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MARCADORES BIOQUÍMICOS E DE ESTRESSE OXIDATIVO NO FÍGADO E NOS RINS DE RATOS SUBMETIDOS A DIFERENTES PROTOCOLOS DE UTILIZAÇÃO DE ESTEROIDES ANABOLIZANTES

Santa Maria, RS
2016

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-graduação em Medicina Veterinária, Área de concentração em Patologia e Patologia Clínica Veterinária, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de **Mestre em Medicina Veterinária**

Orientadora: ProfªDrª Cinthia Melazzo de Andrade

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Santa Maria, RS
2016

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RESUMO

MARCADORES BIOQUÍMICOS E DE ESTRESSE OXIDATIVO NO FÍGADO E NOS RINS DE RATOS SUBMETIDOS A DIFERENTES PROTOCOLOS DE UTILIZAÇÃO DE ESTEROIDES ANABOLIZANTES

AUTOR: Guilherme Lopes Dornelles
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Esteroides anabólicos androgênicos (EAA) são substâncias sintéticas derivadas da testosterona que promovem crescimento muscular e ganho de força. Devido a isso, são utilizadas ilegalmente para melhora da performance atlética de equinos, caninos ou atletas ou para maior produção de carne. As doses variam de 10 a 100 vezes a recomendação terapêutica, o que potencializa os efeitos deletérios em diversos órgãos. O objetivo deste trabalho foi avaliar os efeitos de diferentes protocolos de administração (P1, P2 e P3) de undecilenato de boldenona (UB) e estanozolol (EST) em indicadores da função e lesão, bem como, parâmetros oxidativos hepático e renal. Para isso, foram utilizados 54 ratos Wistar, machos, distribuídos em nove grupos com seis animais cada que receberam por via intramuscular 5 mg/kg de UB ou EST uma vez por semana durante 4 semanas (P1); 2,5mg/kg de UB ou EST uma vez por semana durante 8 semanas (P2) e 1,25mg/kg de UB ou EST uma vez por semana durante 12 semanas (P3). Cada protocolo teve um grupo controle (GC) que recebeu 0,1 mL de azeite de oliva intramuscular. Posteriormente a eutanásia, realizada uma semana após o último tratamento, foi avaliada a atividade sérica da alanina aminotransferase (ALT) e fosfatase alcalina (FA), os níveis séricos de albumina, creatinina, colesterol, proteínas totais, triglicerídeos, ureia, espécies reativas de oxigênio (ERO), substâncias reativas ao ácido tiobarbitúrico (TBARS), glutationa reduzida (GSH) e tiois totais (T-SH). No protocolo P1 obteve-se atividade sérica de ALT e concentração de colesterol significativamente ($p<0,05$) maiores comparando-se o grupo UB com o grupo GC. O grupo EST obteve aumento significativo ($p>0,05$) da ALT em relação ao grupo controle no protocolo P2. No tecido hepático, comparando-se os grupos UB e GC, obteve-se níveis maiores ($p<0,05$) de ERO e TBARS em P1 e P3 e concentração menor ($p<0,05$) de GSH em P3. O grupo EST apresentou valores maiores ($p<0,05$) de ERO no P1 e P3, de TBARS no P3 e níveis menores ($p<0,05$) de GSH no P3 quando comparado ao grupo GC. A concentração hepática de T-SH foi menor ($p<0,05$) no P2 comparando UB e EST ao grupo GC. No tecido renal, ao comparar o grupo UB com o grupo GC obteve-se níveis significativamente maiores ($p<0,05$) de ERO e TBARS nos protocolos P1 e P2 e menores ($p<0,05$) de GSH e T-SH nos protocolos P1, P2 e P3. Comparando-se os grupos EST e GC os níveis de GSH foram menores ($p<0,05$) no P2 e P3. O grupo EST apresentou níveis maiores ($p<0,05$) de ERO nos protocolos P2 e P3, de TBARS no P3, concentração menor ($p<0,05$) de GSH no P2 e P3 e níveis menores de T-SH no P2 e P3 quando comparado ao grupo GC. Neste estudo foi possível concluir que os anabolizantes são prejudicias mesmo quando utilizados em baixas doses ou em poucas aplicações, visto que em todos os protocolos avaliados foi possível observar alterações do balanço redox no fígado e rins dos ratos.

Palavras-chave: Undecilenato de boldenona. Estanozolol. Dano oxidativo. Hepatotoxicidade. Esteroides anabólicos androgênicos.

ABSTRACT

BIOCHEMICAL AND OXIDATIVE STRESS MARKERS IN THE LIVER AND KIDNEYS OF RATS SUBMITTED TO DIFFERENT PROTOCOLS OF ANABOLIC STEROIDS

AUTHOR: Guilherme Lopes Dornelles
ADVISOR: Cinthia Melazzo de Andrade

Anabolic androgenic steroids (AAS) are synthetic substances derived from testosterone that promote greater muscle mass and strenght. Thus, they are used illegally to improve athletic performance of horses, dogs or athletes or to improve meat production. The doses, ranging from 10 to100 times the therapeutic recommendation, enhances the deleterious effects on various organs. The objective of this study was to evaluate the effects of different protocols (P1, P2 and P3) of boldenone undecylenate (BU) and stanozolol (ST) on markers of liver and kidney function and variables of oxidative stress in these organs. For this, 54 male Wistar rats were divided into nine groups of six animals each. Each animal received intramuscularly 5.0 mg kg^{-1} of BU or ST once a week for four weeks (P1); 2.5 mg kg^{-1} of BU or ST once a week for eight weeks (P2); and 1.25 mg kg^{-1} of BU or ST once a week for 12 weeks (P3). For each protocol, a control group was used (CG), and they received 0.1 ml of olive oil intramuscularly. Blood, and fragments of liver and kidney were collected for alanine aminotransferase activity (ALT), alkaline phosphatase (ALP), albumin, creatinine, cholesterol, total protein, triglycerides, urea, reactive oxygen species (ROS), thiobarbituric acid reactive substances (TBARS), total thiols (T-SH), and glutathione (GSH) evaluation. Seric ALT activity and cholesterol concentration were significantly ($p<0.05$) higher compared to CG when BU of protocol P1 was used. ALT activity was significantly higher ($p<0.05$) compared to the CG in protocol P2 when ST was used. Liver samples showed higher levels ($p<0.05$) of ROS and TBARS in protocols P1 and P3 when BU was used, and lower GSH activity ($p<0.05$) on group treated with protocol P3. Rats that have received ST under protocol P1 and P3 showed higher levels ($p<0.05$) of ROS, as well as increased TBARS levels in P3 but lower GSH activity in P3 ($p<0.05$) when compared to the CG. In the liver, the T-SH concentration was lower ($p<0.05$) in P2 when compared BU and ST of the CG. In renal tissues, ROS and TBARS levels were significantly higher ($p<0.05$) in animals that received BU under protocols P1 and P2; and GSH activity and T-SH levels were reduced in the three protocols (P1, P2 and P3). In addition, animals treated with ST occurred showed reduced renal levels of GSH levels ($p<0.05$) in P2 and P3. The treatment with ST also led to higher ROS levels ($p<0.05$) in P2 and P3, and TBARS levels in P3, but reduced concentration ($p<0.05$) of GSH levels in P2 and P3, and T-SH in P2 and P3. In conclusion, anabolic steroids are harmful even when used in low doses or in a few applications, since in all evaluated protocols was possible to observe changes in the redox balance in the liver and kidneys.

Keywords: Boldenone Undecylenate. Stanozolol. Oxidative damage. Hepatotoxicity. Anabolic androgenic steroids.

