UDC 619:636/637 CODEN MVPRE e-ISSN 1857-7415

MACEDONIAN VETERINARY REVIEW

DAYS OF VETERINARY MEDICINE 2015

Proceedings of the 6th International Scientific Meeting
Days of veterinary medicine 2015
Struga, Macedonia
September 24-26, 2015

Mac Vet Rev 2015; Volume 38; Supplement 1; Pages: 1-72

International Scientific Journal

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An Official Publication of

the Faculty of Veterinary Medicine-Skopje
Ss.Cyril and Methodius University in Skopje

e-ISSN 1857-7415

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MACEDONIAN VETERINARY REVIEW
Vol. 38; Suppl. 1, Pages 1-72, 2015

The Mac Vet Rev is an international peer-reviewed, Open Access journal published two times per year.
Mac Vet Rev Online (e-ISSN 1857-7415) offers free access to all articles at http://www.macvetrev.mk.

Indexed/Listed in: AGRIS, Academic Journals Database, AkademicKeys, Bielefeld Academic Search Engine (BASE), CAB Direct, CAS (Chemical Abstracts), CiteFactor, CORE (COnect REpositories), CrossRef, Directory of Open Access Journals (DOAJ), Global Health, Directory of Research Journals Indexing (DRJI), EBSCO, EFITA, Genamics JournalSeek, GetInfo, Google Scholar, INDEX COPERNICUS, Scientific Index Service (SIS), IVIS, Journal Index.com, JournalTOCs, Journal Rate, L-Primo, Open J-Gate, Open Access Library (OALib), PERIODICOS, Ulrich's Periodicals Directory, SCOPUS, ScienceCentral.com, SHERPA/RoMEO, SUNCAT, Veterinary Bulletin, Veterinary Science Database, Virtual Science Library(VLC), Wanfang Data, WorldWideScience.gov.(http://www.macvetrev.mk/Indexed in.html)

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CONTENT

O18

Optimization and validation of LC-MS/MS method for multiresidual analysis of β-agonists in urine

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Introduction: β-agonistsare synthetically produced compounds which are frequently used in animals for treatment of pulmonary diseases.They have been illegally used to improve production performance of food-producing animals in 5- to 10- fold of the therapeutic doses.On the other hand β-agonistsare transmitted in meat and meat products and present a risk for public health. For these reason European Union banned the use of these substances as growth promoters in farm animals. The aim of this study was optimization and validation of LC-MS/MSmethod for determination of four β-agonists: clenbuterol, salbutamol, terbutaline and ractopamine.

Material and methods: The extraction of β-agonists residue from urine samples was performed according the confirmatory method from the reference laboratory for β-agonists from Berlin, Germany. Validation of the method was performed according the Commission Decision 2002/657/EC. Linearity of the method, Decision limit (CCα), Detection capability (CCβ), accuracy, precisionand reproducibility of the method were evaluated.

Results: The calculated values for coefficient of correlation (r^2) were from 0.987 to 0.997. The obtained values for CCα and CCβ for clenbuterol were 0.120 µg/L and 0.139µg/L, respectively. For salbutamol CCα was 0.534 µg/Land CCβ was 0.565 µg/L. The calculated results for CCα for terbutaline and ractopamine were 1.625 µg/Land 0.591 µg/L, and for CCβ were 1.768µg/L and 0.680µg/L, respectively.The accuracy of the method was evaluated by determining the recovery of spiked urinesamples on three concentration level (at 0.5, 1 and 1.5 times the Minimum Required Performance Level (MRPL). The method recovery ranged from 89.83% to 97.40%. The precision of the method ranged from 3.49 to 11.49 (RSD_r, %) and the reproducibility of the method was from 6.40 to 17.64 (RSD_R, %).

Conclusion: According the data obtained from the validation procedure the method showed good linearity, good precision, recovery and reproducibility and the method is applicable for determination of β-agonists in urine.

O19

Effects of caffeic acid phenethyl ester on liver in high-fructose corn syrup drinking rats

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Introduction: High-fructose corn syrup (HFCS), is used as a common sweetener in many beverages and foods over the past few decades largely, induces metabolic syndrome. Caffeic acid phenethyl ester (CAPE), an active component of propolis from honeybee hives, is known to have anti-inflammatory and anti-oxidant properties. Th epurpose of the study was to determine the effect of CAPE on liver in HFCS drinking rats.

Material and methods: Eighteen male 8 week aged Spraque-Dawley rats were randomly divided into three groups (n=6): Control, HFCS and HFCS+CAPE. Metabolic syndrome was induced by HFCS 30% in tap water for six weeks. CAPE applications at the dose of 50 micromol/kg/day/i.p for two weeks, were initiated after four weeks of fructose consumption. After the experimental period of six weeks, rats were decapitated and livers were taken for the evaluation of biochemically and histopathologycally. Liver tissues malondialdehyde (MDA), glutathione (GSH) levels and catalase (CAT) activities were quantified by spectrofotometry. Also liver tissue specimens were embedded in paraffin blocks. Sections obtained from paraffin blocks were used for immune detection of eNOS and HSP70.

Results: In comparison with control group, MDA level was significantly higher, but GSH level and CAT activity were significantly lower in HFCS group. CAPE administration led to a significant decrease in MDA level and an increase in both CAT activity and GSH level when compared to HFCS group. Also, in comparison with control group, HSP70 immunreactivity increased in HFCS group, but decreased in the HFCS+CAPE group than HFCS group. Significant decreases were observed in eNOS activity in HFCS group than control group. On the other hand, no significant differences were found in the eNOS immunoreactivity in HFCS+CAPE group than HFCS group.

Conclusion: These results indicate that the overconsumption of HFCS may promote hepatic damage and lead to oxidative stress which can cause inflammation in the liver. On the other hand CAPE supplementation had beneficial effects and might play an anti-inflammatory and anti-oxidant role on liver in HFCS drinking rats.