UNIVERSIDADE DE UBERABA
MESTRADO ACADÊMICO EM ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOPATOLOGIA.

MARIANA ANDRADE OLIVEIRA

EFEITO DA SUPLEMENTAÇÃO COM EXTRATO DE *AJUGA TURKESTANICA* NO PADRÃO HISTOPATOLÓGICO DE RATOS SUBMETIDOS À NATAÇÃO

UBERABA (MG)
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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia – Mestrado Acadêmico da Universidade de Uberaba, como requisito para a obtenção do título de Mestre em Odontologia, área de concentração Biopatologia.

Orientador: Prof. Dr. Geraldo Thedei Junior

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Área de concentração: Biopatologia

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“Não existe grandeza onde não há simplicidade, bondade e verdade”.

(Leon Tolstoi)

RESUMO

OLIVEIRA, M. A. **Efeito da suplementação com extrato de *Ajuga turkestanica* no padrão histopatológico de ratos submetidos à natação**. 2020. 43f. Dissertação (Mestrado em Odontologia) – Universidade de Uberaba, Uberaba (MG), 2020.

Ajuga turkestanica é uma planta perene originária da Ásia Central cujo extrato apresenta uma série de compostos bioativos denominados coletivamente "fitoecdisteroides", análogos do hormônio ecdisterona, presente nos insetos, e que seriam os responsáveis por inúmeros efeitos metabólicos. Este trabalho tem como objetivo a determinação dos efeitos do extrato comercial de *A. turkestanica* (EAT) no padrão histopatológico de ratos Wistar submetidos a exercício de natação. Foram utilizados 29 ratos Wistar divididos em quatro grupos, sendo: controle (administração de soro fisiológico; n = cinco animais); EAT sem natação (36 mg/Kg de peso corporal; n = seis animais); Natação e EAT+natação (36 mg/Kg de peso corporal; ambos contendo nove animais). Os animais foram alimentados com dieta padrão para ratos e receberam o tratamento (soro fisiológico ou EAT via gavagem – 1 mL/dia) diariamente, seguidos de natação conforme o grupo. O protocolo de treinamento da natação consistiu de três séries de 30 segundos de natação separadas por um período de descanso de 90 segundos e um período de recuperação de três minutos. No final das seis semanas de experimento, os animais foram eutanasiados e seus órgãos, colhidos para as análises histológicas. Utilizou-se o *software* estatístico *GraphPad Prism* 5 *for Windows* para as análises estatísticas por One Way ANOVA, seguido dos testes Kruskal-Wallis e Dun (tecido adiposo e muscular), Dunnett (esteatose), Holm-Sidak (inflamação) e Tukey (desorganização e descamação testicular). O extrato de *A. turkestanica*, sozinho, não possui atividade redutora do tecido adiposo, uma vez que o extrato não induziu a redução na área dos adipócitos quando administrado isoladamente e nem quando foi associado ao exercício ($p>0,05$). Os animais tratados com EAT não apresentaram diferença nos níveis de esteatose e de inflamação em relação aos animais do grupo-controle ($p >0,05$) sugerindo que o EAT não tem atividade prejudicial ao fígado. O mesmo aplica-se à ocorrência de desorganização e descamação testicular ($p>0.05$), sugerindo que o extrato não possui os efeitos indesejáveis dos esteroides anabolizantes. O extrato também falhou em causar hipertrofia nas fibras do músculo quadríceps ($p>0,05$), enquanto a natação teve um efeito positivo nesse parâmetro, levando a um aumento na área de secção transversal, tanto isoladamente quanto

associada ao EAT ($p<0,05$). Os dados obtidos permitem concluir que o extrato de *A. turkestanica*, na dosagem utilizada não possui efeitos anabólicos significativos, nem é capaz de induzir mobilização de gordura dos adipócitos.

Palavras-chave: *Ajuga turkestanica*. Metabolismo Animal. Ratos Wistar. Histopatologia.

ABSTRACT

OLIVEIRA, M. A. **Effect of supplementation with *Ajuga turkestanica* extract on the histopathological pattern of rats submitted to swimming**. 2020. 43f. Dissertation (Master in Dentistry) – University of Uberaba, Uberaba (MG), 2020.

Ajuga turkestanica is a perennial plant originating in Central Asia whose extract presents a series of bioactive compounds collectively called “phytoecdysteroids”, analogues of the hormone ecdisterone, present in insects, and which would be responsible for numerous metabolic effects. This work aims to determine the effects of the commercial *A. turkestanica* extract (ATE) on the histopathological pattern of Wistar rats submitted to swimming exercise. Twenty nine Wistar rats were used, divided into four groups: control (administration of saline; n = five animals); ATE without swimming (36 mg / kg body weight; n = six animals); Swimming and ATE + swimming (36 mg / kg body weight; both containing nine animals). The animals were fed a standard diet for rats and received the treatment (saline or ATE via gavage - 1 mL / day) daily, followed by swimming according to the group. The swimming training protocol consisted of three sets of 30 seconds of swimming separated by a 90 seconds rest period and a three-minute recovery period. At the end of the six-week experiment, the animals were euthanized and their organs were collected for histological analysis. The statistical software GraphPad Prism 5 for Windows was used for statistical analysis by One Way ANOVA, followed by the Kruskal-Wallis and Dun (fat and muscle), Dunnett (steatosis), Holm-Sidak (inflammation) and Tukey (disorganization and testicular desquamation). The extract of *A. turkestanic*a, alone, has no adipose tissue reducing activity, since the extract did not induce a reduction in the area of adipocytes when administered alone or when associated with exercise ($p > 0.05$). The animals treated with ATE showed no difference in the levels of steatosis and inflammation in relation to the animals in the control group ($p > 0.05$) suggesting that the ATE has no harmful activity to the liver. The same applies to the occurrence of testicular disorganization and desquamation ($p > 0.05$), suggesting that the extract does not have the undesirable effects of anabolic steroids. The extract also failed to cause hypertrophy in the fibers of the quadriceps muscle ($p > 0.05$), while swimming had a positive effect on this parameter, leading to an increase in the cross-sectional area, both in isolation and in association with ATE ($p < 0.05$). The obtained data allows one to conclude that the extract of *A.*

turkestanica, in the used dosage does not have significant anabolic effects, nor is it able to induce fat mobilization of the adipocytes.