LISTA DE FIGURAS

INTRODUÇÃO
Figura 1 – Estrutura química da testosterona..11
Figura 2 – Estrutura química da boldenona...13
Figura 3 – Estrutura química do estanozolol. 13
MANUSCRITO
Fig. 1. Oxidative stress markers in the liver of rats treated with different protocols of boldenoneundecylenate (BU) and stanozolol (ST). Reactive oxygen species (ROS) (A, B and C), thiobarbituric acid reactive substances (TBARS) (D, E and F), glutathione (GSH) (G, H e I) and total thiols (T-SH) (J, K e L) in the following protocols: Protocol 1 (P1) (A, D, G, and J), Protocol 2 (P2) (B, E, H, and K) Protocol 3 (P3) (C, F, I, and L). Data were expressed as mean ± standard error. Different letters indicate significant differences between groups (p<0.05; n = 6). 27
Fig. 2. Oxidative stress markers in the kidney of rats treated with different protocols of administration of boldenoneundecylenate (BU) and stanozolol (ST). Reactive oxygen species (ROS) (A, B and C), thiobarbituric acid reactive substances (TBARS) (D, E and F), glutathione (GSH) (G, H e I) and total thiols (T-SH) (J, K e L) in the following protocols: Protocol 1 (P1) (A, D, G, and J), Protocol 2 (P2) (B, E, H, and K) Protocol 3 (P3) (C, F, I, and L). Data were expressed as mean ± standard error. Different letters indicate significant differences between groups (p<0.05; n = 6). 28

LISTA DE TABELAS

MANUSCRITO

Table 1. Seric levels of albumin, cholesterol, creatinine, total protein, triglycerides, urea, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in rats submitted to protocol 1 (P1), which included administration of 5 mg kg^{-1} boldenone undecylenate (BU) and stanozolol (ST) compared to the control group (CG). 24

Table 2. Seric levels of albumin, cholesterol, creatinine, total protein, triglycerides, urea, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in rats submitted to protocol 2 (P2), which included administration of 2.5 mg kg^{-1} boldenone undecylenate (BU) and stanozolol (ST) compared to the control group (CG). 25

Table 3. Seric levels of albumin, cholesterol, creatinine, total protein, triglycerides, urea, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in rats submitted to protocol 3 (P3), which included administration of 1.25 mg kg^{-1} boldenone undecylenate (BU) and stanozolol (ST) compared to the control group (CG). 25

LISTA DE ABREVIATURAS E SIGLAS

INTRODUÇÃO

EAA	Esteroides anabólicos androgênicos
ERN	Espécies reativas de nitrogênio
ERO	Espécies reativas de oxigênio
EST	Estanozolol
Fe^{2+}	Ferro ferroso
Fe^{3+}	Ferro férrico
H_2O_2	Peróxido de hidrogênio
MAPA	Ministério da Agricultura, Pecuária e Abastecimento
O_2	Oxigênio
$O_2^{\bullet -}$	Ânion superóxido
UB	Undecilenato de boldenona

MANUSCRITO

AAS	Anabolic androgenic steroids
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
BU	Boldenone undecylenate
CG	Control group
DCF	2'-7'-dichlorofluorescein
DCFH-DA	2'-7'-dichlorofluorescein diacetate
DTNB	5-5-dithio-bis-(2-nitrobenzoic acid)
GSH	Glutathione
MDA	Malondialdehyde
P1	Protocol 1
P2	Protocol 2
P3	Protocol 3
ROS	Reactive oxygen species
ST	Stanozolol
TBARS	Thiobarbituric acid reactive substances
T-SH	Total thiols

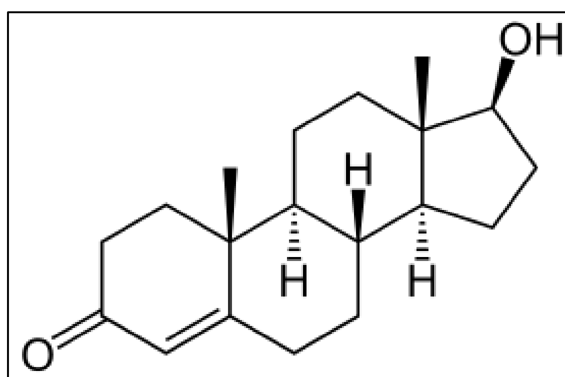
SUMÁRIO

1 INTRODUÇÃO 11
2 MANUSCRITO 18
ABSTRACT. 19
1. Introduction..20
2. Materials and methods 21
2.1 Animals..21
2.2 Experimental procedure 21
2.3 Serum analyses..22
2.4 Preparation of liver and kidney homogenates..22
2.5 Measurement of intracellular reactive oxygen species (ROS) production 22
2.6 Thiobarbituric acid reactive substance (TBARS) measurement 23
2.7 Measurement of reduced glutathione (GSH) 23
2.8 Determination of total thiols 23
2.9 Statistical analyses..24
3. Results 24
3.1 Serum parameters..24
3.2 Liver..26
3.3 Kidney 26
4. Discussion 29
5. References 32
3 CONCLUSÃO 37
4 REFERÊNCIAS..38

1 INTRODUÇÃO

Hormônio é uma substância química, secretada em pequenas quantidades na circulação sanguínea, que produz uma resposta fisiológica nos tecidos-alvo. Quimicamente, os hormônios são classificados como aminas, esteroides ou proteínas e peptídeos (COSTANZO, 1999). Os hormônios esteroides incluem os hormônios adrenocorticais, metabólitos ativos da vitamina D e os produzidos pelas gônadas, que possuem o colesterol como precursor comum (BERNE; LEVY, 2000; SILVA et al., 2002). A testosterona (Figura 1) é o hormônio esteroide androgênico mais importante produzido pelas células de Leydig nos testículos (SMITH et al., 1988).

Figura 1 – Estrutura química da testosterona



Fonte: (KICMAN, 2008).

Os esteroides anabólicos androgênicos (EAA) são hormônios esteroides da classe de hormônios sexuais masculinos (HANDELSMAN, 2001) e incluem a testosterona e seus derivados (THEIN et al., 1995; FERRERA et al., 1997). Entretanto, alguns autores definem os EAA como os derivados sintéticos da testosterona (HOBERMAN; YESAILIS, 1995; GOLDBERG et al., 2000). Essas substâncias foram sintetizadas com o objetivo de aumentar a atividade biológica da molécula *in vivo*, produzir andrógenos oralmente ativos e desenvolver produtos que sejam menos androgênicos e mais anabólicos (SHAHIDI, 2001).

Os mecanismos de ação propostos para os EAA incluem a modulação da expressão dos receptores androgênicos como consequência do (1) metabolismo

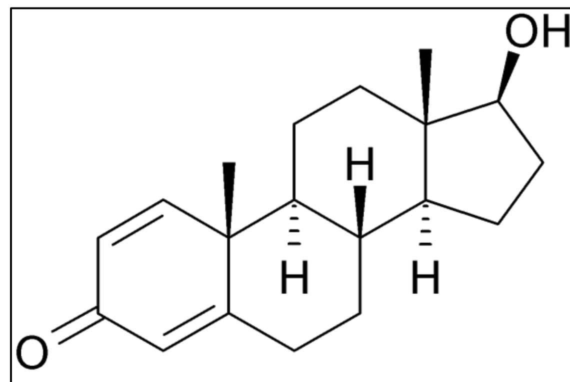
intracelular e (2) afetando diretamente a topologia do receptor androgênico e então subsequente interação com co-ativadores (SHP e DAX-1) e atividade transcripcional. Além de (3) efeito anticatabólico por interferir com a expressão de receptores de glicocorticoides; e (4) por vias não-genômicas e genômicas no sistema nervoso central, resultando em mudanças comportamentais (KICMAN, 2008).

Os EAA exercem seus efeitos anabólicos e androgênicos nos tecidos reprodutivos e não reprodutivos. Suas ações androgênicas são responsáveis pelo desenvolvimento do trato reprodutivo masculino e desenvolvimento de características sexuais secundárias, enquanto que os efeitos anabólicos estimulam a fixação de nitrogênio, aumento da síntese de proteína e eritropoiese (MOTTRAM; GEORGE, 2000; SHAHIDI, 2001). A administração de anabolizantes em doses supra fisiológicas podem induzir hipertrofia das fibras musculares do tipo I e II (KADI, 2008) e são efetivos em aumentar a massa muscular esquelética e a força em machos eugonadais (KICMAN, 2008), o que faz com que essas drogas sejam utilizadas para melhora do desempenho atlético de caninos, equinos e atletas (SCHÄNZER, 1996). Devido a razões de ordem ética e aos efeitos nocivos à saúde, essas substâncias tiveram seu uso em competições proibido a partir de 1976 na Olimpíada de Montreal, onde foi realizado pela primeira vez o controle dessas drogas (MARQUES et al., 2003), atualmente monitoradas pela Agência Mundial Antidoping (WADA), criada em 1999.

