

CAHD
2020

The 1st International Electronic Conference on Antioxidants in Health and Disease

01-15 DECEMBER 2020 | ONLINE

Pre-clinical investigations of *Verbena officinalis* L. tisane effects against induced stress in Wistar male rats

Chaired by PROF. DR. MIHALIS I. PANAGIOTIDIS



Sonia M Oliveira ^{1, 2*}, Elsa Dias ^{1, 3}, and Maria de Lourdes Pereira ^{1, 4}

¹ CICECO - Aveiro Institute of Materials, 3810-193 Aveiro, Portugal

² Hunter Medical Research Institute, NSW 2305, Australia;

³ Centro Hospitalar do Baixo Vouga, Aveiro, Portugal;

⁴ Department of Medical Sciences, University of Aveiro 3810-193 Aveiro, Portugal.



Corresponding author: Sonia.oliveira@ua.pt

Abstract:

Verbena officinalis or vervain is globally used as an herbal medicine and dietary supplement for anti-depressive and anti-convulsive purposes, as well as to treat inflammatory disorders, skin burns, abrasions and gastric problems. In our exploratory research, we investigated the biochemical, antioxidant, and histopathological effects of local *V. officinalis* infusion in rats previously submitted to chronic physical stress. The animals presented significant alterations in several organs' ratio; namely, epididymis and brain ratios with $p=0.003$ and $p=0,013$, respectively. Moreover, tissues like kidney and liver presented relevant histologic alterations due to experimental conditions. Total protein, creatine kinase (CKI), uric acid (URCA), circulating and hepatic alkaline phosphatase (ALP) and Glutathione S-transferases (GSTs), and glucose levels were statistically different between treated and non-treated animals with $p<0.05$. Altogether, biochemical and hematological results indicated significant impacts in antioxidant, lipidic and protein metabolism. Therefore, physical stress and vervain infusion have significant in vivo effects. Chronic stress effects were not counteracted by vervain consumption (e.g., $p=0.5$ for hepatic and renal superoxide dismutase (SOD) levels between the different groups). A correlation between histology and the active components in an herbal extract would enable a better evaluation of herbal medicines. Accordingly, further studies of vervain extracts effects are in progress.

Keywords: *Verbena officinalis*; oxidative stress; male reproductive morphology; toxicology; ethnopharmacology

Verbena officinalis

- Wayside perennial;
- Dietary supplement / cosmetic industry;
- Metabolites that may play important physiological roles:
 - o **isoprenoids** inhibited tumor cell proliferation on human breast adenocarcinoma, leukemia and colon adenocarcinoma (Mo and Elson, 1999; Tatman and Mo, 2002; De Martino et al., 2009);
 - o used for **anti-depressive** and **anticonvulsive** effects (Rashidian et al., 2017);
 - o used in the treatment of **inflammatory** disorders, **skin** burns, abrasions and gastric diseases (Speroni et al., 2007);

BUT bioeffects and reproducibility of therapeutic effects are **affected by** factors such as:

- the proper identification of plants,
- season and collection area,
- extraction and purification method,
- and concentration and dosage uptake (Ardakani et al., 2003).



In www.biorede.pt

Pre-clinical investigation of vervain antioxidant potential

Physical exercise has a paradoxical reaction in health (Schnabel and Blankenberg, 2007).

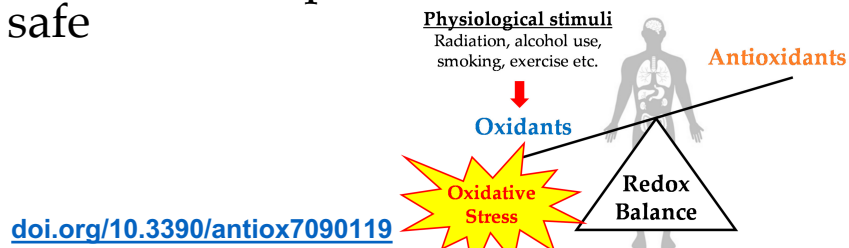
- 1) triggers the augmentation of reactive oxygen species (**ROS**) which react with the cellular structures oxidizing them, and
- 2) acts as a **modulator** to the intracellular **antioxidant** systems, improving the ROS scavenging activity.

Athletes' use of herbal supplements has skyrocketed in the past decades. But clinical testing and understanding of most herbal remedies is lacking. Many regulations do not ensure safe and effective products.