Keywords: *Ajuga turkestanica*. Animal Metabolism. Wistar rats. Histopathology.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

%	Porcentagem
>	Maior
µm	Micrometro
Akt	Proteína
ATE	*Ajuga Turkestanica Extract*
C2c12	Mouse myoblast cell line
CEEA	Comite de Ética em Experimentação Animal
COBEA	Colégio Brasileiro para Experimentação Animal
EAT	Extrato da Ajuga Turkestanica
g	Grama
IRS	Proteína adaptadora
Kg/BW	Body Weight
mg/Kg	Miligrama/kilograma
mg/ml	Miligrama/mililitro
mTORC2	Complexo de proteínas
NaCl	Cloreto de sódio
ºC	Grau Celsius
PDK1	Proteína
PI3K	Fosfatidil Inositol Trifosfato
PIP2	Fosfatidil Inositol Bisfosfato
PIP3	Fosfatidil Inositol Trifosfato
Ser 473	Aminoácido
Thr-308	Aminoácido
TSC1	Complexo proteico
TSC2	Complexo proteico

SUMÁRIO

1 INTRODUÇÃO

Os ecdisteroides, cetosteroides poli-hidroxilados, com longas cadeias laterais de carbono, são produzidos principalmente em insetos e plantas. Embora o papel dos ecdisteroides como hormônios dos insetos e seu envolvimento no desenvolvimento e no processo de muda tenham sido bem estudados, seu papel nas plantas é menos elucidado (GORELICK-FELDMAN, 2008).

Os ecdisteroides encontrados nas plantas, chamados fitoecdisteroides, não provocam nenhuma das respostas clássicas dos hormônios vegetais, no entanto, eles possuem fraca atividade semelhante à giberelina no arroz, além de afetar a diferenciação em embriões de alfafa (MACHACKOVA; VAGNER; SLAMA, 1995). Foi sugerido que as plantas utilizam ecdisteroides como defesa química contra insetos herbívoros, interrompendo o equilíbrio hormonal dos insetos e o processo de muda, pois sua semelhança estrutural com o hormônio ecdisona seria suficiente para induzir alterações fisiológicas nos insetos que se alimentassem de plantas contendo fitoecdisteroides (GORELICK-FELDMAN, 2008).

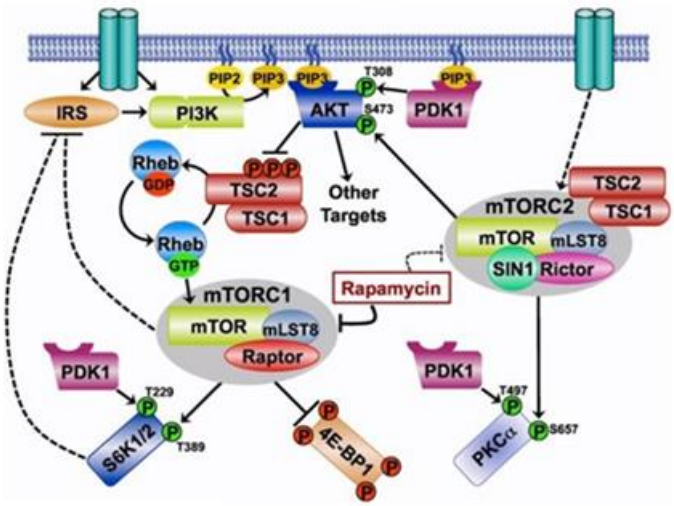
As plantas são fontes naturais de ecdisteroides. Embora a maioria das plantas não contenha quantidades significativas desses compostos, algumas plantas produzem altos níveis dessas substâncias. Entre as plantas comestíveis, apenas a *Spinacia oleracea* (espinafre) contém quantidades consideráveis de ecdisteroides, entre eles, a 20-hidroxiecdisona, um dos ecdisteroides mais comuns (GORELICK-FELDMAN, 2008). Além de seu uso como cultura alimentar, o espinafre também pode ter potenciais qualidades terapêuticas (GORELICK-FELDMAN, 2008).

Os ecdisteroides são amplamente comercializados para atletas como suplemento dietético (PARR *et al*., 2015) com a finalidade de aumentar a força e a massa muscular durante o treinamento de resistência, reduzir a fadiga e facilitar a recuperação. Vários estudos relataram uma ampla gama de efeitos farmacológicos de ecdisteroides em mamíferos, a maioria deles benéfica para o organismo. Nos anos 80, o ecdisteroide mais ativo, a ecdisterona, era suspeito de ser usado pelos atletas olímpicos russos (PARR *et al*., 2015). Os níveis de ecdisteroides na dieta ocidental são geralmente baixos (geralmente na faixa de menos de 1 $g.dia^{-1}$), enquanto as doses usadas pelos fisiculturistas são indicadas na faixa de até 1000 $mg.dia^{-1}$ (PARR *et al*., 2015).

Báthori *et al*. (2008) reportaram que os ecdisteroides influenciam muitas funções fisiológicas e têm uma grande aplicação experimentalmente na farmacologia, com largo emprego sobre os mamíferos, incluindo os seres humanos. Dentre os ecdisteroides, a 20–hidroxiecdisona vem sendo constantemente investigada com ênfase nos eventos metabólicos. Foram relatados, em investigações extensivas, os possíveis efeitos promotores de crescimento da ecdisterona em várias espécies animais (ratos, camundongos) e em seres humanos e muitos vestígios sobre seu uso indevido por atletas estão circulando desde então (PARR *et al*., 2015; SLAMA *et al*., 1995).