O undecilenato de boldenona (17β-Boldenona, 1-dehidrotestosterona ou androsta-1,4-diene-17β-ol-3-one; UB; Figura 2) é classificada como um hormônio anabolizante esteroide androgênico de cadeia carbônica extensa obtida a partir da dehidrogenação da testosterona. Difere desta somente por uma ligação dupla na posição 1 (KUHN, 2002; de BRABANDER et al., 2013).

Assim como os outros anabolizantes, o UB é classificado pela Agência Internacional de Pesquisa sobre o Câncer na classe 2A (promotores de crescimento – esteroides) como um provável carcinogênico. Possui índice carcinogênico maior que outros andrógenos, como nandrolona, testosterona e stanozolol (de BRABANDER et al., 2013). É uma droga de uso veterinário disponível comercialmente com os nomes de Equipoise, Ganabol, Equigan e Ultragan. Promove crescimento muscular por promover balanço positivo de nitrogênio, que estimula a produção e reduz a destruição de proteína, bem como causando retenção corporal de água, nitrogênio, sódio, potássio e cálcio (MOORADIAN et al., 1987). Essa molécula tem uma meia-vida muito longa e pode aparecer em testes anti-dopping por até 1,5 anos (HOFFMANN, 2002).

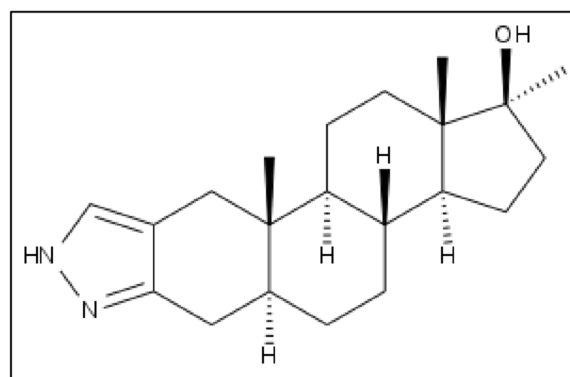
Figura 2 – Estrutura química da boldenona



Fonte: (KICMAN, 2008).

Estanozolol (EST) (Figura 3) é um composto sintético 17α-alquilado, derivado da testosterona que exibe alto potencial anabólico e reduzida degradação hepática quando comparado ao hormônio natural masculino (PEY et al., 2003). Esse composto é um anabolizante popular utilizado por fisiculturistas e por atletas para melhora da performance atlética (MAINI et al., 2014). Devido à sua alta biodisponibilidade e por permanecer inalterado pela primeira passagem no metabolismo hepático o EST pode ser administrado por via oral e, devido a isso, é mais utilizado que os outros EAA (DESHMUKH et al. 2010). O EST é usualmente considerado seguro, mesmo em altas doses (MAINI et al. 2014).

Figura 3 – Estrutura química do estanozolol



Fonte: (KICMAN, 2008).

EAA são utilizados de forma terapêutica para terapia de reposição de andrógenos (devido a desordens no hipotálamo, pituitária ou genéticas) e em pacientes com doenças crônicas para melhora da qualidade de vida através dos efeitos benéficos dos andrógenos (HANDELSMAN, 2001), como estimulação da eritropoiese, aumento da densidade óssea, ganho de peso em pacientes caquéticos (SHAHIDI, 2001).

O abuso de EAA dentro e fora do meio esportivo tem sido uma preocupação para a sociedade e governos, bem como para as agências mais importantes da saúde e esportes, incluindo a Organização Mundial da Saúde (OMS) e o Comitê Olímpico Internacional (COI) (SILVA et al., 2007). No Brasil, onde EAA são drogas ilícitas, resultados de um estudo mostraram que a prevalência da utilização desses compostos varia entre 2,1% a 31,6% de acordo com a região estudada e as características da amostra (ABRAHIN et al., 2014). A maioria dos usuários dessas substâncias utilizam protocolos de administração de oito semanas com doses que variam de 10 a 100 vezes a dose terapêutica, o que pode causar vários efeitos adversos (YERSALIS; BAHRKE,1995). Em adição, as doses de EAA são compostas por mais de um anabolizante, administrado por via oral ou intramuscular (SANCHEZ-OSORIO et al., 2008).

O uso dessa substância é proibido na Europa pelas diretivas 96/22/EC e 03/74/EC para proteger a saúde do consumidor. Nos Estados Unidos, os anabolizantes não são indicados para o uso em humanos e somente podem ser adquiridos em clínicas veterinárias (HOFFMANN, 2002; GABR et al., 2009). No Brasil, a utilização dessas drogas é restrita, conforme estabelecido pela Resolução N°2 de 2/5/2004 do Ministério do Esporte, acompanhando a legislação internacional (Legislação Brasileira de Produtos Controlados):

> Lei Nº9.965 de 27/abril/2000: “A dispensação ou a venda de medicamentos do grupo terapêutico dos esteroides ou peptídeos anabolizantes para uso humano estarão restritas à apresentação e retenção da receita emitida por médico ou dentista” (Legislação Brasileira de Produtos Controlados).

A utilização de promotores de crescimento em animais de produção possibilita melhor conversão alimentar, maior produção de carne, aumento na produção de leite ou redução da gordura corporal (CHIESA et al, 2014). No entanto, a maioria dos

países proíbe a utilização de anabolizantes com o objetivo aumentar a produção de carne (KUHN, 2002; CANNIZZO et al., 2007; SOMA et al., 2007). No Brasil, o uso dessas substâncias em aves de produção e na pecuária bovina é proibida, conforme estabelecido pelo Ministério da Agricultura, Pecuária e Abastecimento (MAPA):

> 1. Instrução Normativa nº17 de 18/junho/2004: ''Proibir a administração, por qualquer meio, na alimentação e produção de aves, de substâncias com efeitos tireostáticos, androgênicos, estrogênicos ou gestagênicos, bem como de substâncias ß-agonistas, com a finalidade de estimular o crescimento e a eficiência alimentar'' (MAPA);
> 2. Instrução Normativa n°55 de 01/dezembro/2011: ''Proibir a importação, a produção, a comercialização e o uso de substâncias naturais ou artificiais, com atividades anabolizantes hormonais, para fins de crescimento e ganho de peso em bovinos de abate'' (MAPA).

Dentre os efeitos colaterais mais comuns decorrentes da utilização de EAA, estão descritos na literatura: neoplasia hepática e carcinoma (VELAZQUEZ; ALTER, 2004), desordens psiquiátricas e comportamentais (CLARK; HENDERSON, 2003), fertilidade reduzida (DOHLE et al., 2003), coagulação sanguínea (PÄRSSINEN; SEPPÄLÄ, 2002), falência renal (YOSHIDA et al, 1994) hipertensão (FERENCHICK, 1990) e aterosclerose (COHEN et al., 1988). Estudos em modelos animais demonstraram que a maioria dos efeitos dos EAA observados nos humanos também ocorrem nos animais, como aumento da massa muscular (LIONIKAS; BLIZARD, 2008); lesões patológicas no fígado e rim (TOUSSON, 2013) e coração de coelhos (VASILAKI et al., 2016); aumento de agressão em ratos e alterações neurodegenerativas (NAMJOSHI, 2016), bem como alterações reprodutivas (ODA; EL-ASHMAWY, 2012).

