Evaluation of the Effect of Refined Extracts of *Matricaria recutita L* and *Chrysanthemum coronarium L*, Proposed as Bio-Pesticides for Agricultural Use, Determining the Variation of Biomarkers of Oxidative and Hepatotoxic Stress in Acutely Exposed Rats

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Abstract

Chamomile (*Matricaria recutita L*) and Chrysanthemum (*Chrysanthemum coronarium L*. (Garland)) are plants belonging to the family *Asteraceae*. The pharmacological properties of these herbs result from interactions among their many components. Of these, over 120 secondary metabolites with pharmacological and/or potential pesticide activity have been identified. The diverse chemical and biological characteristics of this set of compounds, *i.e.*, essential oils and organic extracts of genera *Matricaria* and *Chrysanthemum*, have been shown to have particular pesticidal effects, especially those rich in pyrethroids. This work characterizes chamomile and chrysanthemum extracts for their pesticidal properties and their effects following acute exposure in rats. Results show hepatotoxic and oxidative stress-inducing effects in the livers of rats exposed to *C. coronarium* extracts, but not those of *M. recutita*.

Keywords

Chamomile, Chrysanthemum, Toxicity, Pyrethroid

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1. Introduction

Chamomile (*Matricaria recutita L*) is a plant belonging to the family *Asteraceae*, considered by many as one of the most important medicinal plants in the world for its anti-inflammatory, antispasmodic, antiallergic, antibacterial, and fungicidal properties [1] [2]. Also of *Asteraceae*, Chrysanthemum (*Chrysanthemum coronarium L*. (Garland)) is an annual herbaceous weed originating in Mediterranean regions. Phytochemical investigations of some members of the genus *Chrysanthemum* have revealed the presence of several odoriferous precursors (cis-chrysanthenyl acetate, trans-chrysanthenyl acetate, α-pinene) and phenolic components (mainly derived from quinic acid) [3]. Previous analyses of *C. coronarium* essential oils have indicated different composition patterns based on origin and genotype [4].

Pesticides are grouped into sets of compounds with different chemical and biological characteristics, classified by activity into herbicides, insecticides, fungicides, etc. These are useful both in the agricultural industry and at the domestic level, where they are frequently used to eliminate insects or other pests [5] [6]. The indiscriminate use of chemical pesticides, however, has been shown to affect humans and the environment, further contributing to reduced crop production [7]. Additionally, several types of pests have developed resistance to synthetic insecticides: more than 500 species of insects and mites no longer respond to conventional pesticides [8]. An alternative for this is the use of biopesticides.

Essential oil and organic extracts of genera *Matricaria* and *Chrysanthemum* [9] have been shown to have different fungicidal and pesticide effects [10]. In the case of the genus *Matricaria*, organic extracts have been shown to have anti-microbial activity [11] and insecticidal properties [10]. These properties have motivated the study of both genera [9] [12] for different purposes, both as medicinal and as possible pesticides.

On the basis of the above, the insecticidal properties of hexanic extracts of flowers of *M. recutita* and *C. coronarium* have been studied in search of new alternatives such as biopesticides. In addition, experimental studies of their toxicity, induced by *Chrysanthemum coronarium* extract against house flies and red spider mites, points to sub-lethal and lethal effects of both, in addition to anti-feeding behavior [13]. Research has also been performed on lethal toxic effects of chamomile against *Tetranychus urticae Koch*, with extract obtained by Soxhlet extraction, where this extract and its purified fraction showed, like *C. coronarium* extracts, both lethal and sublethal effects [14].

Of these, the main biological components with insecticidal properties are derived from Pyrethrum, an oleoresin extract from the flowers of *Chrysanthemum cinerariaefolium* that contains approximately fifty percent of active insecticidal ingredients. Known as pyrethrins and pyrethroids, these have been used as insecticides for nearly a hundred years [15]. The latter insecticides are chemically similar to natural pyrethrins, but are modified to increase potency and stability [16] [17]. Pyrethroid use has been estimated at 23% (relative) of all insecticides

used in the world [18]. Their uses, both in agriculture and in homes, have increasingly been replacing old insecticides [19].