Foi demonstrado que a ecdisterona aumenta a síntese de proteínas no músculo esquelético (SYROV, 2000). Gorelick-Feldman *et al*. (2008) propuseram a estimulação direta ou indireta da via de sinalização Fosfatidil Inositol Trifosfato/ Proteína (PI3K/Akt) como mecanismo para esse aumento da síntese proteica (GORELICK-FELDMAN, 2008) A PI3K é ativada por fatores de crescimento por meio da interação direta com receptores, na presença de proteínas adaptadoras(scaffolding), como as proteínas adaptadoras (IRS). Estas interações recrutam a PI3K para seu substrato, o fosfatidil-inositol 4,5-bifosfato (PIP2) permitindo a geração do segundo mensageiro lipídico fosfatidil-inositol 3,4,5-trifosfato (PIP3). A Akt e a PDK1 são recrutadas para a membrana plasmática pela associação com PIP3. Isto permite que a Akt seja ativada por meio da fosforilação no resíduo do aminoácido (Thr-308) pela proteína PDK1 e aminoácido Ser-473 pelo complexo de proteínas (mTORC2). Uma vez ativa, a Akt fosforila muitos alvos, incluindo múltiplos sítiosna – complexo proteico (TSC2), que forma um complexo funcional com o complexo proteico (TSC1). Conforme figura 1 abaixo:

Figura 1. Via de sinalização PI3K.



Fonte: Ferreira (2010).

Recentemente, a ligação da ecdisterona ao Receptor Estrogênico Beta humano pôde ser demonstrada em experimentos de cultura de células e a indução de hipertrofia nas células *Mouse myoblast cell line* (C2c12) mostrou ser mediada pela ativação do Receptor Estrogênico Beta (PARR *et al*., 2015). Esse mecanismo seria, portanto, diferente do mecanismo de ação dos esteroides anabolizantes-androgênicos, que aumentam a massa muscular principalmente por meio da ligação ao receptor androgênico (PARR *et al*., 2015).

Também foi relatado que os ecdisteroides têm outros efeitos em mamíferos, incluindo a redução dos níveis de colesterol e glicose no sangue (YOSHIDA *et al*., 1971), além de ação imunomoduladora, antiarrítmica e hepatoprotetora (KURMUKOV; SHARAPOVA; SYROV, 1976).

Após a administração diária de 20-hidroxiecdisona na dose de 2,5 mg/kg, para os animais com hipercolesterolemia, em três, seis e oito semanas, o nível de colesterol no plasma sanguíneo diminuiu em 7%, 16,9% e 29%, respectivamente (BATHORI *et al*., 2008).

A suplementação com 200 mg de em homens adultos jovens, durante 8 semanas, associada ao treinamento de resistência, não evidenciou diferença significativa em comparação ao grupo placebo quanto aos parâmetros, força muscular, muscular, capacidade anabólica, nível de testosterona livre, cortisol, relação TL/C, ureia e creatinina (WILBORN, 2006). No entanto, seu análogo 20-

hidroxiecdisona induziu um acréscimo seletivo de massa no músculo tríceps braquial de ratos após cinco dias de infusão contínua por meio de bombas osmóticas implantadas subcutaneamente no músculo tríceps braquial (5mg/kg/dia).

Ajuga turkestanica (Figura 2) é uma planta perene que cresce na Ásia Central, tratando-se de uma fonte de substâncias bioativas, entre as quais um hormônio ecdisteroide conhecido como turkesterona, que apresentaria efeito positivo no metabolismo de lipídios, colaborando para o aumento da performance esportiva, muito utilizada, também, para o tratamento de doenças cardíacas, dores estomacais, síntese de proteínas, manutenção do metabolismo anabólico, aumento da massa muscular, enquanto diminui o tecido adiposo (LAFONT; DINAN, 2003).

A alta concentração deste potente ecdisteroide torna a *A. turkestanica* uma planta medicinal potencialmente útil. Cheng *et al.* (2008) citaram, em seus estudos, que a *A. turkestanica* é indicada por apresentar propriedades tônicas, pois estimula o crescimento muscular, porém, desde que o fornecimento de proteínas seja o recomendado.

Figura 2. Aspecto geral da planta *Ajuga turkestanica*.

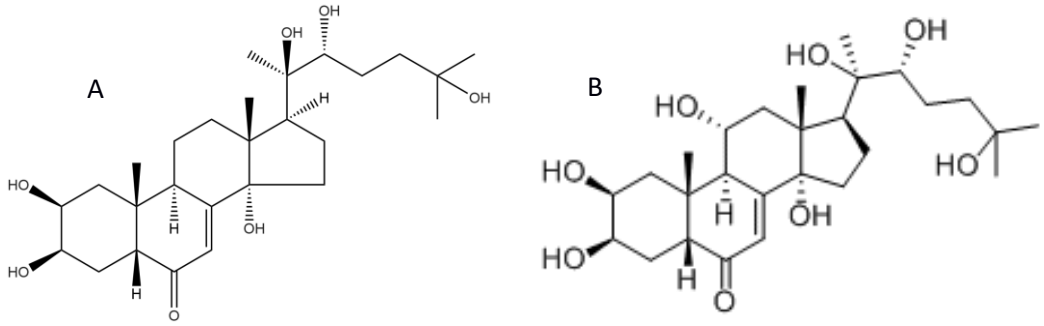
Fonte: Wikispecies... (2019).

Do ponto de vista estrutural, há uma aparente semelhança entre a turkesterona e a β-ecdisterona (Figura 3), uma vez que ambas apresentam a presença do núcleo esteroidal, três anéis de seis membros e um de cinco membros e são compostos quirais, com vários centros assimétricos ou estereocentros. Além

disso, os dois apresentam uma cetona insaturada (grupamento cetônico conjugado com uma dupla ligação) no segundo anel do núcleo esteroidal.

Embora ambos sejam polióis, os dois compostos diferem estruturalmente, pela presença de um grupo hidroxila adicional no anel 3 do núcleo esteroidal da turkesterona. Isso faz com que a turkesterona tenha um centro quiral a mais que a B- Ecdisterona. Essas diferenças nas estruturas químicas, mesmo que pareçam discretas, podem promover diferentes propriedades biológicas ou farmacológicas a essas moléculas.