Apesar de serem substâncias de uso ilegal, essas drogas são facilmente obtidas. O abuso de EAA pode levar a danos sérios e irreversíveis em diversos órgãos (MARAVELIAS et al. 2005), como fígado e rins (CERRETANI, 2013). O fígado é responsável pelo metabolismo de carboidratos, lipídeos, proteínas, hormônios e vitaminas; detoxificação e excreção de produtos e substâncias tóxicas; digestão de gorduras; e produção da maioria dos fatores de coagulação. É um órgão altamente vascularizado que recebe sangue pela artéria hepática e pela veia portal, a qual é responsável por 70-75% do sangue que chega ao fígado (ALLISON, 2012). A função

e estrutura hepática são severamente alteradas por altas doses de EAA, o que aumenta as atividades séricas das enzimas hepáticas alanina aminotransferase, aspartato aminotransferase e lactato desidrogenase além de poder induzir o aparecimento de desordens severas, como peliose hepática, hiperplasia celular e adenoma hepatocelular (SHAHIDI, 2001).

Os rins são responsáveis por excretar a maior parte dos produtos terminais do metabolismo corporal e controlar as concentrações da maioria dos constituintes dos líquidos orgânicos (GUYTON; HALL, 2009). Os anabolizantes são comumente excretados na urina principalmente como conjugados glicurônicos. Essa formação é catalisada pela enzima uridina difosfato-glicuronosil-transferase (UGT). A glucuronidação dos esteroides e seus metabólitos de fase I é uma importante via metabólica de detoxificação e desativação que pode explicar, em parte, os efeitos renais dos EAA (DESHMUKH et al., 2010). O abuso de EAA pode provocar aumento da creatinina, ureia e ácido úrico. Além disso, usuários dessas substâncias possuem altos ricos de desenvolverem tumor de Wilms (JUHN, 2003), neoplasia renal maligna rara em adultos (ALBUQUERQUE et al., 2004).

Os efeitos colaterais dos anabolizantes, como alterações hepáticas e renais, estão envolvidos com o estresse oxidativo, que é caracterizado pelo desbalanço redox (FRANKENFELD et al., 2014). O organismo produz constantemente diversas espécies reativas (ER), tais como por exemplo as espécies reativas de oxigênio (ERO) e de nitrogênio (ERN), entre outras as quais atuam fisiologicamente em funções como: fagocitose; sinalização celular; controle da pressão sanguínea; apoptose; e envelhecimento (FERNANDEZ et al., 2007). O termo ''espécies reativas'' refere-se a radicais livres e outras moléculas que são igualmente reativas, como por exemplo o peróxido de hidrogênio (H_2O_2), ozônio (O_3), nitritos (NO_2^-) e nitratos (NO_3^-) (BARREIROS et al., 2006). Os radicais livres são átomos ou moléculas que possuem número ímpar de elétrons em sua última camada, o que confere alta reatividade a esses átomos ou moléculas (FERREIRA; MATSUBARA, 1997).

Metais de transição, como ferro ou cobre, podem doar ou aceitar elétrons livres durante reações intracelulares, catalisando a formação de radicais livres. H_2O_2 pode reagir com o ferro (reação de Fenton) na sua forma ferrosa (Fe^{2+}) produzindo ferro na forma férrica (Fe^{3+}) e radical hidroxila ($^{\cdot}OH$), que é o radical livre mais reativo e lesivo e para o qual o organismo humano não possui nenhum mecanismo de defesa. A maior parte do ferro intracelular é Fe^{3+}, e por isso ele primeiro precisa ser reduzido a Fe^{2+}

para participar da reação de Fenton (FERNANDEZ et al., 2007; VASCONCELOS et al., 2007).

Para equilibrar a produção de espécies reativas, o organismo possuiu mecanismos antioxidantes que são classificados como antioxidantes enzimáticos e não enzimáticos. Os enzimáticos são compostos pela superóxido dismutase (SOD), que catalisa a dismutação do ânion superóxido ($O_2^{\bullet -}$) a H_2O_2 e oxigênio (O_2); a catalase (CAT) que decompõe H_2O_2 a O_2 e água (H_2O); e a glutationa peroxidase (GPx) que atua sobre peróxidos utilizando glutationa como co-fator. O sistema antioxidante não enzimático é formado por diversas substâncias, dentre elas: glutationa (GSH); tiois totais (T-SH); tocoferóis; ascorbato; proteínas de transporte de metais de transição, como a transferrina (transporte de ferro) e a apoferritina (transporte de cobre) (VASCONCELOS et al., 2007).

Quando houver excesso de produção destas espécies reativas ou depleção do sistema antioxidante, ocorrerá o estresse oxidativo, o qual resultará em lesões celulares e consequentemente no surgimento de doenças crônicas (FERREIRA; MATSUBARA, 1997; FERNANDEZ et al., 2007). O dano celular resulta do ataque de ERO e ERN sobre as macromoléculas, tais como açúcares $(CHOH)_n$, DNA, proteínas e lipídeos (VASCONCELOS et al., 2007). Todos os organismos aeróbios estão suscetíveis ao estresse oxidativo, pois durante a respiração mitocondrial pequenas porções do oxigênio consumido (aproximadamente 2%) são convertidas em espécies altamente reativos: $O_2^{\bullet -}$ e H_2O_2 (PAPA; SKULACHEV, 1997).

Desta forma, sabendo-se que os efeitos colaterais dos EAA estão relacionados ao estresse oxidativo e que essas drogas podem causar danos em órgãos vitais do organismo, o objetivo deste trabalho foi avaliar os marcadores hepáticos e renais, bem como parâmetros de estresse oxidativo, no fígado e rins de ratos tratados com diferentes protocolos de administração de UB e ST.

Os resultados serão apresentados na forma de um artigo científico intitulado “Biochemical and oxidative stress markers in the liver and kidneys of rats submitted to different protocols of anabolic steroids”.

2 MANUSCRITO

Biochemical and oxidative stress markers in the liver and kidneys of rats submitted to different protocols of anabolic steroids

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ABSTRACT. The objective of this study was to evaluate the effects of different protocols (P1, P2 and P3) of boldenone undecylenate (BU) and stanozolol (ST) on markers of liver and kidney function and variables of oxidative stress in these organs. For this, 54 male Wistar rats were divided into nine groups of six animals each. Each animal received intramuscularly 5.0 mg kg^{-1} of BU or ST once a week for four weeks (P1); 2.5 mg kg^{-1} of BU or ST once a week for eight weeks (P2); and 1.25 mg kg^{-1} of BU or ST once a week for 12 weeks (P3). For each protocol, a control group was used (CG), and they received 0.1 ml of olive oil intramuscularly. Blood, and fragments of liver and kidney were collected for alanine aminotransferase activity (ALT), alkaline phosphatase (ALP), albumin, creatinine, cholesterol, total protein, triglycerides, urea, reactive oxygen species (ROS), thiobarbituric acid reactive substances (TBARS), total thiols (T-SH), and glutathione (GSH) evaluation. Seric ALT activity and cholesterol concentration were significantly ($p<0.05$) higher compared to CG when BU of protocol P1 was used. ALT activity was significantly higher ($p<0.05$) compared to the CG in protocol P2 when ST was used. Liver samples showed higher levels ($p<0.05$) of ROS and TBARS in protocols P1 and P3 when BU was used, and lower GSH activity ($p<0.05$) on group treated with protocol P3. Rats that have received ST under protocol P1 and P3 showed higher levels ($p<0.05$) of ROS, as well as increased TBARS levels in P3 but lower GSH activity in P3 ($p<0.05$) when compared to the CG. In the liver, the T-SH concentration was lower ($p<0.05$) in P2 when compared BU and ST of the CG. In renal tissues, ROS and TBARS levels were significantly higher ($p<0.05$) in animals that received BU under protocols P1 and P2; and GSH activity and T-SH levels were reduced in the three protocols (P1, P2 and P3). In addition, animals treated with ST occurred showed reduced renal levels of GSH levels ($p<0.05$) in P2 and P3. The treatment with ST also led to higher ROS levels ($p<0.05$) in P2 and P3, and TBARS levels in P3, but reduced concentration ($p<0.05$) of GSH levels in P2 and P3, and T-SH in P2 and P3. Thus, it is concluded that the BU in doses of 5 (day 30) and 2.5 mg/kg (day 60) changes the seric ALT activity, possibly showing a hepatotoxic effect. High doses of BU may lead to increased levels of cholesterol (protocol P1) possibly due to inhibition of the normal steroid biosynthesis process. All protocols used have caused changes in the redox balance of the organs studied (except in the liver, protocol P2), which indicates that these drugs might be harmful even at low doses.

