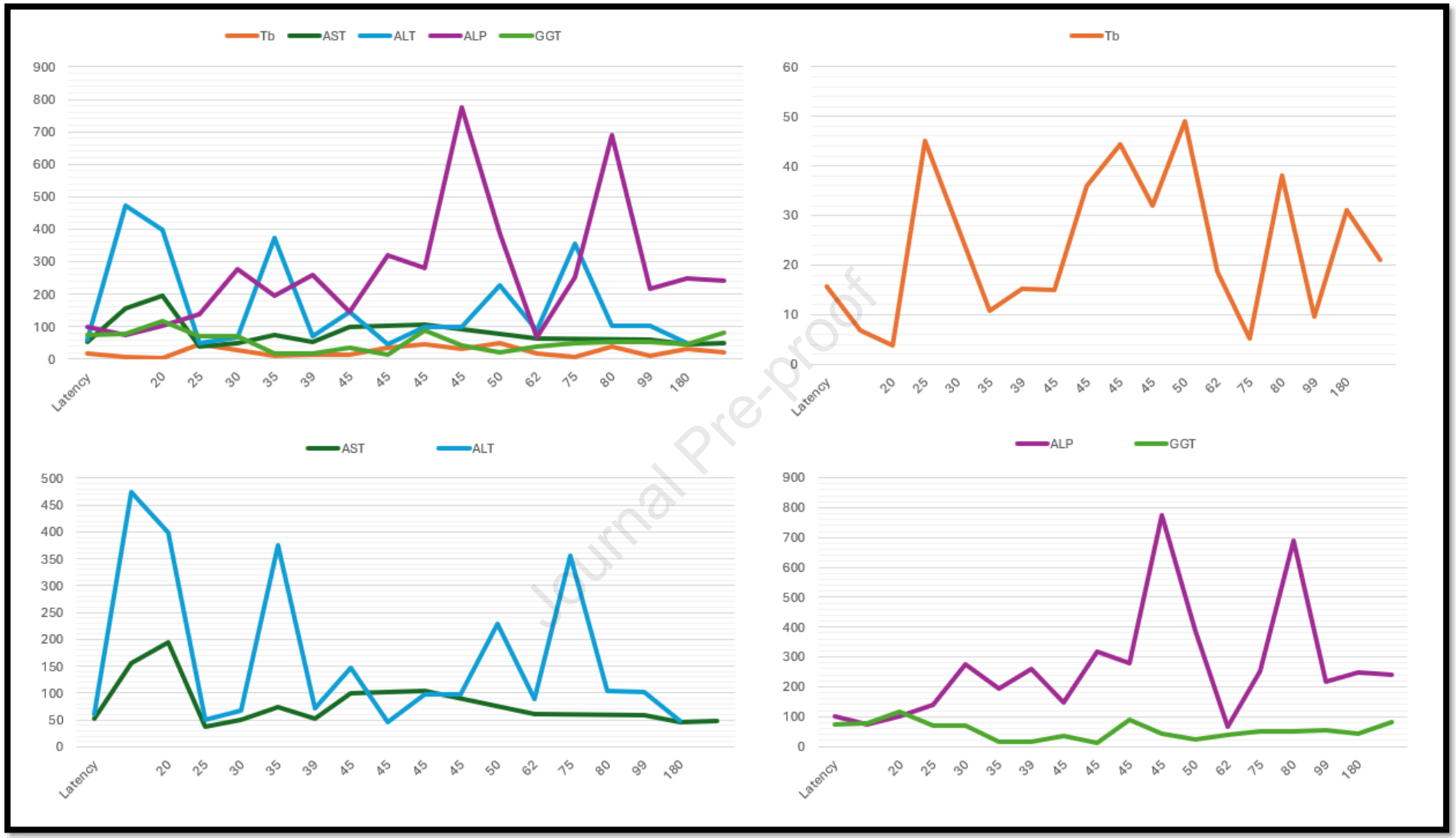


Figure 2. The pattern of enzymatic changes found in DILI by Stanozolol: significant increase in total bilirubin in almost twenty times the upper limit of normality, with GGT close to normal values
TB: total bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl-transferase



