Current Trends in Natural Sciences Vol. 6, Issue 12, pp. 248-252, 2017

Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521

Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

STUDY ON THE HEPATOPROTECTIVE EFFECT OF *PLANTAGO MAJOR* EXTRACT ON EXPERIMENTAL POISONING BY DICLOFENAC IN NMRI ALBINO MICE

Mădălina Elena Scarlat [1,*], Monica Bucă [1], Magdalena Cristina Zagardan [1], Alina Păunescu [1], Cristina Maria Ponepal [1], Monica Popescu [1], Alexandru Gabriel Marinescu [1]

[1] University of Piteşti, Faculty of Sciences, Physical Education and Informatics, Târgu din Vale St. 1, 110040, Piteşti, Romania

Abstract
Medicinal plants are alternative medicine's key weapons. Thanks to the medicinal effects some plants turned out to have, they have been chosen by people to heal or at least ameliorate various affections. Nature is continuously changing: some species become extinct, others are born and some cross time. Despite the trend to enhance plant-based medicines, research data in this field is still incomplete.
The experimental research in this paper aimed at evaluating the hepatoprotective capacity of the Plantago major (plantain) species in albino mice –NMRI stain, after experimental poisoning by Diclofenac. Using physiological and biochemical methods and techniques, we researched potential structural and functional changes ocurred further to the experimental poisoning by Diclofenac.

Keywords: Diclofenac, Hepatoprotection, Plantago major

1. INTRODUCTION

The goal of the experimental research in this paper was to evaluate the hepatoprotective capacity of the *Plantago major* species under experimental conditions of Diclofenac poisoning, a non-steroidal anti-inflammatory drug, administered in a dose of 0.16 ml.
It is common knowledge that Diclofenac determines rare, but significant cases of toxicity, usually with late onset (Boesterli, 2003).
Numerous studies worldwide research the effect of plant extracts of various plants in order to subsequently use them to treat various afflictions.
Bedecean (2015) researched the hepatoprotective effect of *Origanum vulgare* and *Rosmarinus officinalis* extract in the work titled "*Effects of some phytotherapeutic hydro-alcoholic extracts on experimental template of chronic hepatitis in mice"*
To do this, 78 Swiss line mice were used, divided into 13 batches of 6 mice each, and a dose of 1ml/kg CCl_4 was used to induce hepatitis; to evaluate the hepatoprotective effect of the *Origanum vulgare* extract, three therapeutic doses of 7 mg/kg, 3, 5 mg/kg and 1 mg/kg respectively were administered three times per week in alternative days from CCl_4 administration.
Haematologically, normal range values in leukocytes were reported after 4 weeks in all batches treated with *Origanum vulgare* extracts, as compared to batch 2, which received 1ml/kg CCl_4,