Keywords: boldenone undecylenate, stanozolol, oxidative damage, hepatotoxicity, anabolic androgenic steroids, rats.

1. Introduction

Anabolic androgenic steroids (AAS) are synthetic compounds derived from testosterone that have been modified to enhance the anabolic activity, preferably the androgenic effect which is responsible for the development of male sexual characteristics. There are some medical applications for these compounds such as muscular atrophy, growth retardation, anemia, hypogonadism, and bone demineralization (Shahidi 2001).

Due to the anabolic properties of AAS, specially on muscle growth and strength, they have been used to maximize athletic performance in humans and animals (Kicman 2008, Guan et al. 2010). AAS doses 10 to 100 times the therapeutic dose are often used causing severe side effects (Yersalis and Bahrke 1995) that may include kidney (Yoshida et al. 1994, Cerretani et al. 2013, Riezzo et al. 2014) and hepatic problems, masculinization, testicular aplasia, cancer predisposition, and behavioral changes (Shahidi 2001). Thus, in order to improve athletic performance in competitions the use of AAS was prohibited and regulated by the World Anti-doping Agency (WADA) in 1999.

Boldenone undecylenate (BU; androsta-1,4-dien-17β-ol-3-one) is a veterinary AAS with intense anabolic properties and moderate androgyny. This anabolic has been used illegally in race horses, humans, and cattle to enhance performance and promote weight gain through improved feed conversion (Soma et al. 2007 Verheyden et al. 2007 Gryglik et al. 2010). Another AAS widely used by humans and racing horses is stanozolol (ST) since it can be administered orally due to their high bioavailability, remaining unaffected by first pass hepatic metabolism (Deshmukh et al. 2010) in addition to the fact that it is safe even at high doses (Maini et al. 2014).

The abusive use of AAS can cause lesions in many organs (Frankenfeld et al. 2014), including liver (Neri et al., 2011, Ding et al. 2013), and kidneys (Cerretani et al. 2013 Riezzo et al. 2014). The liver is actively related to several metabolic and detoxifying functions processes, while the kidneys are organs responsible for excretion of compounds, such as AAS. As a result, these organs are continually exposed to high levels of endogenous and exogenous oxidants (Bejma 2000). Reactive oxygen species

(ROS) are normally produced by almost every cell in the body, and act in diverse cellular functions such as apoptosis, cell signaling, angiogenesis, and cell proliferation (Giorgio et al. 2007, Fernandez et al. 2007). Either the increase of ROS production or decrease on its detoxification ability by antioxidants such as glutathione (GSH) and total thiols (T-SH) may lead to increased amounts of these reactive species which rapidly react with cellular components, causing changes in DNA, proteins and lipids, resulting on changes in the cellular function and processes (Jones 2008). By reacting with lipids, ROS may promote lipid peroxidation. The assessment of the thiobarbituric acid reactive substances (TBARS) levels is a common method for measuring lipid peroxidation (Esterbauer 1993).

Considering that anabolic steroids are widely used in humans and animals to improve athletic performance and knowing that their overuse may cause lesions in many organs, we have designed this study to evaluate biochemical and oxidative stress markers in the liver and kidneys of rats under different protocols of anabolic steroids (BU and ST).

2. Materials and methods

2.1 Animals

This study used 54 Wistar rats, 60 day-old with approximately 250 to 300g of body weight from the Central Animal Facility of *Universidade Federal de Santa Maria (UFSM)*. The rats were maintained on an experimental room under constant temperature (23±1°C), with 12 hours of alternating periods of light and dark, as well as feed and water *ad libitum*.

2.2 Experimental procedure

The animals were subjected to three administration protocols (P1, P2, and P3) of boldenone undecylenate (BU) (Equipoise® 50 mg mL^{-1} - Fort Dodge) and stanozolol (ST) (Nabolic Strong® 25 mg mL^{-1}Chinfield Veterinary Products). The rats were divided into six groups of six animals each that received intramuscular injections of 5.0 mg kg^{-1} of BU or ST once a week for 4 weeks (P1); 2.5 mg kg^{-1} of BU or ST once a week for 8 weeks (P2); and 1.25 mg kg^{-1} of BU or ST once a week for 12 weeks (P3). There

was a control group (CG) for each protocol that have received injections of 0.1 mL of olive oil, adding up to nine groups of animals for this experiment. The therapeutic protocol recommended by the manufacturers in equine practice is 2.5 mg kg^{-1} once per week for 8 weeks. Based on this protocol, we sought to investigate the effects double doses in half time, as well as half the dose for extra four weeks. All procedures were approved by the Research Ethics Committee of the institution (UFSM) under protocol number 032/2014.

2.3 Serum analyses

At the end of the experimental period all rats were anesthetized with isoflurane followed by exsanguination via intracardiac puncture. Blood samples were collected and stored in tubes without anticoagulant, and the sera obtained by centrifugation (3200 rpm for 10 min) was stored at -18°C until analysis. Seric albumin, cholesterol, creatinine, total protein, triglycerides, urea, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured using commercial kits (Labtest®) in a semi-automatic device (Bioclin 100®).

2.4 Preparation of liver and kidney homogenates

Liver and kidneys were collected, excised, and homogenized 1:10 (weight/volume) in 10 mM solution of Tris-HCl, pH 7.4. Procedures were carried out at 0-4°C. The homogenate was centrifuged at 1,800 rpm for 10 min in a refrigerated centrifuge, and the supernatant was used for the determination of reactive oxygen species (ROS), thiobarbituric acid reactive substances (TBARS), and glutathione (GSH).

2.5 Measurement of intracellular reactive oxygen species (ROS) production

The production index of ROS by cellular components was determined from the levels of 2'-7'-dichlorofluorescein (DCF) (Myhre et al. 2003). Aliquots of 50 µL of supernatant of liver and kidneys were added separately in medium containing distilled water and 2'-7'-dichlorofluorescein diacetate (DCFH-DA) to 1mM. After addition of DCFH-DA, the medium was incubated in the dark for 1h until the evaluation of

fluorescence (excitation at 488 nm and emission at 525 nm). DCF levels were determined using a standard curve and results were corrected for protein levels, which was evaluated by the Brilliant Blue binding method of protein Coomassie (Bradford 1976).

2.6 Thiobarbituric acid reactive substance (TBARS) measurement

The lipid peroxidation in liver and kidney was determined according to the method described by Ohkawa et al. (1979) by examining the concentration of malondialdehyde (MDA). A solution containing 200 μL of standard (0.03 mM MDA) or samples (liver and kidney), 500 μL of 0.8% thiobarbituric acid, 200 μL of sodium dodecyl sulfate, 8.1% sodium dodecyl sulfate (SDS), and 500 μL of acetic acid (2.5 M CH3COOH, pH 3.4) was heated at 95°C for 120 min. The absorbance was measured at 532 nm. The amount TBARS was expressed as nmol MDA/g tissue.

2.7 Measurement of reduced glutathione (GSH)

GSH activity was measured by spectrophotometry using Ellman's reagent (Ellman, 1959). An aliquot of 200 μL of supernatant (liver and kidney) was added to a final volume of 900 μL solution. The product of the reaction was measured at 412 nm after addition of 50 μL of 5-5-dithio-bis-(2-nitrobenzoic acid) (DTNB) at 10 mM. A standard curve using cysteine was used to calculate the activity of the enzyme, and these were expressed as nmol GSH/g tissue.

2.8 Determination of total thiols

The total thiols (T-SH) levels were measured by a spectrophotometer by the modified method of Boyne and Ellman (1972). For that, an aliquot of 200 μL of supernatant (liver and kidney) was used in a final volume of 900 μL solution. The product of the reaction was measured at 412 nm after addition of 50 μL of DTNB at 10 mM. A standard curve using cysteine was used to calculate the content of thiol groups in the samples, and these were expressed as nmol T-SH/g tissue.

2.9 Statistical analyses

The statistical program Graph Pad PRISM® 6.01 was used. The results for each group were expressed as mean ± standard error. Data were analyzed by one-way ANOVA. When the differences between both groups were significant, the Tukey test was used as post hoc analysis with a $p<0.05$.