In their classifications, pyrethroids are either Type I or Type II, depending on their chemical structures and their biological effects with acute dosage exposures [20] [21]. Pyrethrins lacking an *α*-cyan group in their chemical structure produce characteristic toxic effects, such as tremors and aggressiveness (Type I or T Syndrome). The presence of this cyan group, in contrast, leads to a syndrome characterized by salivation and choreoathetosis (Type II or S Syndrome) [20] [22]. Pyrethroids are strongly lipophilic, penetrating very quickly into many insects and paralyzing the nervous system. Mammalian hepatitis enzymes are capable of very efficiently hydrolyzing pyrethrins and pyrethroids into inert products. This accelerated degradation, in addition to the relatively low bioavailability, largely explains why toxicity in mammals is relatively low. However, the 1996 Food Quality Protection Act proposed and requested that the EPA consider possible cumulative toxic effects of pyrethroids with common modes of action [10]. The median lethal dose ($LD50_s$) for pyrethroids in rats varies depending on the type or mixture of pyrethroids. Thus, some type I pyrethroids, such as Permethrin and Resmethrin, have $LD50_s$ of 1200 and 2000 mg/Kg in weight, respectively; while type II pyrethroids, such as Deltamethrin and Esfenvalerate, have $LD50_S$ of 250 and 87 mg/Kg in weight, respectively [23].

And so, in addition to attacking their intended target organisms, pesticides can also affect humans and/or animals. Once insecticides enter the body, they are transported to different parts of the body by the circulatory system. The liver is one such highly irrigated organ, especially through the portal vein transporting materials absorbed in the gastrointestinal tract [24], which increases its concentration of toxins. Insecticides, like organochlorines, have measured hepatotoxicity in both human and animal studies, possibly associated with the release of liver enzymes [25]. Hepatocellular damage may be related to changes of liver enzyme levels in plasma, which are indicative of liver tissue releasing enzymes when damaged or injured. Indeed, increased values of liver enzymes in plasma are commonly taken as indicators of liver damage [26]. For this reason, liver function tests can be used as markers of cell damage.

Common liver function tests include alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TB), and others. These are biomarkers of different hepatic functions, such as excreting anions (bilirubin), hepatocellular integrity (transaminases), and formation and subsequent flow of bile (bilirubin and alkaline phosphatase) [27]. Bilirubin is formed from red blood cell heme lysis within the reticuloendothelial system. Bilirubin in serum reflects a balance between hepatobiliary production and excretion [27]. Bilirubin is captured by the liver for conjugation and excretion in the bile. Hepatocellular alterations or biliary obstructions may be the cause of an increase in circulating bilirubin. Alanine transaminase (ALT), a cytosolic enzyme, is found in higher concentrations in the liver, and is more specific to liver damage [27]. Destruction or changes in

permeability, of cell membranes provokes the release of ALT, increasing plasmatic concentration. It has been demonstrated [25] that workers exposed to a wide variety of insecticides can suffer liver damage. This is due to accidental exposures to high levels of toxic substances, occasional low-dose exposures over long periods of time, or improper use of protective equipment while performing their work. Tomei *et al.* (1998) found that subjects with increased levels of alanine transaminase, alkaline phosphatase, and total bilirubin were correlated with liver damage and disruption of liver functions.

Evidence of liver alterations in toads poisoned with a pyrethroid insecticide, fenvalerate, was demonstrated by Sakr *et al.* (2002) [28]. Batrachians were administered the pyrethroid at a dose of 0.5 mg/kg weight for three weeks. Results showed significant increases in circulating transaminases. This result, together with histological evidence of abundant leukocyte infiltration, blood vessel congestion, and other abnormalities, indicated that fenvalerate exposure caused liver stress in toads.

Evolution between generations of living beings is dependent upon various environmental and chemical factors, including exposure to various foreign chemical substances of artificial origin, known as xenobiotics. In the presence of chemicals, organisms do not generate degradation and excretion pathways for each molecule; rather, these tend towards a general mechanism that removes the maximum amount of molecules from the organism at the same time [29]. As the organ responsible for xenobiotic metabolization processes, every drug, artificial chemical, pesticide, and/or hormone is metabolized by the liver. Many of the toxic products that are introduced to the body are lipophilic, which, due to high affinity with cell membranes, inhibits their excretion by the body.