Figura 3. Estrutura da β-ecdisterona (A) e da turkesterona (B)



Fonte: Gao, Cai e Xi (2008).

Diante do exposto, pode-se assumir que os fito/ecdiesteroides podem oferecer uma alternativa promissora na substituição dos esteroides anabólicos androgênicos, inclusive, como agente terapêutico no tratamento da atrofia muscular, mas devem, como já ponderado anteriormente (BATHORI *et al*., 2008), ser mais estudados.

Além disso, a livre comercialização do extrato de *A. turkestanica* também motiva a realização de mais estudos acerca de seus potenciais efeitos benéficos e também visando a identificar algum efeito potencialmente tóxico, pois a maioria dos estudos disponíveis na literatura foi realizada com extratos parcialmente purificados ou com o composto turkesterona (ou seus análogos) isolado. Isso justifica estudar os efeitos do extrato comercial de *A. turkestanica* e comparar os resultados aos descritos na literatura para o composto total ou parcialmente purificado.

Diante disso, este trabalho tem como objetivo estudar o efeito de um extrato comercial de *A. turkestanica* no padrão histopatológico do tecido adiposo, fígado, testículos e músculo quadríceps de ratos submetidos à natação.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Estudar o efeito do extrato comercial de *A. turkestanica* (EAT) no padrão histopatológico do tecido adiposo, fígado, testículo e tecido muscular (músculo quadríceps) de ratos Wistar adultos submetidos à natação.

2.2 OBJETIVOS ESPECÍFICOS

Compreendem determinar o efeito da suplementação com extrato comercial de *A. turkestanica* nos seguintes parâmetros:

a) área das células adiposas;
b) padrão histopatológico do fígado: ocorrência de esteatose e inflamação;
c) padrão histopatológico do testículo: ocorrência de desorganização e descamação;
d) área de fibra muscular dos músculos quadríceps.

3 ARTIGO

Effect of the *ajuga turkestanica* extract on the histopathological pattern of rats subject to swimming

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ABSTRACT

Ajuga turkestanica is a perennial plant originating in Central Asia whose extract presents a series of bioactive compounds collectively called “phytoecdysteroids”, analogues of the hormone ecdisterone, present in insects, and which would be responsible for numerous metabolic effects. This work aims to determine the effects of the commercial *A. turkestanica* extract (ATE) on the histopathological pattern of Wistar rats submitted to swimming exercise. Twenty nine Wistar rats were used, divided into four groups: control (administration of saline; n = five animals); ATE without swimming (36 mg / kg body weight; n = six animals); Swimming and ATE + swimming (36 mg / kg body weight; both containing nine animals). The animals were fed a standard diet for rats and received the treatment (saline or ATE via gavage - 1 mL / day) daily, followed by swimming according to the group. The swimming training protocol consisted of three sets of 30 seconds of swimming separated by a 90 seconds rest period and a three-minute recovery period. At the end of the six-week experiment, the animals were euthanized and their organs were collected for histological analysis. The statistical software GraphPad Prism 5 for Windows was used for statistical analysis by One Way ANOVA, followed by the Kruskal-Wallis and Dun (fat and muscle), Dunnett (steatosis), Holm-Sidak (inflammation) and Tukey (disorganization and testicular desquamation). The extract of *A. turkestanic*a, alone, has no adipose tissue reducing activity, since the extract did not induce a reduction in the area of adipocytes when administered alone or when associated with exercise ($p > 0.05$). The animals treated with ATE showed no difference in the levels of steatosis and inflammation in relation to the animals in the control group ($p > 0.05$) suggesting that the ATE has no harmful activity to the liver. The same applies to the occurrence of testicular disorganization and desquamation ($p > 0.05$), suggesting that the extract does not have the undesirable effects of anabolic steroids. The extract also failed to cause hypertrophy in the fibers of the´~quadriceps muscle ($p > 0.05$), while swimming had a positive effect on this parameter, leading to an increase in the cross-sectional area, both in isolation and in association with ATE ($p < 0.05$). The obtained data allows one to conclude that the extract of *A. turkestanica*, in the used dosage does not have significant anabolic effects, nor is it able to induce fat mobilization of the adipocytes.

Keywords: *Ajuga turkestanica*. Animal Metabolism. Wistar rats. Histopathology.

1. Introduction

Ecdysteroids, polyhydroxylated ketosteroids, with long carbon side chains, are produced mainly in insects and plants and are widely marketed to athletes as a dietary supplement[1] in order to increase strength and muscle mass during resistance training, reduce fatigue and facilitate recovery. However, ecdysterone supplementation had no significant effects on changes in body mass, body composition, muscle strength and anabolic status.[2]

Ajuga turkestanica is a perennial plant that grows in Central Asia, being a source of bioactive substances, including an ecdysteroid hormone known as turkesterone, which would have a positive effect on lipid metabolism, helping to increase sports performance. The plant is also widely used for the treatment of heart disease, stomach pain, protein synthesis, maintenance of anabolic metabolism, increase in muscle mass, while decreasing fat tissue.[2,3] The high concentration of this potent ecdysteroid makes *A. turkestanica* a potentially useful medicinal plant. In studies that *A. turkestanica* is indicated for having tonic properties, as it stimulates muscle growth, however, provided that the supply of proteins is recommended.[4]

In view of the above, it can be said that phyto/ecdiestoids may offer a promising alternative in the replacement of anabolic androgenic steroids, including as a therapeutic agent in the treatment of muscle atrophy, but they should, as already considered above,[4] be more studied. In addition, the free commercialization of *A. turkestanica* extract also motivates further studies about its potential beneficial effects and also in order to identify any potentially toxic effects, since most of the studies available in the literature have been carried out with partially purified extracts or with the compound turkesterone (or its analogs) isolated. This justifies studying the effects of the commercial extract of *A. turkestanica* and comparing the results to those described in the literature for the fully or partially purified compound.