3. Results

3.1 Serum parameters

Seric ALT activity was significantly ($p<0.05$) higher in the protocols P1 and P2 comparing to animals treated with BU and control (CG), as well as in the protocol P2 comparing to rats treated with ST and CG. However, seric levels of cholesterol were higher ($p<0.05$) in rats that received BU when compared to healthy (CG), but only in protocol P1. Other parameters (albumin, creatinine, alkaline phosphatase, total protein, triglycerides, and urea) showed no significant differences in any of the protocols used. These results were showed in tables 1, 2 and 3.

Table 1. Seric levels of albumin, cholesterol, creatinine, total protein, triglycerides, urea, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in rats submitted to protocol 1 (P1), which included administration of 5 mg kg^{-1} boldenone undecylenate (BU) and stanozolol (ST) compared to the control group (CG).

	Groups		
	CG	BU	ST
Albumin (mg/dL)	4.27 ±0.08[a]	4.12 ±0.15[a]	4.19 ±0.11[a]
ALT (U/L)	38.08 ±1.52[a]	55.73 ±5.40[b]	45.53 ±3.23[a]
Cholesterol (mg/dL)	78.33 ±5.25[a]	102.8 ±6.14[b]	93 ±3.20[a]
Creatinine (mg/dL)	0.68 ±0.04[a]	0.7 ±0.06[a]	0.71 ±0.01[a]
ALP (U/L)	182 ±8.42[a]	170.3 ±18.42[a]	158.7 ±9.53[a]
Total protein (g/dL)	5.65 ±0.16[a]	5.98 ±0.21[a]	5.68 ±0.11[a]
Triglycerides (mg/dL)	97.33 ±7.79[a]	102.5 ±7.21[a]	83.33 ±5.38[a]
Urea (mg/dL)	38.43 ±1.50[a]	35.19 ±2.08[a]	36.51 ±1.92[a]

Data were expressed as mean ± standard error. Different letters in the same row indicate significant differences between groups ($p<0.05$; $n = 6$).

Table 2. Seric levels of albumin, cholesterol, creatinine, total protein, triglycerides, urea, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in rats submitted to protocol 2 (P2), which included administration of 2.5 mg kg^{-1} boldenone undecylenate (BU) and stanozolol (ST) compared to the control group (CG).

	Groups		
	CG	BU	ST
Albumin (mg/dL)	4.64 ±0.11[a]	4.49 ±0.13[a]	4.56 ±0.12[a]
ALT (U/L)	46.62 ±2.74[a]	59.85 ±3.58[b]	65.77 ±3.56[b]
Cholesterol (mg/dL)	141.8 ±8.12[a]	145.3 ±10.40[a]	171.3 ±15.79[a]
Creatinine (mg/dL)	0.71 ±0.02[a]	0.73 ±0.04[a]	0.77 ±0.01[a]
ALP (U/L)	230 ±19.58[a]	230.8 ±11.93[a]	225.5 ±22.38[a]
Total protein (g/dL)	7.01 ±0.16[a]	6.81 ±0.12[a]	7.38 ±0.17[a]
Triglycerides (mg/dL)	139.3 ±13.86[a]	139 ±11.85[a]	190.8 ±16.48[a]
Urea (mg/dL)	50.58 ±2.84[a]	51.17 ±1.92[a]	58.06 ±1.93[a]

Data were expressed as mean ± standard error. Different letters in the same row indicate significant differences between groups ($p<0.05$; n = 6).

Table 3. Seric levels of albumin, cholesterol, creatinine, total protein, triglycerides, urea, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in rats submitted to protocol 3 (P3), which included administration of 1.25 mg kg^{-1} boldenone undecylenate (BU) and stanozolol (ST) compared to the control group (CG).

	Groups		
	CG	BU	ST
Albumin (mg/dL)	5.01 ±0.13[a]	5.03 ±0.15[a]	4.93 ±0.09[a]
ALT (U/L)	59.28 ±4.37[a]	52.85 ±2.84[a]	52.98 ±4.92[a]
Cholesterol (mg/dL)	15.08 ±7.73[a]	156.7 ±8.14[a]	141.2 ±8.64[a]
Creatinine (mg/dL)	0.66 ±0.07[a]	0.60 ±0.09[a]	0.63 ±0.04[a]
ALP (U/L)	185.8 ±7.13[a]	176.7 ±9.38[a]	207.5 ±10.03[a]
Total protein (g/dL)	7.68 ±0.10[a]	7.78 ±0.19[a]	8.1 ±0.09[a]
Triglycerides (mg/dL)	204.3 ±23.34[a]	167.8 ±24.62[a]	135.3 ±15.08[a]
Urea (mg/dL)	52.83 ±2.37[a]	52.15 ±3.24[a]	52.79 ±2.67[a]

Data were expressed as mean ± standard error. Different letters in the same row indicate significant differences between groups ($p<0.05$; n = 6).

3.2 Liver

In the hepatic tissue, ROS levels were significantly ($p<0.05$) higher in rats treated with BU and TS compared to CG for protocols P1 and P3 (Fig.1A and 1C, respectively). However, the results for the protocol P2 were not significantly different ($p>0.05$) to the ROS, TBARS, and GSH (Fig. 1B, 1E and 1H). TBARS levels in liver samples were higher ($p<0.05$) in animals treated with BU compared to the control group in the protocols P1 and P3 (Fig. 1D and 1F). Rats treated with ST showed higher ($p<0.05$) TBARS levels compared to the CG in protocol P3 (Fig. 1F). In the hepatic tissue, GSH activity was lower ($p<0.05$) in animals treated with ST and BU compared to CG, but only for protocol P3 (Fig. 1I). In addition, T-SH levels were lower ($p<0.05$) in rats that have received BU and TS compared to CG in P2 (Fig. 1K and 1L).

3.3 Kidney

The ROS levels in the kidneys were significantly ($p<0.05$) higher in rats that have received BU protocols P1 and P2 (2A and 2B), as well as in animals treated with ST in protocols P2 and P3 when compared to the control group (Fig.2B and 2V). In addition, renal samples showed higher ($p<0.05$) TBARS levels in rats treated with BU compared to healthy animals in the protocols P1 and P2 (Fig. 2D and 2E). Protocol P3 resulted on an increase ($p<0.05$) in TBARS levels only in animals that were administered ST when compared to CG (Fig. 2F). In all protocols (P1, P2 and P3) compared to the control group, GSH activity in the kidney was lower ($p<0.05$) in rats treated with BU (Fig. 2G, 2H and 2I). Only in protocols P2 and P3, GSH activity in animals treated ST were lower ($p<0.05$) compared to CG (Fig. 2H and 2I). In the kidneys, T-SH content was lower ($p<0.05$) in animals receiving ST compared to GC in protocols 1, 2 and 3 (Fig. 2J, 2K and 2L). In addition, it was observed lower levels of T-SH in treated rats with BU compared to CG in P3 (Fig. 2L).