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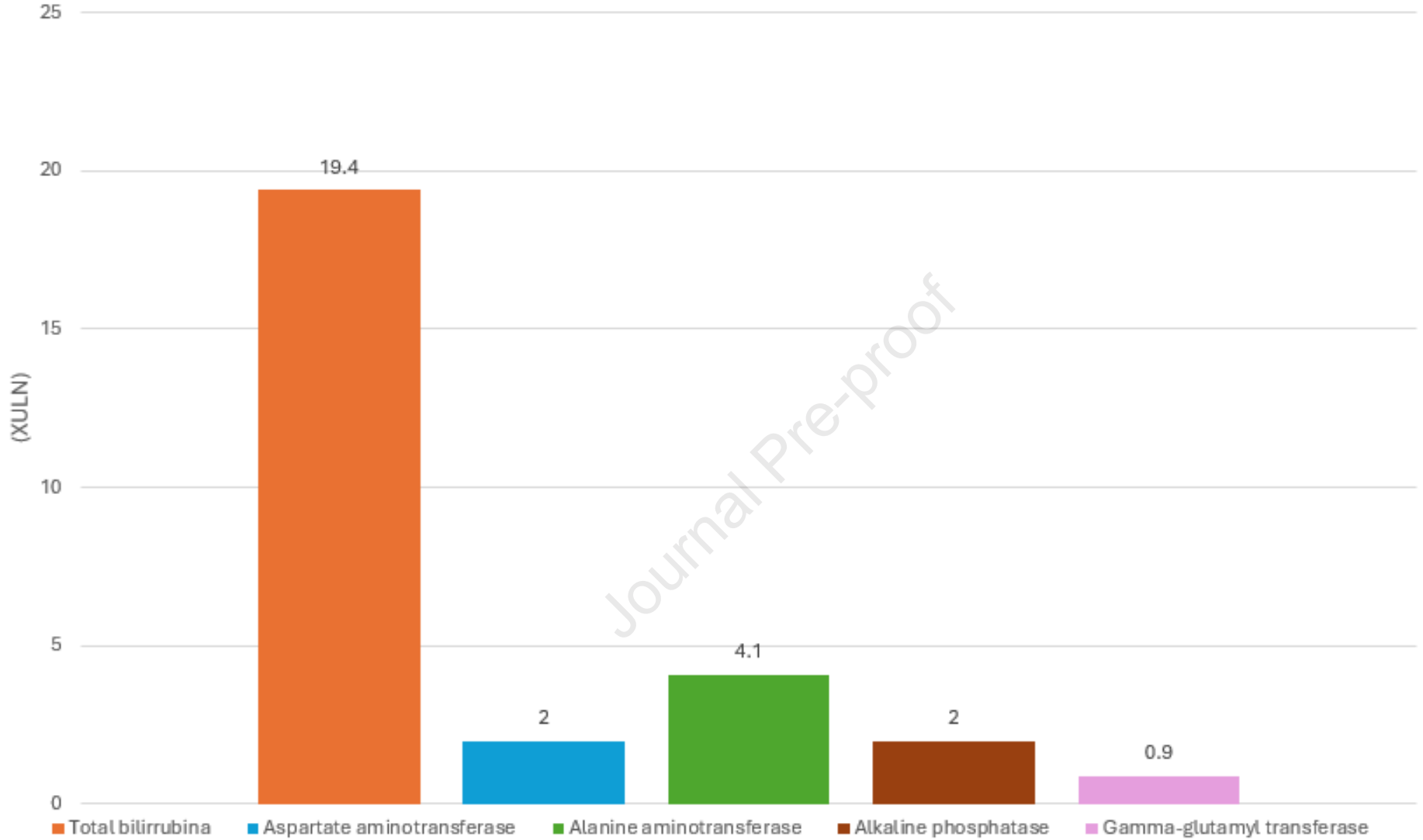

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Dear Editor in chief,

The authors declare that there is no conflict of interest regarding the topic of the work.

Sincerely,

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