Some **herbal extracts** may prove useful in the athletic setting however, its safety is not ensured (Winterstein A. P. and Storrs C. M., 2001)

Since oxidative stress induces cell injuring (e.g. DNA inactivation, lipidic peroxidation, denaturation of proteins) is important to carry out studies to **detect changes in the oxidative stress** of individuals because of oral ingestion of antioxidants and health foods.

Vervain has been used in folk medicine against several ailments like burns and inflammatory conditions. But its pharmacological potential is still under-exploited.



CAHD
2020

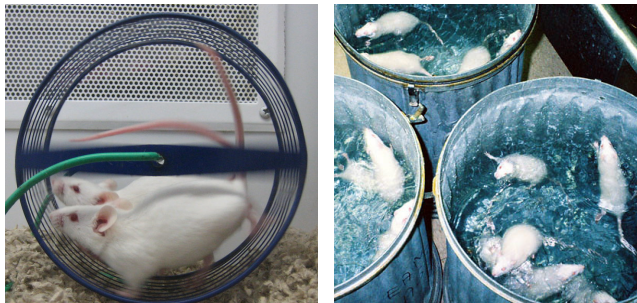
Methods



Oral administration
200 mg/kg



Male Wistar rats



Daily stimuli to simulate acute exercise



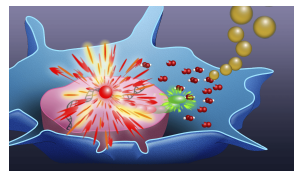
Body weight monitored



Liver, kidney, spleen, testis, epididymis, heart, skeletal muscle and brain collected, weighted and prepared for histology.



Biochemistry: cholesterol, LDH, bilirubin, creatinine kinase, amongst others.



Oxidative stress: Glutathione peroxidase (GPox) and superoxide dismutase (SOD) antioxidant activity

OBJECTIVE: To investigate the **biochemical**, antioxidant, and **histopathological potential** of an extract of **vervain** (*V. officinalis*) **against chronic physical stress**, in male Wistar rats.

CAHD
2020

Findings

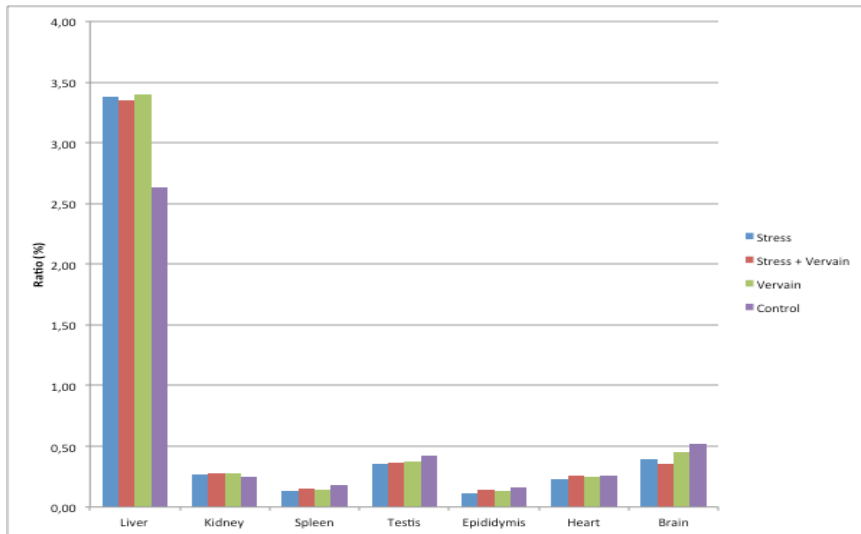


Fig. 1. Organ/body mass ratio (%) in the several groups. ^α statistically different from control ($p \leq 0.05$); ^β statistically different from stress group ($p \leq 0.05$).

- The animals presented significantly different weight ratios of **epididymis**, **brain** and **heart**.