Therefore, this work aims to study the effects of a commercial extract of A. turkestanica on the histopathological pattern of adipose tissue, liver, testicles and muscle (quadriceps) of rats submitted to swimming.

2. Material and methods

2.1. Animals

29 Wistar rats with an average weight of 250g were used, supplied by Federal University of Uberlândia. The animals were kept in a controlled environment, with 12-hour light-dark cycles, temperature ranging from 25 to 27ºC, with water and standard diet for rodents ad libitum, in polypropylene boxes containing four animals/box.

This experiment was conducted according to the rules of the Brazilian College for Animal Experimentation. The project was approved by the Animal Experimentation Ethics Committee of the University of Uberaba, process 016/2018, as per ANNEX B.

2.2. Treatment of animals

The entire experiment was conducted in accordance with the rules of the Brazilian College for Animal Experimentation - COBEA and the project was approved by the Ethics Committee on Animal Experimentation at the University of Uberaba (protocol CEEA – 016/2018).

The ATE was obtained in the city of Uberaba and the solution for administration was prepared in a 12.6 mg/mL concentration in sterile saline (0.9% NaCl) and administered daily via gavage, in the afternoon, immediately before the exercise of swimming. The animals were divided into four groups: control group (n = 5, gavage with 1 mL of water), ATE (n = 6, gavage with 36mg ATE/ Kg BW), swimming (n = 9, gavage with water) and ATE + SWI (n = 9, gavage with 36mg ATE / Kg BW).

The swimming exercise was performed according to the previously described.[6]

The exercise consisted of three to six swimming sessions, on six consecutive days, for six weeks. Each session consisted of three periods of 30 seconds of swimming, each exercise being separated by a rest period of 90 seconds. Each session was followed by a three-minute recovery period. In the first week, the animals performed the exercise without weight and, in the others, a lead weight was attached to the animal, increasing each week (1%, 2%, 4%, 6% and 7%, respectively for weeks two, three, four, five and six). The animals in the control group and those in the group that received ATE were only wetted and dried immediately.

2.3. Swimming exercise

The exercise performed in the study consisted of short periods of high intensity muscle activity. Three to six swimming sessions were held six days a week for six

weeks. The sessions were divided as follows: Monday - three sessions, Tuesday - three sessions, Wednesday - four sessions, Thursday - five sessions, Friday - five sessions, Saturday - six sessions, Sunday- rest. Each session lasted three periods of 30 seconds of swimming, and each swim was separated by a rest period of 90 seconds. Each session was followed by a three-minute recovery period. In the exercise program, small lead weights were tied on the rats' backs to increase the intensity of the exercise. In the first week, the animals performed the exercise without weight, in order to adapt to the proposed training. In the second week, the rats performed sessions with weights equal to 1% of their body mass. In the third week, with weights equal to 2% of your body mass. In the fourth week, with weights equal to 4% of your body mass. In the fifth week, rats weighing 6% of their body weight. In the last week, the weight of the vest represented 7% of the animals' body mass. After the end of the swimming session, the animals were dried with clean cloths and a hairdryer.

2.4. Euthanasia and histopathological analysis

At the end of the experiment, the animals were fasted overnight and euthanized by administration of sodium pentobarbital (30 mg/ kg BW). After verifying the absence of pupillary and caudal reflexes, the animals were submitted to laparotomy and section of the diaphragm.

Liver, testicles, periepididimal and perirenal adipose tissues and quadriceps femoris muscle were dissected, fixed in 10% buffered formaldehyde and subjected to routine histological processing and staining (Hematoxylin/Eosin). The cuts were cross-sectional, with a thickness of 6 µm.

The sections were visualized under a common light microscope (ZEISS-Berlin-Germany, model AXIO SCOPE) and the images, captured with an Axion Cam/Cc 1 digital camera. Quantitative and qualitative analyses were carried out, using 64 - fields per slide.

a) The area of adipocytes (500 fields / group, 20 x increase);
b) Liver sections (500 fields / group, 40 x magnification) were evaluated for the presence of steatosis and inflammation. These values were expressed in% of the total;
c) Testis sections (500 fields / group, 40x magnification) were evaluated for the presence of disorganization and flaking;

d) The area of the cells of the quadriceps muscles (transverse sections, 500 fields / group, 20x magnification) was measured using the Axion Vision Rel. 4.8.2 software. These measurements were presented in square micrometers.

2.5. Statistical analysis

The individual results of all animal parameters were entered into an electronic spreadsheet for statistical analysis using the GraphPad Prism 7 for Windows® software. The D´Agostino & Pearson or Shapiro-Wilk test was used to verify the normality of the data. When the assumption of normality was satisfied, the One Way ANOVA test was used, followed by the Dunnett tests, Tukey or Holm-Sidak, for comparison between groups, and data were expressed as mean ± SD. When the results did not pass the normality test, non-parametric analysis of variance was used using the Kruskal-Wallis test, followed by Dunn's or Tukey's multiple comparison tests, and the results were expressed in median (min / max). In all cases, differences with $p < 0.05$ were considered significant.