Thus, the liver has two mechanisms designed to convert liposoluble chemicals into hydrosoluble chemicals: Phase I Biotransformation and Phase II Biotransformation. In some cases, biotransformation produces metabolites that are more toxic than the original compound, a process called bioactivation. If these more toxic metabolites accumulate and overcome the body's defenses, then they can produce damage that manifests itself in a toxic response [30] [31]. In general, xenobiotics are biotransformed in phase I, where a functional group is added to causes a small increase in solubility in water (which serves as substrates for Phase II enzymes). The enzymatic system used in Phase I liver biotransformation is called cytochrome system P450-or mixed-function oxygenases [32] which have the function of introducing an oxygen atom to the substrate. The transformation of xenobiotics by phase I enzymes can generate reactive metabolites like electrophiles and free radicals, which can damage cellular components including proteins and DNA [33].

Cytochrome P450 (CYPs) are expressed ubiquitously in different life forms, animals, plants, fungi, and bacteria [34] [35] and are indispensable for eukaryotic life (though not so for prokaryotic life, some of which lack CYPs [35]). Most CYPs are expressed in the liver [36]. CYPs catalyze a wide variety of reactions,

including N-alkylation, O-alkylation, S-oxidation, and hydroxylation of aromatic and aliphatic substrates, and, depending on the bioavailability of oxygen in the tissue, redox reactions [37]. Most of these reactions require an initial step involving the transfer of an oxygen atom from molecular oxygen (O_2) to various substrates. There is evidence that the process may generate reactive oxygen species, such as superoxide anions and hydrogen peroxide, in addition to the radical substrate R*, which, when bound to a hydroxyl radical, may generate an ROH hydroxylated product [38].

Regulation of CYP 450 enzymes may influence how certain drug results are administered regarding increases in the transcription of one or more CYP isoforms [39]. In many cases, inducers are also substrates of the induced enzymes. Consequently, CYP activities remain elevated only when needed [40]. Because the liver is the primary biotransformation site for xenobiotics, there is an abundant expression of several CYPs isoforms in this organ [41].

The presence of other biomarkers may also be indicative of hepatic functions that are activated and/or altered during xenobiotic detoxification processes, such as Superoxide Dismutase (SOD), the largest antioxidant enzyme in aerobic cells. A Cu-Zn form of SOD is found in the cytoplasm, and another Mn form in the mitochondria. SOD reduces superoxide radicals to hydrogen peroxide with high specificity [42]. Next, Glutathione Peroxidase (GPx), part of a group of proteins containing Selenium (Se) known as selenoproteins, are involved in protecting against oxidative damage. GPx is located both in the cytosol and mitochondria, as well as the cell membrane. It is thus an important cellular protection mechanism against oxidative damage in membrane lipids, proteins and nucleic acids [43]; and is essential in GSH pathways that reduce lipid hydroperoxides to water for removal. In short, GPx converts glutathione from a reduced GSH state to an oxidized GSSG state. Here, reduced glutathione levels are essential for enzyme activity, and so cells are equipped with NADPH-dependent glutathione reductase (GR) to reduce oxidized glutathione [42] [44].

The objective of this work, then, is to analyze parameters and biomarkers that would indicate if Chamomile and Chrysanthemum extracts present hepatoxic and/or stress effects associated with acute exposure (24 hours), and determine, if any, non-lethal and/or slightly toxic concentrations in biological models, according to the parameters established by Kennedy *et al.* (1986) [45].

2. Materials and Methods

2.1. Chamomile and Refined Chrysanthemum Extracts

100 g of dried and finely ground chamomile flower and 0.8 g of activated carbon are added to a batch reactor equipped with a condenser. Add 2.5 L of Hexane (Merck) and heat to reflux continuously for 8 hrs. Cool to room temperature. Filter to separate insoluble material. Solvent is extracted at reduced pressure and 50˚C temperature by Rotavapor. A dark yellow viscous residue corresponding to a **Semi-Refined Chamomile Extract** is obtained. Add 40 mL of Ethanol

(Merck) to the semi-refined extract. The mixture is agitated for 15 minutes at room temperature and filtered by separating the insoluble material. Solvent is extracted at reduced pressure and 50˚C Rotavapor temperature. A low viscosity residue of dark yellow coloring corresponding to a *Refined Chamomile Extract* is obtained.