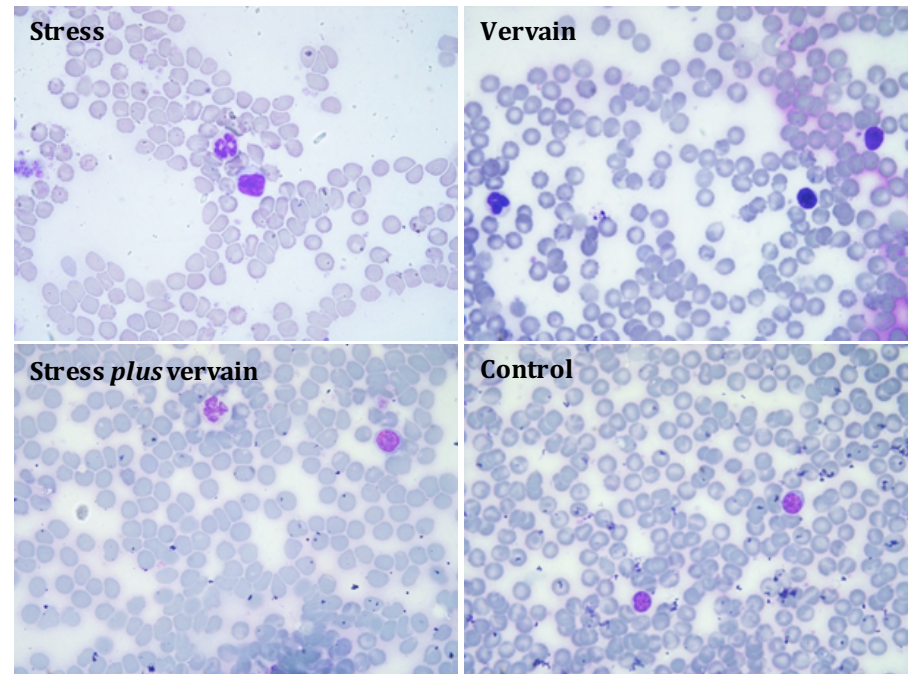


Fig. 2. Wright's stain from blood from animals of each group at the end of the protocol.