*Corresponding author, E-mail address: gabriel_mda8487@yahoo.com

Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521

Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

which presented leukocytopenia. Preserving leukocytic values under normal ranges may be an indicator of the extract's immunostimulatory effects.
Liver enzymes values, Alanine Aminotransferase, Aspartate Aminotransferase and Gamma – Glutamyl – Transferase register significant increase after 6 weeks in all the batches treated, after which they decrease and return to normal ranges after 8 weeks, irrespective of the extract dose received. The same numerical change is registered by total proteins and albumins, values being elevated after 6 weeks in all batches and decreasing after 8 weeks, irrespective of the extract dose administered.
To assess the hepatoprotective effect of *Rosmarinus officinalis* extract, 54 mice received a dose of 1ml/kg CCl_4, then they were divided into batches of 6 animals each. Of these, 3 batches received extract in a dose of 500mg/body, and other 3 batches received extract in a dose of 100mg/body, sacrificed after 4, 6 and 8 weeks.
Tetrachloride and the extract were administered in different days, 3 times per week.
The hydroalcoholic extract of *Rosmarinus officinalis* in doses of 250mg/body and 100mg/body turned out to have a poor hepatoprotective effect, he hepatoprotective action owing to the content of 1,8- cineol; the active ingredients in the hydroalcoholic extract of *Rosmarinus officinalis* has mildly synergic and polyvalent properties.
Also, the work called "*Hepatoprotective effect of certain plants in the spontaneous flora of Arad County"* (Suciu, 2013) deals with researching the hepatoprotective action of *Lycopodiun cavatum, Equisetum aarvense* and *Gentiana asclepiadea* species under experimental poisoning by Paracetamol in Swiss albino mice (NMRI). To conduct a functional assessment of the liver in the mice poisoned by Paracetamol and those treated by plant extracts, serum transaminase tests were conducted (Alanine and Aspartate Transaminase); they provided information about the functional integrity of hepatocytes, transaminase level being reported 8 times higher after 24 and 48 hours from the intraperitoneal injection, representing peak moments of toxic hepatitis onset. Ethanolic extracts of *Equisetum arvense* administered to mice exerted a hepatoprotective effect in all concentrations under research; in return, an increase in these enzymes were ascertained in the ethanolic extracts of *Lycoodiun clavatum* and *Gentiana asclepiadea* especially, in concentration of 3g/kg body administered before the paracetamol poisoning.
Plantain – *Plantago major* is a herbaceous plant that can easily adapt to various soil, humidity, temperature and light conditions; it is more likely to grow in the lowland, plain area to alpine areas. Plantain leaves contain an iridoid called aucibin and flavonoids, as well as mucilage, volatile oils and antibiotic substances, phytonocides, phylloquinone, carotenoids and minerals.
The antioxidant potential of the *Plantago major* extract has been researched and demonstrated by Mello (2015), highlighting its protective action against oxidative stress.
Also, Idris et al (2009) demonstrated in the study called "Hepatoprotective and anti-inflammatory activities of Plantago major species" that the Plantago major species has an anti-inflammatory and hepatoprotective effect after experimental poisoning by CCl_4.

2. MATERIALS AND METHODS

Swiss- albino mice- NMRI strain were used for this research, weighing over 30g; they were housed under standard lab conditions, into plastic and metal cages with special wood chips, Lignocel 3-4, Rettenmaier & Sohne, Germany, receiving special ration (combined granulated fodder for mice and rats) and water ad libitum, in a controlled, 12 hours light/12 hours dark Circadian rhythm, and an ambient temperature of 22±2°C.

*Corresponding author, E-mail address: gabriel_mda8487@yahoo.com

Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521

Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

The animals were provided by SPF Animal House, Băneasa Resort, Cantacuzino Institute of Bucharest.
The experiment consisted of four experimental batches (Table 1), each batch being made up of 6 albino mice –NMRI strain, as follows: a control group, a batch of mice intraperitoneally injected by 0.16 ml of Diclofenac in a single dose, a batch of mice injected by 0.16 ml Diclofenac and treated by 0.2 ml plantain extract for 14 days and a batch treated only with plantain plant extract in a concentration of 0.20 ml.

Table 1. Experimental batches

Batch		Intraperitoneal injection	Gavage
Control group – one batch (6 mice)			
Diclofenac- one batch		**x**	
Plantain	**Plantain extract+ Diclofenac- one batch**	**x**	**x**
	Plantain extract- one batch		**x**

Plantain plant extracts were administered by gavage and they were obtained via two processing cycles: primary and advanced; ethyl alcohol was used as solvent in various concentrations (Lupuleasa, Fica, Emese, 2005).
Blood samples were collected by venipuncture at the basis of mice's tail, and the following indices were determined: cholesterol, triglycerides.

3. RESULTS AND DISCUSSIONS

Seven days after diclofenac had been administered in a concentration of 0.16 ml to the mice batch tested, significant increase was reported by cholesterol values, as compared to the values registered by the control group (Figure 1).
Orinya et al (2016) also reported elevated cholesterol values in their paper called "*Haematological and biochemical studies on the effect of diclofenac sodium on Wistar Rattus Norvegicus*" after 7 days since the administration of a diclofenac dose in a concentration of 49.05mg/kg.
According to the statistical interpretation (Duncan's range test), no significant changes have been found as regards this physiological index further to the administration of two doses of *Plantago major* plant extract in a concentration of 0.25mg to the batch poisoned by diclofenac.
With regard to the variation of triglycerides under the influence of diclofenac, they show significant changes in all three experimental batches, as follows: further to diclofenac administration in a concentration of 0,16ml, triglycerides level increased by 164% as compared to the values reported by the control group, and seven days after two doses of *Plantago major* plant extract was administered at a concentration of 0,25 mg, decrease in triglycerides value by 21% was reported, as compared to the diclofenac-poisoned batch.