2.2. Characterization of Refined Extracts

General experimental procedures

Gas chromatographic analysis was performed on a Hewlett-Packard 5790-A chromatograph, equipped with a split injector, without divider; and a flame ionization detector, connected to an LDC Analytical D-2500 integrator to store and replicate the data. A fused silica capillary column (30 m, 0.25 mm dia.) coated with a 0.25 mm film of 5% cross-linked methyl phenyl silicone (Supelco SE-54) with helium (1 mL/min) as carrier gas was used. In a typical analysis, the column furnace was maintained for 2 minutes at 80˚C, then programmed at 10˚C/min at 250˚C and maintained for 10 minutes. The temperature of the injector and detector was 250˚C. Identification of the extract components was achieved using a selective mass detector (Shimadzu MS-QP5050-A) coupled to a gas chromatography system (Shimadzu GC-17A Series) under conditions as described above. Structural clarification was achieved by searching a database (Masslab, NIST, Wiley) of spectra using a matching algorithm based on probability.

2.3. Rat Assays

Assays were undertaken to determine LD50 and effects after acute exposure of chamomile and chrysanthemum extracts in rats [46]. Five Sprague-Dawley rats of each sex and of similar weights (150 - 200 grams) were treated with respective extracts. 2000 mg/Kg was administered orally over one day. 24 hours after the doses of refined extracts, the rats were euthanized by cervical dislocation, and livers were extracted, weighed, and frozen with liquid nitrogen until study, with the exception of samples for GSH assays, carried out the same day.

2.4. Determination of Enzymatic Activity and Oxidative Stress

2.4.1. Super Oxide Dismutase (SOD)

Weigh 1 gram of liver, homogenize in a Potter-Elvehjem with sodium phosphate buffer solution pH 7.7 (0.1 M)-EDTA (1 mM) 1:3; then add 5 µL PMSF and mix gently. Centrifuge 10 minutes at 340xg, then dilute supernatant with distilled water to 50 times.

In the case of the test control, incubate 1475 µL Glycine buffer solution pH 10.0 (0 mM) for 2 minutes at 37˚C; add Epinephrine 25 µL (1 mM); mix, and measure absorbance at 480 nm (ε = 4.0 $mM^{-1} \cdot cm^{-1}$) after 30, 60, 90, and 120 seconds. For the sample, add 10, 25, and 50 µL epinephrine (1 mM) at 1465, 1450, and 1425 µL of glycine buffer pH 10.0 (50 mM) respectively; in each case,

preincubate 2 minutes at 37˚C; then add 50, 25, and 10 µL of sample (respectively); and measure absorbance at 480 nm at 30, 60, 90, and 120 seconds. Enzyme activity is expressed in SOD units. A SOD unit is defined as the amount of enzyme that inhibits the reaction rate by 50% [47].

2.4.2. GPx Assay

After weighing 1 gram of liver, homogenize in a Potter-Elvehjem with sodium phosphate buffer solution pH 7.7 (0.1 M)-EDTA (1 mM) 1:3; subsequently add 5 µL PMSF, mixing gently. Centrifuge 10 minutes at 340 ×g, then dilute supernatant with distilled water to 50 times.

Add to the quartz spectrophotometer cell (3.0 mL) 1.63 mL of Phosphate buffer solution pH 7.7 (100 mM); add 20 µL of t-BOOH (30 mM), 100 µL GSH (4 mM), 30 µL NADPH (8 mM), 50 µL GSR (50 UI/mL); and read absorbance at λ = 340 nm (ε = 6.22 $mM^{-1} \cdot cm^{-1}$) on thermoregulated spectrophotometer (35˚C) every 10 seconds for 1 minute. Then add 50 µL sample, mix and measure every 10 seconds for 1 minute.