- No abnormalities were identified in the blood smears or hemograms

Findings

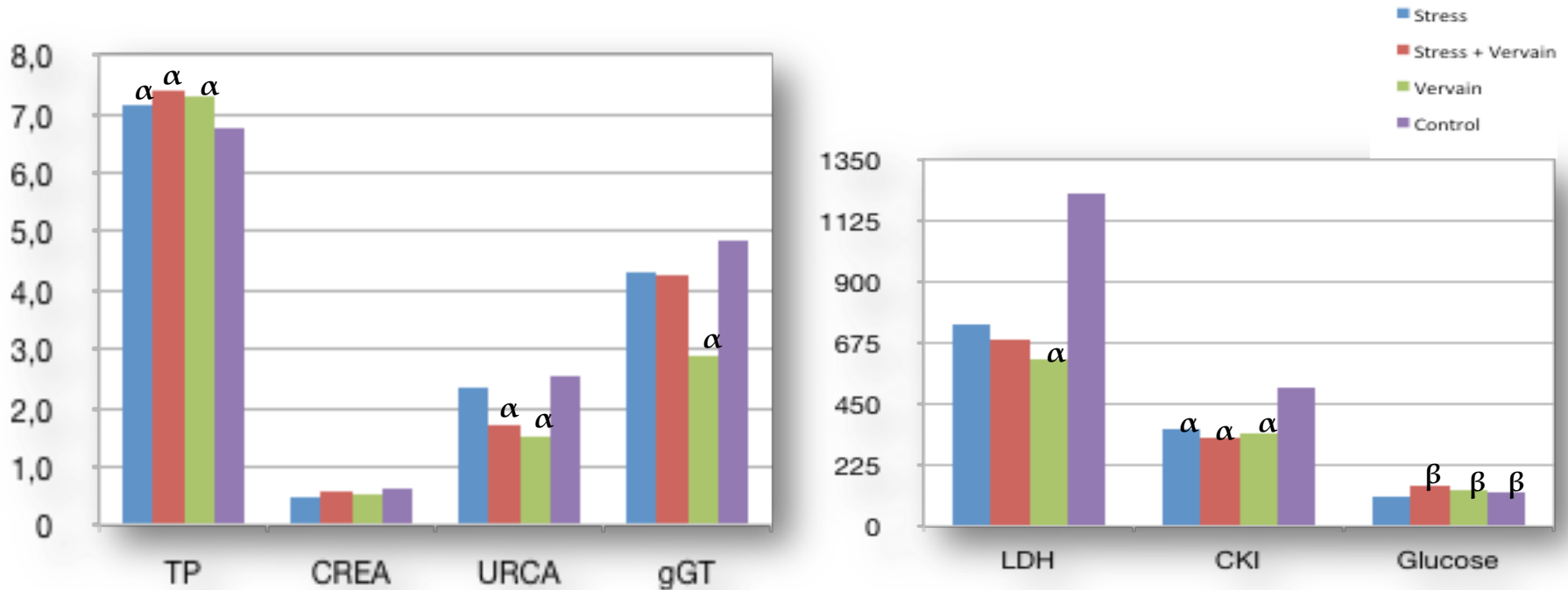


Fig. 3 & 4 – Blood parameters results. α – Statistically different from control ($p \leq 0.05$); β – Statistically different from stress group ($p \leq 0.05$)

- Biochemical and hematological markers **CREA**, **LDH**, **TP**, **CKI**, **URCA**, **γ GT**, and **glucose** were significantly different amongst the groups.
- Additionally, we detected alterations in blood glutathione peroxide (GPox) and superoxide dismutase (SOD) activity.

Findings

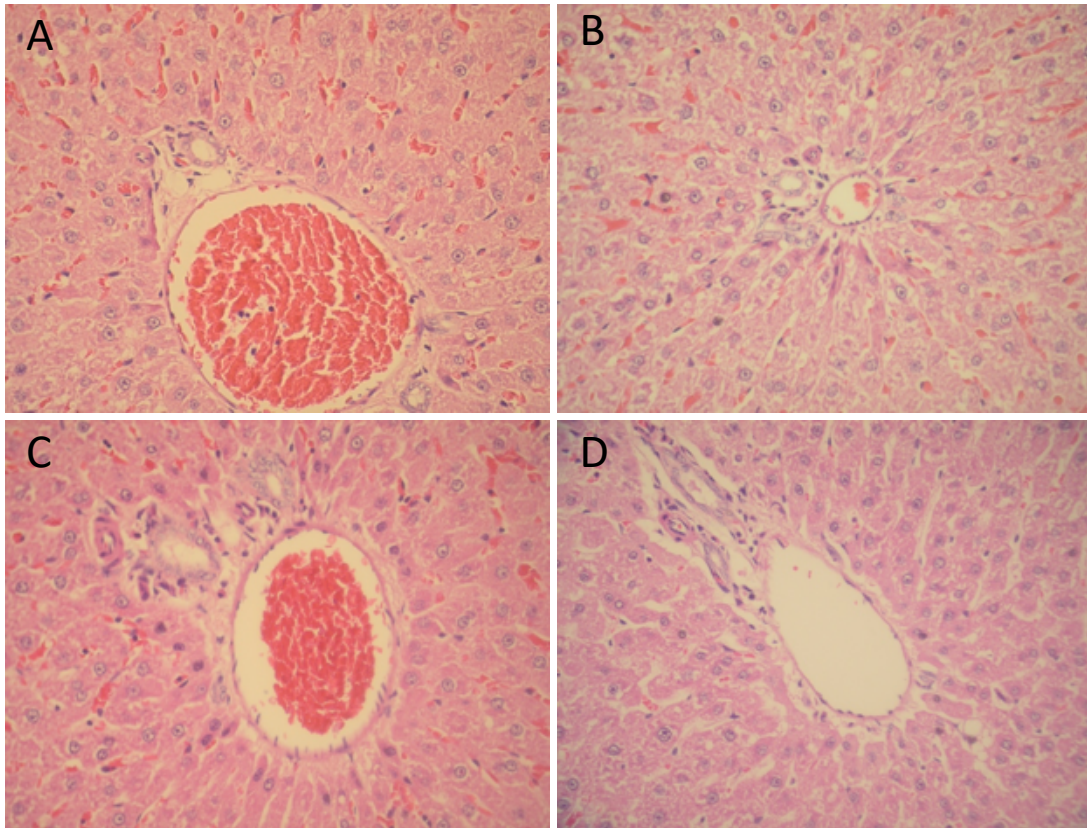


Fig. 5. Liver histology. A – stress; B – vervain; C – Stress + vervain; D – negative control. At=400x

- Rats submitted to chronic **stress** presented some inflammation and altered hepatic structure.
- No significant improvement was observed in liver morphology due to the administration of the *vervain* infusion.