3. Results and discussion

3.1 Adipose tissue

Figure 3 - Adipose tissue

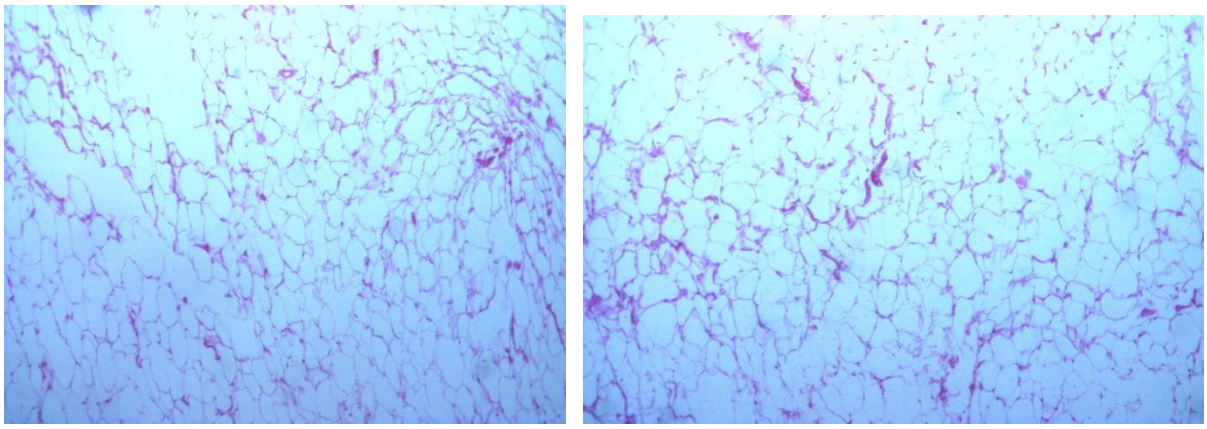

Plate. 1. Cross section areas of adipose tissue cells 6µm thickness, Magnification, 20X. Coloring HE 20X increase.

Regarding the periepididimal adipose tissue, the group treated with ATE did not show a reduction in the area of adipocytes, a fact observed when the control group was compared with the SWI and SWI ATE groups (Plate 1). The comparison of the AET group with ATE + SWI did not reveal any significant difference, suggesting that the reduction in the area of periepididimal adipocytes is due to swimming and not to its

association with ATE Padronizar: SWI ATE ou ATE+SW. When the perirenal adipose tissue was analyzed, a slightly different situation was found, in which the group treated only with ATE did not have hypertrophied adipocytes and, in this case, an effect of swimming (associated or not with ATE) in reducing the adipocytes (Plate 1).

These data suggest that swimming is effective, at least in part, in reducing the volume of the adipocyte, confirming previous studies,[7] and that the extract of *A. turkestanica*, alone, has no adipose tissue reducing activity, since the extract did not induce a reduction in the area of adipocytes when administered alone or when it was associated with exercise (Table 1).

The failure of ATE to reduce the area of adipose cells and even the increase of this value in the perirenal adipose tissue can be explained by the inhibitory action of fat mobilization in adipose tissue induced by ecdisterone, an analog of turkesterone. This ecdysteroid reduces the formation of camp within adipocytes, which reduces the activation of lipase, thus reducing the mobilization of lipids.[8]

Table 1

Area of adipocytes (µm2) after six weeks of swimming exercise associated or not with the administration of ATE.

		Control	ATE	Swimming	ATE+SWI
Periepididimal	Minimum	1055	254	536	286
	Median	3632[a]	3690[a]	2968[b]	2761[b]
	Maximum	8164	7796	8424	8326
Peri renal	Minimum	503	549	242	615
	Median	2584[a]	3177[b]	2820[a]	2669[a]
	Maximum	7711	7170	8383	7590

Values are expressed in median, minimum and maximum. The Kruskal-Wallis test was used, followed by Dunn's multiple comparison test ($p < 0.05$). *The values carrying different superscript letters in the same lane were statistically different*.

3.2. Liver

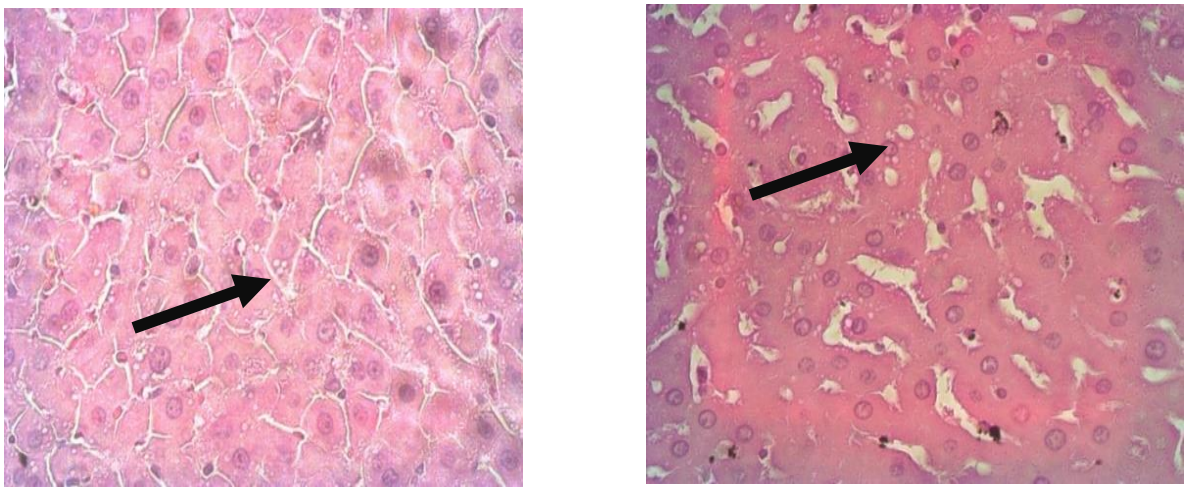

Plate. 2. Liver. Histological cross section of the liver. Areas indicated by the arrows represent hepatic steatosis grade I. 6µm thickness Magnification, 40X. Coloring HE.

Table 2 steatosis was the most frequently observed among experimental animals, having been found in 23.5% of the fields in group C, 17.8% in group swimming, 26.2% in group SWI + ATE and 21.9% in the SWI + ATE group, with no statistical difference between the groups (Plate 2). The occurrence of inflammation was much less than that of steatosis, and the statistical analysis also showed that there was no statistical difference in the presence of inflammation in the comparison between groups (Table 2).

Table 2

Occurrence of steatosis and inflammation (%) in the liver of the animals after six weeks of swimming exercise associated or not with the administration of ATE.

		Control	ATE	SWI	ATE + SWI
Steatosis 1	Average	24[a]	26[a]	18[a]	22[a]
	SD	3.3	8.8	4.4	7.1
Inflamation	Average	5[a]	5.3[a]	4.6[a]	4.8[a]
	SD	2	2.4	1.7	1.9

Values are expressed as mean and standard deviation (SD). One Way ANOVA was used, followed by Dunnett's multiple comparison test (steatosis) and Holm-Sidak (inflammation) ($p < 0.05$). *The values carrying different superscript letters in the same lane were statistically different*.