Unit Definition: 1 unit of GPx is the oxidation/min of 1 µmol of GSH in the system, which is also equal to 1 mmol of NADPH oxidized/min at pH 7.7 and 35˚C [48].

2.4.3. Determining GSH

Prepare a protein free extract by weighing 1 gram of liver homogenized in 49 volumes of 0.5 M $HClO_4$, then centrifuge at 2430 ×g for 10 min at 4˚C. Collect supernatant (acid extract). Prepare 5 mL of a 0.5 M pH 6.8 phosphate buffer solution with 25 mg of Glyoxylic acid and 50 mg of sodium ascorbate. Put aside 1.2 mL into an aliquot and incubate at 60˚C for 5 minutes. Put immediately into ice bath and add 200 µL DNTB 3.7 mM, mix and centrifuge at 21,890 ×g for 2 minutes. Yields a white supernatant. For the control case, 1.12 mL of the same buffer solution is used and 80 µL of standard GSH (diluted 10 times) added, incubated at 60˚C for five minutes. Put immediately into ice bath and add 200 µL DNTB 3.7 mM, mix and centrifuge at 21,890 ×g for 2 minutes, and read the absorbance at 412 nm. For samples, add 1 mL of buffer solution and 200 µL of acid extract, incubate at 60˚C for 5 minutes. Put immediately into ice bath and add 200 µL DNTB 3.7 mM, mix and centrifuge at 21,890 ×g for 2 minutes; and measure the absorbance at a wavelength of 412 nm [49].

2.4.4. Determination of tBARS

Procedure: Weigh 1 g of liver, homogenize in a Potter-Elvehjem with buffer solution Phosphate 30 mM pH 7.4 KCl 120 mM. Centrifuge 10 minutes at 700 ×g, and extract the supernatant. Add 1 mL target sample to the previous buffer solution, 100 µL of BHT, and 1 mL of TCA to precipitate the proteins, chill for 30 minutes and then centrifuge for 10 minutes at 2400 ×g. Extract 1 mL of supernatant and add 1 mL of TBA. Incubate the mixtures for 60 minutes at 100˚C. After cooling, measure absorbances at 535 nm [50].

2.5. Obtaining Microsomal Fractions of Rat Liver

Liver tissues were macerated and washed with 0.15 M NaCl, and homogenized in a Potter-Elvejmen with a buffer (1:3 P/V) containing 0.25 M sucrose and 10 mM Tris-HCl (pH 7.4). The resulting sample was centrifuged at 10,000 ×g for 10 minutes. The obtained supernatant (3 mL) was applied to a Sefarose 4B column (1914.7 cm), recompacted, balanced and eluted with 10 mM Tris-HCL (pH 7.4), 0.01% NaN_3. Flow was regulated at 0.7 mL/min. The eluted volume was collected into 20 tubes, where each tube contained a 2.5 mL sample fraction. Microsomal fractions appearing between 10 - 16 mL volumes were added to 0.25 M solid sucrose. All operations were carried out at 4˚C. The quality of these microsomal preparations is similar in composition with respect to concentrations and activities of certain microsomal enzymes that are obtained by ultracentrifugation [51] [52]. Microsomal protein concentration and microsomal enzyme activity were measured for each collected tube.

2.6. Determination of CYP-450 Activity

2.6.1. Microsomal Protein Concentration of Rat Liver

The concentration of proteins in the microsomal fractions was measured by the Lowry spectrophotometric method [53], using BSA (bovine serum albumin) as the standard protein. Combine BSA, Water and ACR (Alkaline Copper Reagent), mixing well. After 10 minutes, add FR (Folin Reagent), mix and heat for 5 minutes at 55˚C. Cool the tubes, and read the Absorbance at 650 nm in spectrophotometer, leading to zero with the target. To measure microsomal proteins, proceed in the same way, only instead of adding BSA, add 0.25 mL microsomal fraction diluted in a factor of 50. The concentration of microsomal protein is obtained by interpolating absorbance from the BSA calibration curve.