Findings

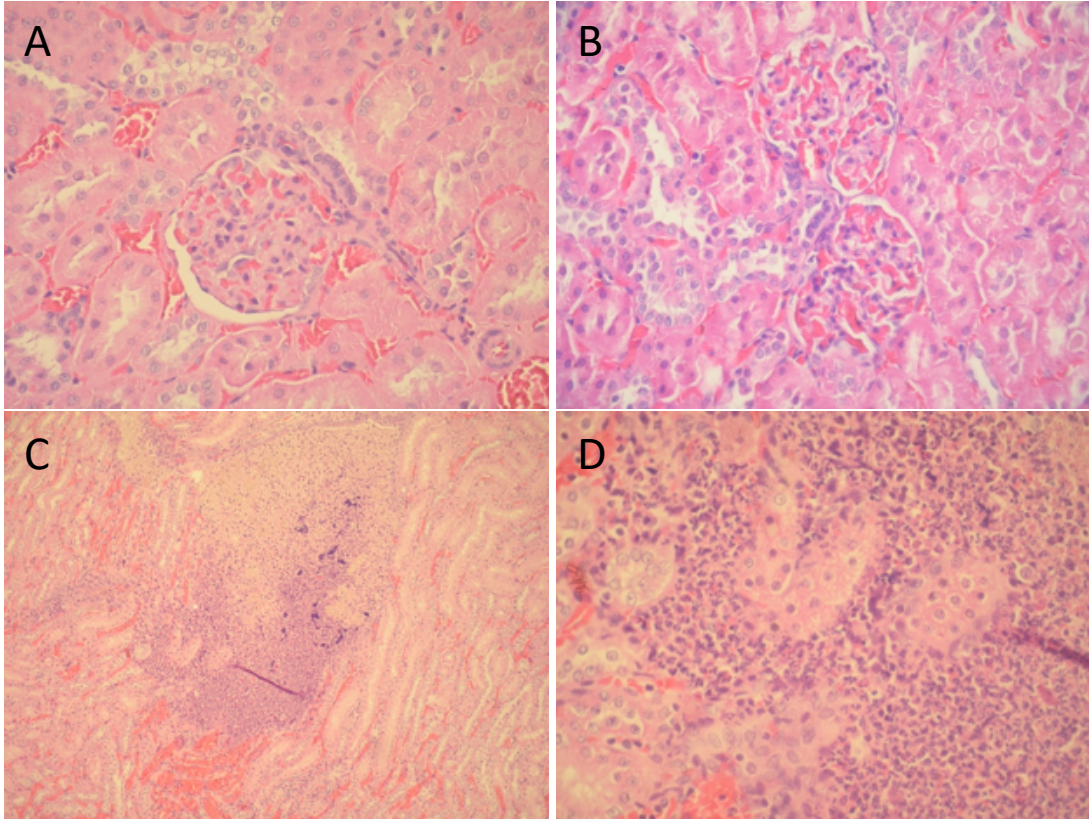


Fig. 6. Kidney histology. A – stress; B – vervain; C – Stress + vervain; D– negative control. At=400x

- Rats submitted to **stress** presented inflammation in the tubules, and dilatation of epithelia.
- Kidneys of rats orally administered **vervain** showed damaged renal structure (haemorrhagic areas, reduced Bowman's space, dilated epithelia)