It is known that the practice of physical activity improves the liver condition, preventing the onset of non-alcoholic liver steatosis[9] (Plate 2) and also has positive effects on process regression in rats induced to steatosis by high-fat diet.[10] However, the animals that underwent treatment with swimming, associated or not with the

administration of ATE, did not have a reduction in the occurrence of steatosis. This fact may be associated with the intensity of the exercise or even with the low occurrence of this pathological process in all animals. In addition, as already mentioned above, compounds present in the ATE are able to reduce fat breakdown in adipocytes and also in hepatocytes,[8] but this inhibitory effect was not detected, which may raise the hypothesis that the amount of active compounds present in the extract used was not sufficient to induce this inhibitory effect.

3.3. Testicles

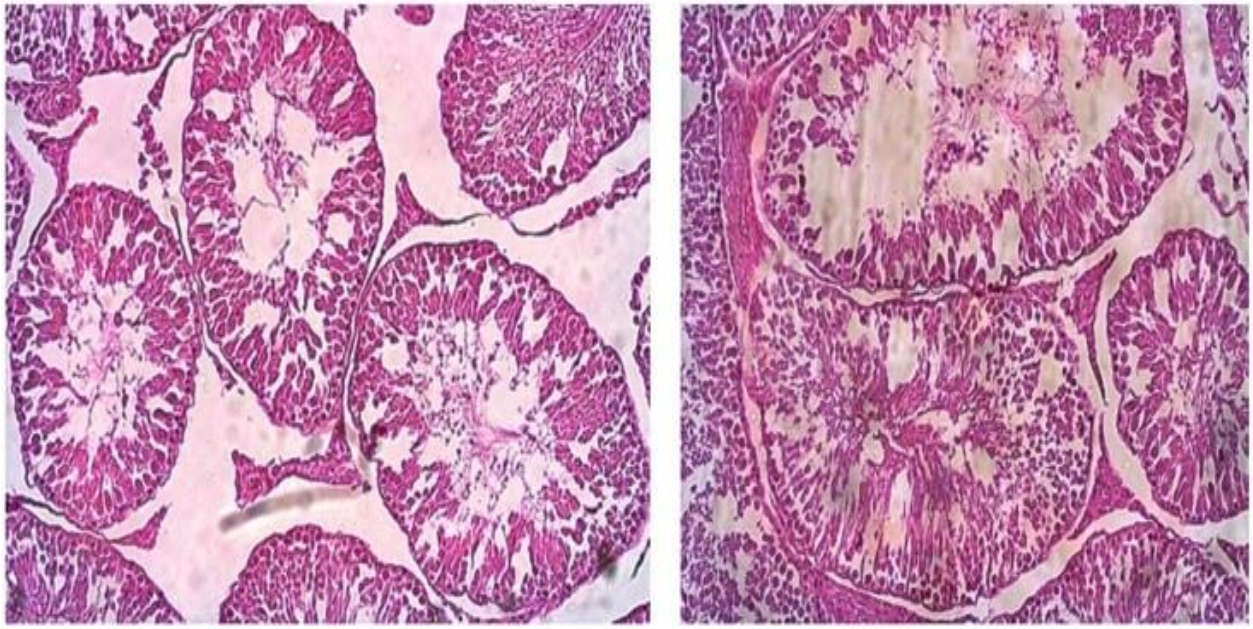

Plate. 3. Testicles. Histological cross section of the testis. The images represent áreas with testicular disorganization; 6µm thickness Magnification, 40X. Coloring HE.

In the histological evaluation of the testicles, it was observed that there was a predominance of normality, with about 88.6% of the fields evaluated. When analyzing the disorganization parameter, its presence was found in 9.2% of the fields evaluated, but without statistical difference between the groups ($p > 0.05$). The same occurred with desquamation, which was present in 2.2% of the fields. The presence of tissue degeneration was not observed in the analysis of the testicles (Table 3).

Table 3

Occurrence of disorganization and flaking (%) in the testis of the animals after six weeks of swimming exercise associated or not with the administration of ATE.

		Control	ATE	Swimming	ATE+Swimming
Disorganization	Minimum	3,3	2	2,6	4,6
	Median	4,7[a]	9,7[a]	8,2[a]	8,8[a]
	Maximum	6,2	16	27	29
Desquamation	Median	4,1[a]	1,8[a]	2[a]	1,8[a]
	DP	2	2,2	1,4	2

Values are expressed as median, minimum and maximum (disorganization) and average and standard deviation (SD) (desquamation). The Kruskal-Wallis test was used, followed by the Tukey post-test (disorganization) and One Way ANOVA, followed by the post-test and Tukey (desquamation), for the comparison between groups ($p < 0.05$). The values carrying different superscript letters in the same lane were statistically different.

The absence of effects on the tissue structure of the testicles may suggest that ATE, in fact, did not exert the adverse effects associated with the use of anabolic steroids such as testosterone.[11] In fact, there is evidence that (phyto) ecdysteroids would exert their effects through another route, which would involve the estrogen β receptor,[1] which would not have the undesirable effects of anabolic steroids.