2.6.2. Pentoxyresorufin O-Dealkylase Activity in Microsomal Fractions of Rat Liver

Pentoxyresorufin O-dealkylase (PROD) catalytic activity is mainly related to the activity of P-450 cytochrome isoforms of subfamily 2B. The activity assay is based on the ortho dealkylation of the Pentoxyresorufin substrate—also called Resorufin pentyl ether—which yields Resorufin. This latter compound has emission spectrum of maximum excitation λ_{max} 530 nm, and maximum emission λ_{max} at 580 nm [54] [55] [56].

PROD activity was measured following Nims & Lubet in 1995 [57], with some modifications. Briefly, after a calibration curve is made for resorufin, the fluorescence intensity (FI) of each cell is measured via Fluorimax-2 fluorometer, calibrated with a thermostated cell at 37˚C containing 30 µL of resorufin 1 µM. In another cuvette, reagents are pipetted in the following order: 400 µL of microsomal fraction; 1515 µL of Tris-HCL 50 mM Buffer (pH 7.5); 10 µL of Dicumarol; and 250 µL of pentoxyresorufin 80 µM. The combination is mixed and pre-incubated for 2 - 3 minutes in a thermostatized bath at 37˚C. The reaction is started with 2 µL of NADPH 10 mM and the fluorescence over time measured.

The PROD Activity is expressed in pmol resorufin formed per minute per milligram of protein (pmol resorufin/min mg protein).

2.7. Statistical Analysis

Kruskal-Wallis test: Used to determine the statistical differences among SOD, GPx, GSH and tBARS assays. Multiple comparison ANOVA, Tukey procedure: Used for CYP 450 determined by PROD, ALT and TB. Data were processed by SPSS software version 14.0.

3. Results and Discussion

3.1. Characterization of the Refined Extracts of Chamomile and Chrysanthemum

Active principles of botanical extracts of both Chamomile and Chrysanthemum were isolated and identified (Table 1), in which the main active principles were dioxaspiros.

The characterization of both refined extracts generated show the presence of dioxaspiros, such as *camphor; β*-farnesene*; α-farnesene;* 1,6-*dioxaspiro*(4,4)-*non*-3-*ene*-2-(2,4-*hexadinylidene*) (also known as spiroether) and 1,6-*dioxaspiro*-(4,4)-*nona*-2-8-*dien*-7-(2,4-*hexadynilidene*), which are known to have biocidal properties.

Both extracts share some of the molecules characterized as camphor, *β*-farnesene, and molecules of the same family as dioxapiros, which could explain some of the shared properties between both extracts, in particular what could refer to toxic properties for organisms.

3.2. The Effects of Chamomile and Chrysanthemum Extracts on Biomarkers

Biomarkers for effects of Chamomile and Chrysanthemum extracts were, for

Table 1. Chemical composition of chamomile and chrysanthemum extracts. The table shows the main components of chamomile and chrysanthemum extract obtained by continuous hexane Soxhlet extraction. Active dioxaspiro principles of Chamomile were 32.37% of the total extract; and for that of Chrysanthemum, 17.21% of the total extract.

Chamomile		Chrysanthemum	
Compound	**% Relative**	**Compound**	**% Relative**
2- Methyl-1-hepten-6-one	2.32	Camphor	5.21
3,3,6-trimethyl-1,5 heptadien-4-one	0.86	*β*-farnesene	2.21
Camphor	0.66	*α*-farnesene	0.7
β-Farnesene	18.6	3-7- dimethyl-1,5-octadien-3,7-diol	1.47
3,5-dimethoxy-4-hydroxy-acetophenone	3.31	1,6-dioxaspiro-(4,4)-nona-2-8-dien-7-(2,4-hexadynilidene)	2.00
7-methoxy-2H-1benzopyran-2-one (coumarin)	1.74	1,6-dioxaspiro(4,4)-non-3-ene-2-(2,4-hexadinylidene)	15.21
2-(2,4-hexadinylidene)-1,6-dioxaspiro(4,4) non-3-ene	32.37	N.N.	15.21
5-Methyl-6-Heneicosan-11-one	21.18	N.N.	10.21

hepatic toxicity, CYP P450; hepatic stability, ATL and TB; and stress, Lipoperoxidation, SOD, GPx and GSH. Results differed significantly from corresponding controls.