http://www.natsci.upit.ro
*Corresponding author, E-mail address: gabriel_mda8487@yahoo.com

Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521

Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

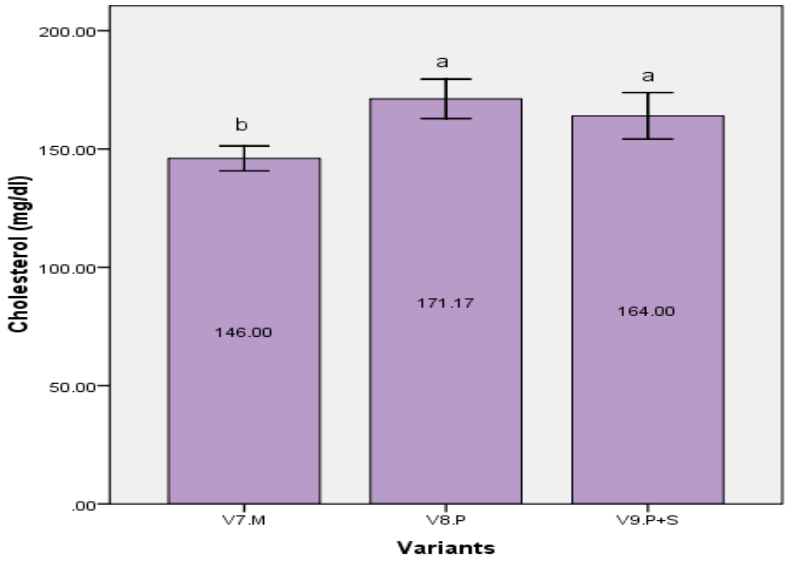

Figure 1. Plantago major extract's influence over cholesterol for Diclofenac- induced toxicity
(V1.M = control variant; V2.P = batch to have received 0.16 ml diclofenac; V3.P+S = batch which received diclofenac and was then treated by Plantago major extract)
(letters represent Duncan's range test interpretation: different letters show significant differences, p<0.05)

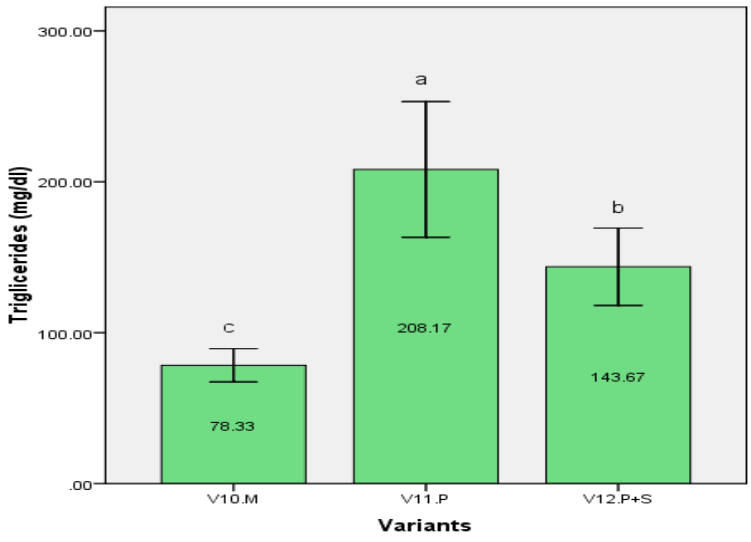

Figure 2. Influence of Plantago major extract over triglycerides for diclofenac-induced toxicity

(V1.M = control variant; V2.P = batch to have received 0.16 ml diclofenac; V3.P+S = batch which received diclofenac and was then treated by Plantago major extract)
(letters represent Duncan's range test interpretation: different letters show significant differences, p<0.05)

Diclofenac is the most used non-steroidal anti-inflammatory drug in 15 countries and is listed on the national lists of essential drugs in 74 countries. However, specialists say that diclofenac is a medicine whose use implies significant risks to the cardiovascular system (McGettigan and Henry, 2013).