Findings

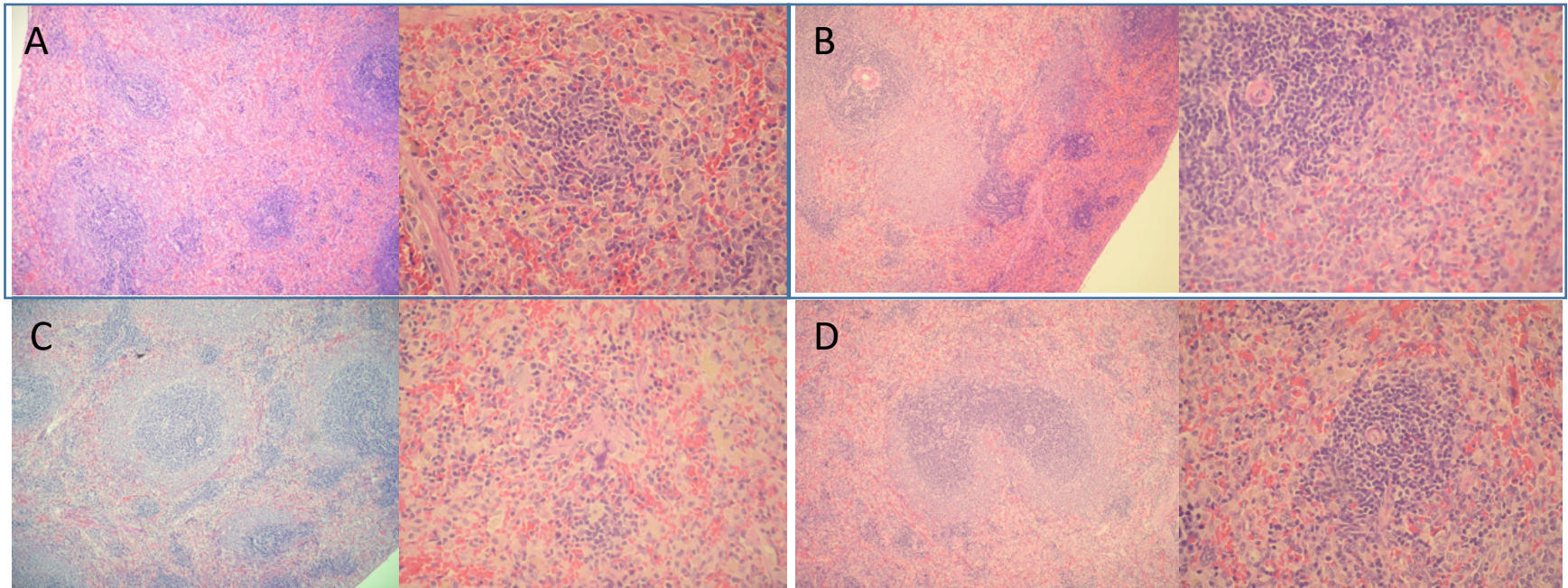


Fig. 7. Spleen histology. A – stress; B – vervain; C – Stress + vervain; D– negative control. At=100x and 400x

- **Vervain** extract appears to improve splenic morphology
- Slight response to **stress** (giant cells appear and possible iron enrichment)
- No histological changes were observed in the sections of brain, heart or muscle.

Findings

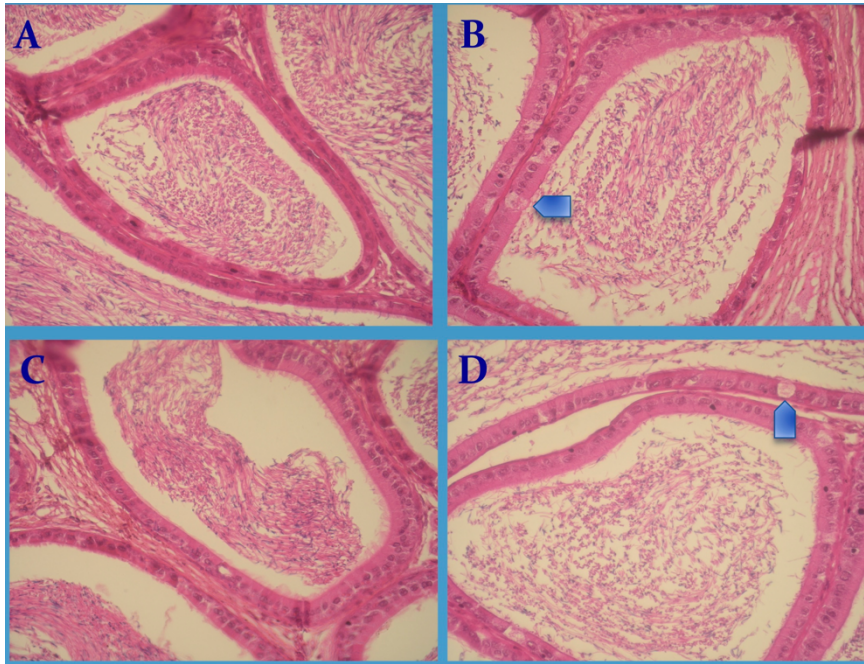


Fig. 8. Epididymis histology with H.E. A – control; B – Stress; C – Vervain; D– Stress + vervain. At=400x