PROD activity related to Cytochrome P450 (or CYP) in both extracts presented a statistically significant increase, in relation to the control: for chamomile, the extract was correlated with an increase of almost three times the activity of CYP, rising to 15.17 ± 4.66 pmol/(min*mg_{prot}); and for that of chrysanthemum, a little over double, to 11.76 ± 0.54 pmol/(min*mg_{prot}) (**Figure 1**). The large increase in PROD activity may be due to some CYP isoenzymes presenting nonhyperbolic kinetics, characteristic of the result of an allosteric effect from substrates [58]. Based on this evidence, the study cannot discard that some chamomile and chrysanthemum extract constituent may be a potential substrate for CYP 450, exerting a positive feedback on the enzyme by increasing its catalytic efficiency. Such a situation could explain the low correlation between increased PROD activity and increased apoprotein.

Liver damage biomarkers alanine aminotransferase (ALT) and total bilirubin (TB) presented distinct behavior between extracts. In rats treated with refined chamomile extract, ALT levels do not significantly differ from control, increasing only by 10.84%; but for chrysanthemum extract, ALT increases by 131.72% (**Figure 2**). This elevated ALT within 24 to 48 hours suggests acute liver injury, and particularly, due to its longer plasma half-life, continuous damage, evident in the case of rats treated with refined chrysanthemum extract.

Rats treated with Chamomile extract do not show TB significantly differing from control, even dropping slightly by 6.58% (**Figure 2**). In the case of TB in rats treated with refined Chrysanthemum extract, there is a statistically significant increase of 53.16%, indicating hepatotoxic effects of the extract. These elevated levels of TB may indicate liver damage (jaundice).

Stress biomarkers reduced glutathione (GSH), glutathione peroxidase (GPx), Super Oxide Dismutase (SOD) and tBARS did not present statistically significant differences in the rats treated with chamomile in relation to the control (**Figures 3(a)-(d)**). However small, measurements did indicate slight increases

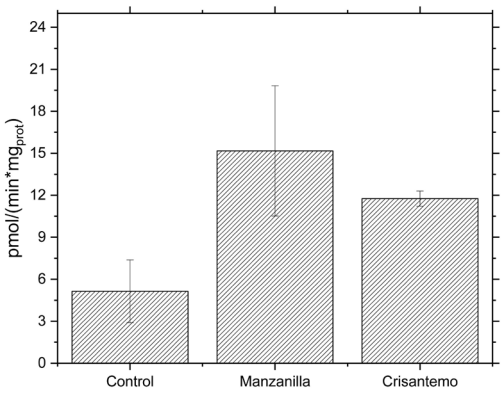

Figure 1. PROD activity in microsomal fractions of rat liver. Significant differences between control and treated rats ($p < 0.05$, $n = 5$).

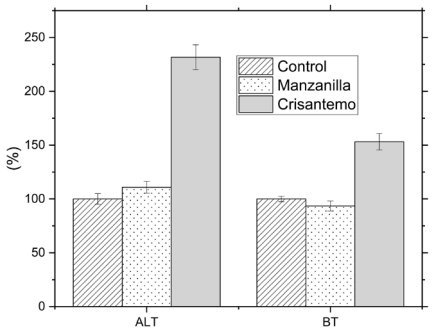

Figure 2. Hepatic biomarkers, alanine transaminase (ALT) and Total bilirubin (TB). Differences expressed as percentage variation. In the case of samples from rats exposed to Chrysanthemum, the differences are statistically significant ($p < 0.05$, $n = 5$).