*Corresponding author, E-mail address: gabriel_mda8487@yahoo.com

Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521

Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

A serious cause for concern was highlighted further to reports according to which numerous deaths due to myocardial infarction were associated with Diclofenac administration, especially in high-risk patients, aged over 50 years (Moodley, 2008).

Yapar et al. (2008) researched, in a study called "*Protective effect of L carnitine against diclofenac toxicity in mice"* the potential protective effects L – carnitine could have against renal and liver lesions caused by high doses of diclofenac in mice. The study was conducted on 32 Swiss Albino mice, divided into four equal groups, depending on the medicine's dose. Biochemical serum parameters were measured (BUN concentrations, creatinine and AST, ALT and ALP activities), as well as GSH and MDA content in the liver and kidney at the end of treatment. The two higher doses of diclofenac induced significant increases of serum markers and MDA build-up in tissues, while the GSH content of the liver and kidney was down in parallel.

4. CONCLUSIONS

Administering Diclofenac in concentrations of 16 ml causes significant increase both in cholesterol values, and triglycerides in the mice tested 7 days after administration.

Treatment by *Plantago major* extract in two doses, in a concentration of 0.25 mg, after seven days, leads to a decrease in triglycerides values by 20% as compared to the values reported by the control group.

5. REFERENCES

Bedecean, I. (2015). *Efectele unor extracte hidroalcoolice fitoterapeutice pe model experimental de hepatita cronica la soareci* [Effects of Phytotherapeutic Hydroalcoholic Extracts on Experimental Model of Chronic Hepatitis in Mice], Cluj: U.S.A.M.V.

Boesterli, U.A., Diclofenac - induced liver injury: a paradigm of idiosyncratic drug toxicity*, Toxicol Appl Pharmacol,* 192, 307- 22.

Kittisupamangkol, W. (2009), Liver injury from diclofenac of acetaminophen? *, Am J Gastroenterol,* 104, 1862.

Lupuleasa, D., Fica, C., Emese, S. (2005). *Tehnologie Farmaceeutica. Manual pentru studenti si asistenti de farmacie [*Farmaceutic Technology. Manual for students and pharmacy assistants]. Bucuresti: Carol Davila.

McGettigan, P, Henry, D. (2013). Use of Non-Steroidal Anti-Inflammatory Drugs That Elevate Cardiovascular Risk: An Examination of Sales and Essential Medicines Lists in Low-, Middle-, and High-Income Countries*, PLoS Med*, 10, 1001388.

Mello, J.C., Gonzalez, M.V., Moraes, V.W., Prieto, T., Nascimento, O.R., Rodrigues, T. (2015). Protective effect of Plantago major extract against t-BOOH-induced mitochondrial oxidative damage and cytotoxicity, *Molecules,* 20, 17747-59.

Moodley, I. (2008). Review of the cardiovascular safety of COXIBs compared to NSAIDS, *Cardiovasc. J. Afr*, 19, 102-107.

Orynia, O. A., Adenkola, A.Y., Ogbe, R.J. (2016). Haematological and biochemical studies on the effect of diclofenac sodium on Wistar Rattus norvegicus*, International Journal of Biological and Chemical Sciences*, 10, 2231-2242.

Suciu, M.(2013*), Efectul hepatoprotector al unor plante din flora spontana a judetului Arad* [The hepatoprotective effect of some spontaneous flora plants in Arad County], Arad: Universitatea de Vest „Vasile Goldis".

Türel, I., Özbek, H., Erten, R., Öner, A.C., Cengiz, N., Orhan, Y. (2009). *Hepatoprotective and anti-imflammatory activities of Plantago major L.,*https://www.ncbi.nlm.nih.gov/pubmed/20442819 Turkey: Universty of Kafkas.

Zhang , X. (2001*).* Selected medicinal plants*, World Health Organization monographs, 3,* 1-7.

*Corresponding author, E-mail address: gabriel_mda8487@yahoo.com