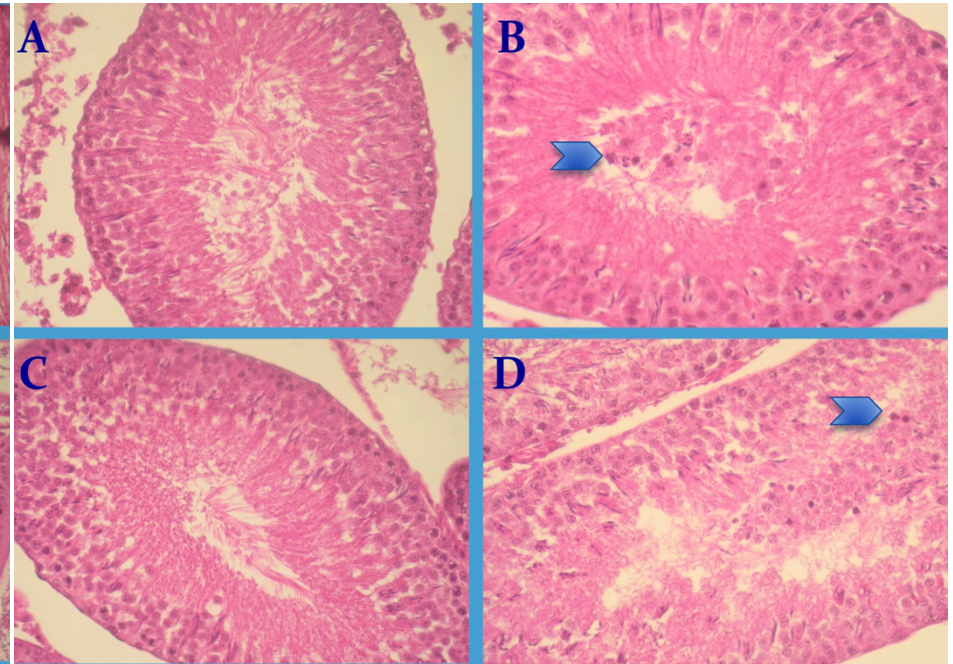
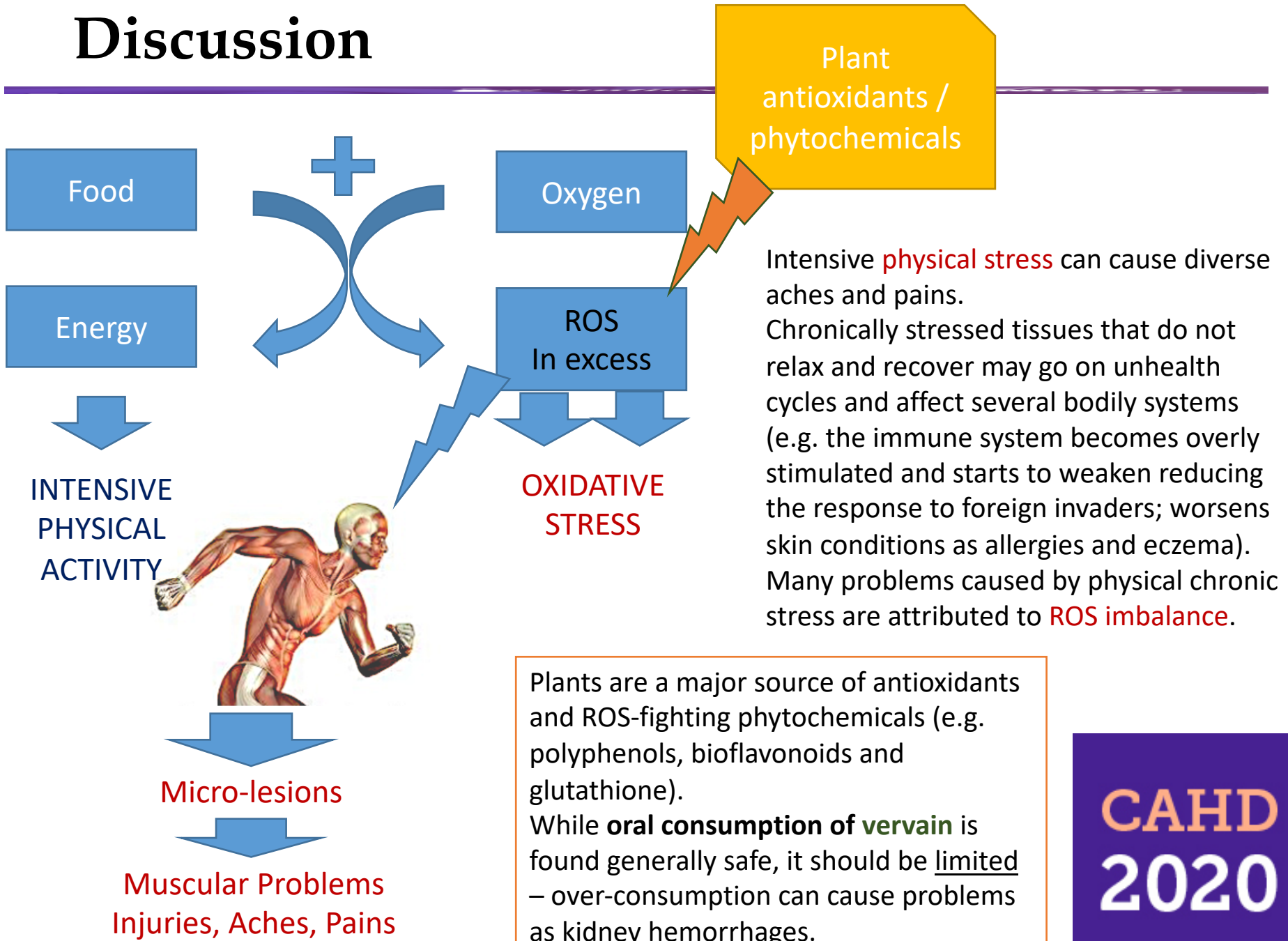