3.4. Muscle tissue

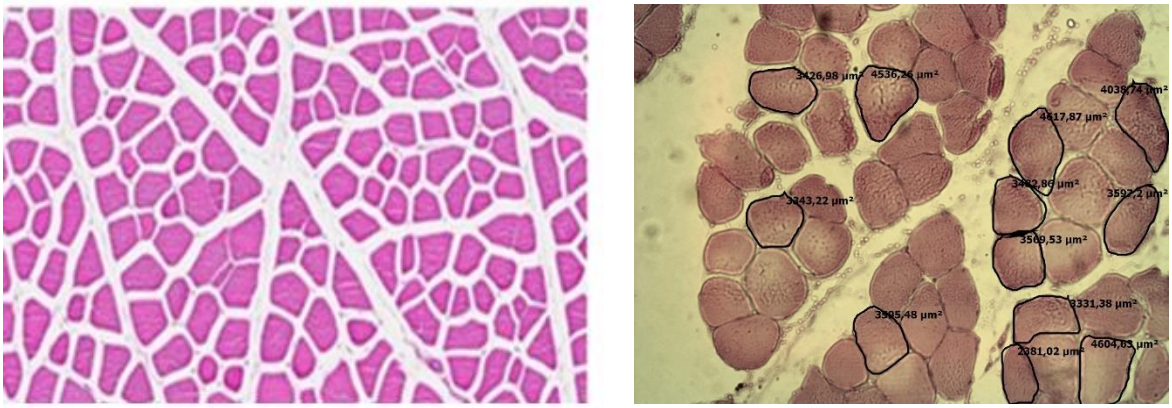

Plate. 4. Muscle tissue. Histological cross section of the quadriceps muscle, where areas of muscle fibers were measured; 6µm thickness Magnification, 20X. Coloring HE.

Finally, treatment with ATE did not induce a significant effect on the cross-sectional area of the quadriceps muscle fibers. However, swimming had a positive effect on this parameter, leading to an increase in cross-sectional area, both in isolation and in association with ATE (Table 4).

Table 4

Cross-sectional area of the quadriceps muscle fibers (μm^2) after six weeks of swimming exercise associated or not with the administration of ATE.

	Control	ATE	Swimming	ATE+Swimming
Minimum	567	548	519	674
Median	1779[a]	1877[a]	2548[b]	2377[b]
Maximum	5213	8195	9265	8324

Values are expressed in median, minimum and maximum. The Kruskal-Wallis test was used, followed by the Dunn test, for the comparison between groups ($p < 0.05$). *The values carrying different superscript letters in the same lane were statistically different*.

The positive effect of swimming on the muscle fiber area corroborates other studies in the literature.[12] However, the absence of a significant effect with the use of the extract allows us to deduce that the extract alone has no effect of increasing the muscle because, alone, did not increase the area of muscle fiber and, when associated with swimming, does not increase this parameter compared to swimming alone.

4. Conclusion

The obtained data allows one to conclude that the extract of *A. turkestanica* (in the tested conditions) is not able to induce the fat mobilization of the adipocytes, has no harmful effect on the liver and testis and does not have significant anabolic effects on the muscle tissue.

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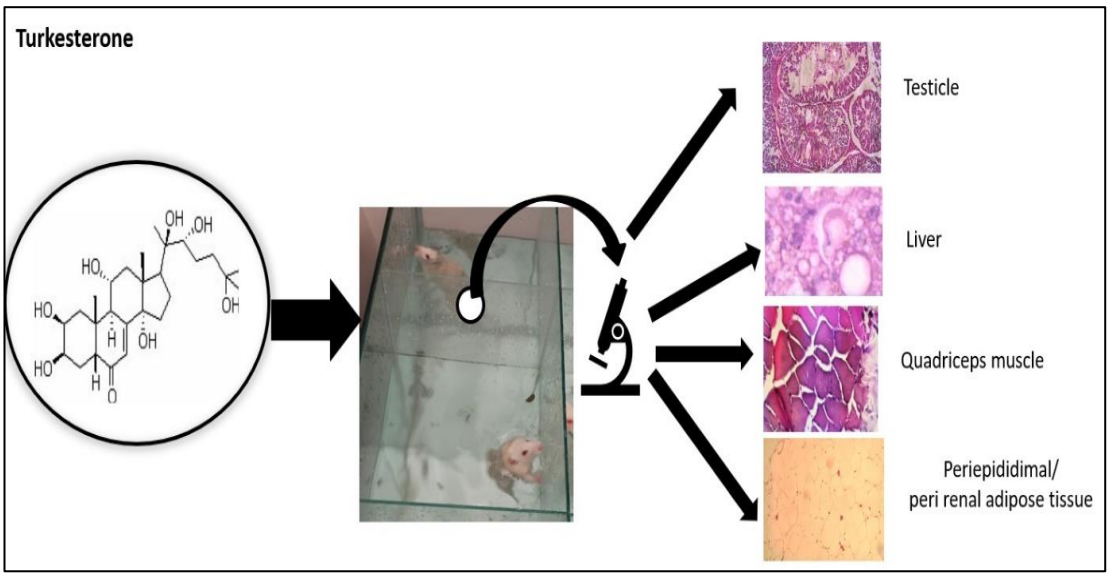
Graphical abstract



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ANEXOS

ANEXO A – Parecer do Comitê de Ética

Uniube Comitê de Ética em Experimentação Animal

Ofício CEEA–027/2018 Uberaba, 29 de junho de 2018

Ilmo. Prof.
Geraldo Thedei jr

Assunto: Encaminha processo nº 016/2018, sobre o protocolo de pesquisa "*Efeito da Turkesterona no metabolismo de ratos Wistar adultos submetidos ao treinamento de natação*".

Prezado(a) Professor(a),

Em resposta a sua solicitação, informo que o protocolo acima referido foi submetido avaliação do CEEA-UNIUBE, em reunião no dia 25/06/2018, sendo considerado **aprovado.**

Atenciosamente,

Profa. Joely F. Figueiredo Bittar
Coordenadora do CEEA-UNIUBE

Av. Tutunas, 720 – Campus FAZU – Bairro: Tutunas CEP: 38061-500– Uberaba, MG – Fone: (34) 3319-8787 –
e-mail: ceea@uniube.br

ANEXO B – Normas de publicação na Journal of Traditional and Complementary Medicine

JOURNAL OF TRADITIONAL AND COMPLEMENTARY MEDICINE

Guide for Authors

PREPARATION

Peer review

This journal operates a single blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More information on types of peer review.

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork. To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2,...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices
If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**

• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract
A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Keywords
Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

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2. Strunk W Jr, White EB. The Elements of Style. 4th ed. New York, NY: Longman; 2000.

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