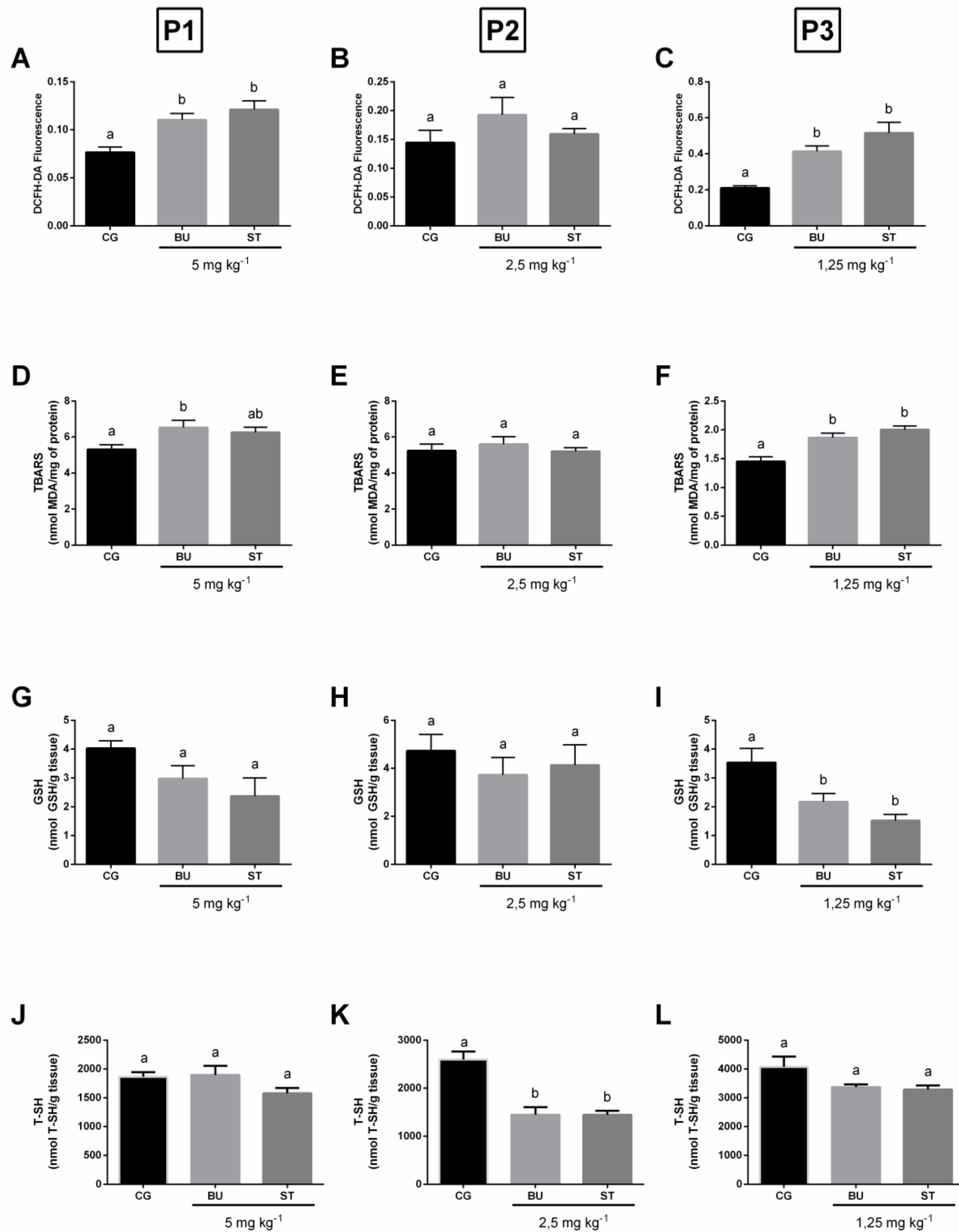

Fig. 1. Oxidative stress markers in the liver of rats treated with different protocols of boldenoneundecylenate (BU) and stanozolol (ST). Reactive oxygen species (ROS) (A, B and C), thiobarbituric acid reactive substances (TBARS) (D, E and F), glutathione (GSH) (G, H e I) and total thiols (T-SH) (J, K e L) in the following protocols: Protocol 1 (P1) (A, D, G, and J), Protocol 2 (P2) (B, E, H, and K) Protocol 3 (P3) (C, F, I, and L). Data were expressed as mean ± standard error. Different letters indicate significant differences between groups ($p<0.05$; n = 6).

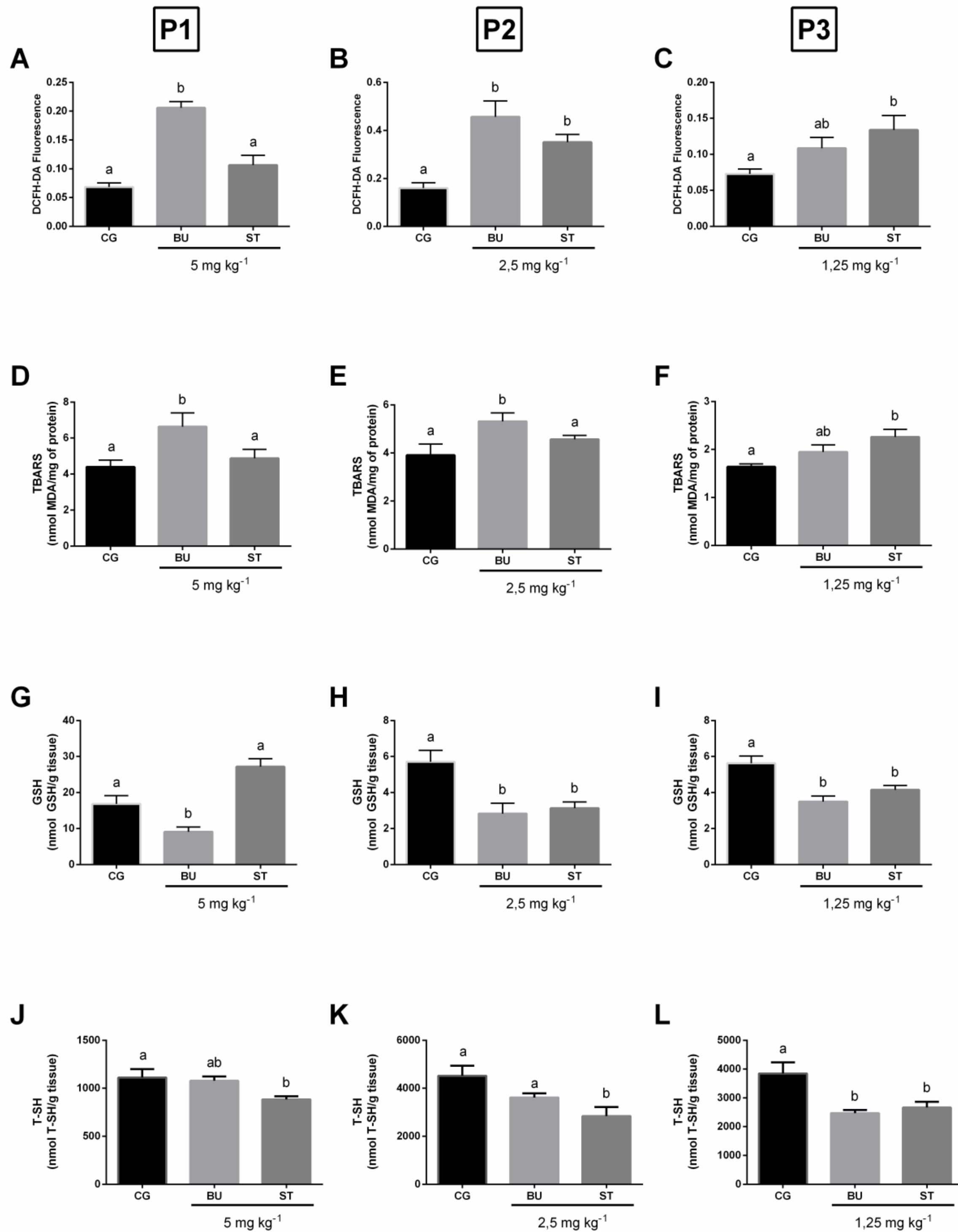

Fig. 2. Oxidative stress markers in the kidney of rats treated with different protocols of administration of boldenoneundecylenate (BU) and stanozolol (ST). Reactive oxygen species (ROS) (A, B and C), thiobarbituric acid reactive substances (TBARS) (D, E and F), glutathione (GSH) (G, H e I) and total thiols (T-SH) (J, K e L) in the following protocols: Protocol 1 (P1) (A, D, G, and J), Protocol 2 (P2) (B, E, H, and K) Protocol 3 (P3) (C, F, I, and L). Data were expressed as mean ± standard error. Different letters indicate significant differences between groups ($p<0.05$; n = 6).