Fig. 9. Testis histology with H.E. A – control; B – Stress; C – Vervain; D– Stress + vervain. At=400x

- Cellular **disruption** and **vacuolization** were observed in the testes and epididymis of the animals submitted to stress.

Discussion



Intensive **physical stress** can cause diverse aches and pains.

Chronically stressed tissues that do not relax and recover may go on unhealthy cycles and affect several bodily systems (e.g. the immune system becomes overly stimulated and starts to weaken reducing the response to foreign invaders; worsens skin conditions as allergies and eczema).

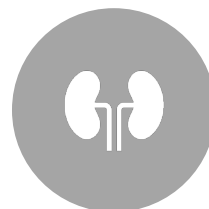
Many problems caused by physical chronic stress are attributed to **ROS imbalance**.

Plants are a major source of antioxidants and ROS-fighting phytochemicals (e.g. polyphenols, bioflavonoids and glutathione). While **oral consumption of vervain** is found generally safe, it should be limited – over-consumption can cause problems as kidney hemorrhages.

Recommendations



Stress and vervain influenced tissue mass namely, of the epididymis and testis.



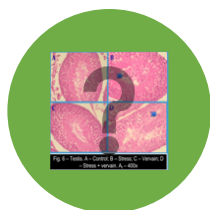
Blood biochemical markers were altered by both physical stress and vervain consumption.



Spermatogenesis was affected by chronic physical stress and by the vervain extract.

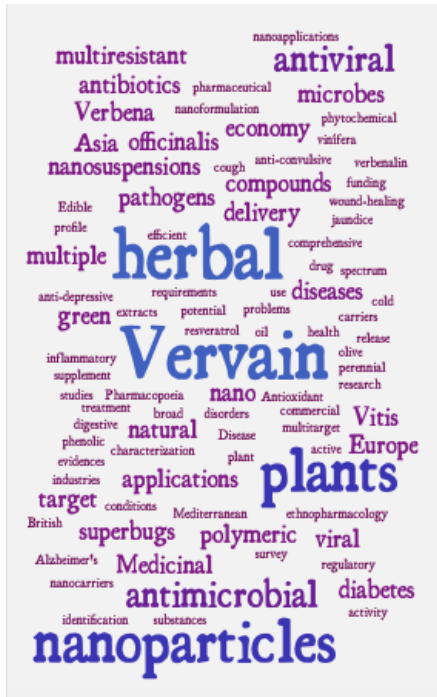


The herbal extract of vervain did not counteract chronic stress effects.



Mechanism of action of the vervain extract should be further explored

Acknowledgments



Dr. Sonia Oliveira
Sonia.oliveira@ua.pt



Elsa Dias
elsdias@ua.pt



Maria de Lourdes Pereira
mlourdespereira@ua.pt

Obrigada!



CAHD
2020