4. Discussion

AAS cause toxic effects to hepatocytes (Welder et al., 1995, Boada et al., 1999), which can be highlighted by increasing hepatic markers in serum (Sileri et al. 2005). In this study, animals treated with BU from protocols 1 and 2 showed increased seric ALT activities, that can be attributed to the release of enzymes from the cytosol of liver cells into the bloodstream as a result of hepatocytes injury (Navarro et al. 1993). El-Moghazy et al. (2012) administered 5 mg $kg^{-1}$of BU in rabbits for three weeks (3, 6 and 9 weeks) which resulted on hepatic histopathological changes, periportal necrosis, and inflammatory cell infiltration. The authors also reported progressive increase on injuries after AAS administrations. In that regard, it has been suggested that an increase in seric ALT activity observed on protocols 1 and 2 might be related to the dose and time of BU exposure for 4 and 8 weeks, respectively. There were no significant differences in ALT levels in the protocol P3, and this is possibly related to the low doses used. However, it is unclear why the ST caused enzymatic changes only in the protocol P2.

In the protocol P1, cholesterol levels increased only in animals receiving BU. Many types of AAS may inhibit the normal process of steroid biosynthesis (Rone et al. 2009), i.e. the cholesterol is not converted to pregnenolone and consequently is stored (Neri et al. 2011), reflecting on high seric levels. Further, it is suggested that low doses of AAS (P2 and P3 protocols) may be the reason for no change in cholesterol levels in the current study, whereas in studies by Gårevik et al. (2012, 2014), when the increase of this parameter was observed only when high doses of AAS were used. According to Neri et al. (2011), prolonged use of EAA causes an increase in hepatic lysosomal hydrolases activity, and decreased in some components of the system microsomal metabolism of drugs and mitochondrial respiratory chain activity without modifying the classic indicators of liver function. This could explain the results of the present study, the absence of significant changes in albumin, TP, and triglycerides in animals treated with steroids. Moreover, the absence of changes in these biochemical parameters confirms the results from studies with rats treated with ST for a short period (12/24/48/72/96 hours) (Boada et al., 1999) or for a long period (56 to 90 days) (Boada et al., 1999, Pey et al. 2003).

In this study, there was an increase of ROS and TBARS levels in liver in rats treated with BU, as well as an increase of ROS in animals receiving ST (P1 protocol). These results corroborate to those found by Mayada et al (2015), where 4.5 mg $kg^{-1}$of

BU in rabbits for four weeks led to an oxidative stress situation. In protocol P2, there were no changes in the TBARS and ROS levels, as well as GSH activity, and we speculate that the antioxidant system of the animals was able to neutralize the deleterious effects of ROS, whereas there was significant reduction in the levels of T-SH. In addition, the host response to AAS may vary depending on frequency, route of administration and dose used (Skogastierna et al. 2013 Sadowska-Krepa et al. 2013). The results obtained with the P3 protocol, where rats were treated with BU and ST, showed that prolonged and continuous use of ST in rats cause increased levels of lipid peroxidation and ROS in the liver of these animals, similarly as described by Pey et al. (2003).

It is known that liver cells need energy to perform several functions and the high metabolic rate of the liver is directly associated with a high flow of electrons in the mitochondrial respiratory chain. However, some of these electrons are deflected, producing further ROS leaving the body susceptible to oxidative stress process (Ogonovszky et al. 2005). When ROS production exceeds the levels of enzymatic and non-enzymatic antioxidants, as well as hepatic repair capacity, oxidative stress occurs in this organ (Carvalho et al. 2010). The oxidative damage resulting from ROS production observed in the animals treated with anabolic are confirmed by assessment of membrane lipid peroxidation, resulting in an increase in TBARS levels, as observed in current study.

An experimental study showed that prolonged administration of AAS causes dysfunction on mitochondrial respiratory chain and mono-oxygenase system, which leads to increased generation of ROS and, thus, the lipid peroxidation (Molano et al. 1999). ROS cause damage to double or triple bonds of polyunsaturated fatty acids of cell membranes by altering their initial chemical conformation, these reactions after commencing self-perpetuating. As a result, there were changes of cohesion, fluidity, permeability and metabolic functions of cells (Chihuailaf et al. 2002). Final products of lipid peroxidation decomposition are numerous and include highly cytotoxic aldehydes, such as malondialdehyde (MDA), acrolein, 4-hydroxy-2-trans-nonenal (HNE) and trans-2,4-decadienal (DDE) (Martinez et al., 2003). In addition, ROS and MDA have been implicated in the pathogenesis of many types of hepatic injury, mainly in liver damage induced by drugs (Uchida et al. 1999). In this study occurred the reduction of GSH activity (protocol P3), and similar results were reported by Welder et al. (1995), who administered AAS in hepatocyte culture and observed depletion of the antioxidant.

In addition, the constant increase of ROS, as observed in this study, could lower the effectiveness of the antioxidant response (Halliwell and Cross 1994 Chihuailaf 2002), since during situations of very intense oxidative stress, GSH can be consumed in an irreversible way, staying in the oxidized form (Gul et al. 2000). Thus, it suggests that changes occur in the redox state of hepatic tissue, and although these are not the cause of the anabolic toxicity, they may influence cellular toxicity.

The increased levels of ROS and TBARS, and reduced content of T-SH and GSH in kidney tissue was observed in this study, indicating that the kidneys suffered depletion of antioxidant defenses, and therefore, its ability to combat ROS has been compromised, resulting in oxidative damage evidenced by lipid peroxidation. Riezzo et al. (2014) administered 3.5 mg kg^{-1} of nandrolonedecanoate in rats (twice a week, for forty-two days) and found that there was an increase in lipid peroxidation and reduction of antioxidant enzymes, which corroborates to the results found in rats treated with BU (protocols P1 and P2) and ST (protocol 3). The oxidative damage mechanism may be related to the possible compounds derived from aromatization of testosterone, with subsequent conversion of 17-β-estradiol, which is highly toxic, genotoxic and carcinogenic (Torres-Bugarín et al., 2007). The genotoxic action of steroid occurs due to metabolic activators and indirect processes that occur in the redox cycle, as well as the production of ROS (Fischer 2001). Thus, activation of the metabolic derivatives of testosterone leads to the formation of ROS, depletion of the antioxidant system and, consequently, the inducing oxidative stress. Side effects reported in the kidney due to the use of AAS are sporadic (Yoshida et al., 1994, El-Moghazy et al., 2011, Cerretani et al. 2013). Therefore, it is suggested that the increase in oxidative damage markers, and depletion of the antioxidant system (P2 and P3 protocols) occurred due to prolonged exposure to AAS, which was also observed by Frankenfeld et al. (2014). As noted in this study, prolonged exposure to AAS, despite the low dose (1.25 mg kg^{-1}), is able to generate an oxidative stress process, which can contribute to liver and kidney damage.

In conclusion, anabolic androgenic steroids (BU and ST) altered serum markers of liver injury at doses of 5 mg kg^{-1} and 2.5 mg kg^{-1} when administered once a week for 30 and 60 days, respectively, demonstrating that these doses and times are capable of changing functions of the liver, or even injure the organ. Prolonged administration of AAS caused change components of liver tissue, even without modifying classical serum indicators of function of this organ. Furthermore, as seen

from the oxidant/antioxidant markers, all protocols (except at P2 in liver) caused changes in redox balance of the evaluated organs. It is known that the increase of pro-oxidants and the depletion of the antioxidant system predispose to the development of various diseases. With this, it is suggested that the use of AAS, even at low doses, should be avoided unless they are used for therapeutic purposes.

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3 CONCLUSÃO

Neste estudo foi possível concluir que os anabolizantes são prejudicias mesmo quando utilizados em baixas doses ou em poucas aplicações, visto que em todos os protocolos avaliados foi possível observar alterações do balanço redox no fígado e rins dos ratos.

Devido a estreita relação entre a patofisiologia dos órgãos estudados com o estresse oxidativo, sugere-se que se os tratamentos fossem extendidos, seriam observadas alterações nos marcadores bioquímicos hepáticos e renais. Estes, no entanto, só apresentaram alterações nos protocolos 1 e 2 para os animais que receberam boldenona e no protocolo 2 para os que receberam estanozolol.

Por fim, recomenda-se que essas substâncias não sejam utilizadas para fins estéticos ou para melhora de performance atlética.

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