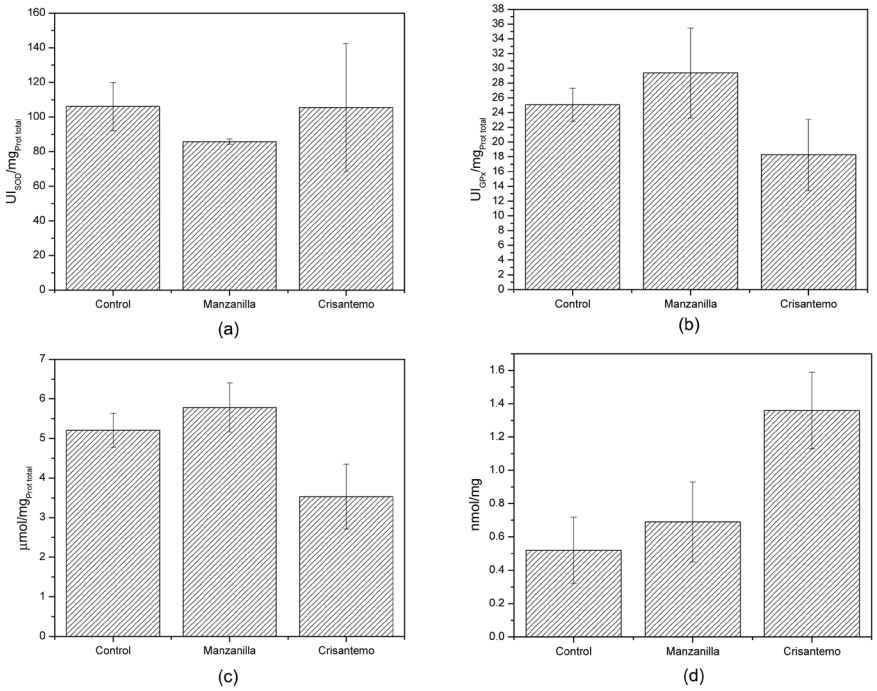

Figure 3. Statistical comparisons of stress biomarkers among controls and rats with acute exposure (24 hours) to refined extracts of chamomile or Chrysanthemum. (a) Super Oxide Dismutase (SOD); (b) Glutathione Peroxidase (GPx); (c) Reduced Glutathione (GSH); and (d) tBARS (lipid oxidation).

in GPx and GSH, perhaps due to acute ROS detoxification events. Any such events were likely caused by CYP 450, reinforced by the slightly increased tBARS level, indicating low lipoperoxidation of hepatocytes. Behavior of SOD was lower than the control, perhaps due to decreased O^{2-} resulting from antioxidant molecules present in Chamomile extract, or reactivity induced between Nitrous Oxide (NO) and the formation of peroxynitrites.

4. Conclusions

The present work characterized extracts and found pyrethroid-type molecules

likely responsible for the biocide activities of the products. This builds on previous studies that had identified such properties against mites (*Tetranychus urticae K.*) and insects (*Musca domestica L.*) at the Entomological Centre, INIA Chile. The study found that acute exposure of non-lethal doses of *Matricaria recutita L* extract presented minor hepatotoxic impact, given the lack of significant alteration to biomarkers. For that of *Chrysanthemum coronarium L*, hepatic functions were shown to be affected by acute exposure, given the significantly altered ALT and total bilirubin levels; as was the redox status of liver cells, given the variation of GSH and tBARS levels.

Neither extracts affected SOD or GPx enzyme activities. The nonreactivity of these antioxidant enzymes may be due to the short time of exposure (24 hours)—longer exposure may more significantly alter enzyme activities.

Finally, both extracts generated significant increases in PROD activity, representative of the CYP-450 function, suggesting that both extracts are metabolized as xenobiotics in the liver. In the case of chamomile, there is a greater increase CYP-450 activity, associated with a greater metabolization of this extract, and thus minimized toxic effects as correlates with other biomarkers; and the PROD activity of rats exposed to chrysanthemum extract does not present lower levels, though still remain higher than the control. This may be associated with low metabolism of extract molecules, and therefore more toxic effects on hepatocytes.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Sharafzadeh, S. and Alizadeh, O. (2011) German and Roman Chamomile. *Journal of Applied Pharmaceutical Science*, **1**, 1-5.

[2] Formisano, C., Delfine, S., Oliviero, F., Tenore, G.C., Rigano, D. and Senatore, F. (2015) Correlation among Environmental Factors, Chemical Composition and Antioxidative Properties of Essential Oil and Extracts of Chamomile (*Matricaria chamomilla* L.) Collected in Molise (South-Central Italy). *Industrial Crops and Products*, **63**, 256-263. https://doi.org/10.1016/j.indcrop.2014.09.042

[3] Buono-Core, G.E, Nuñez, V., Lucero, A., Vargas, R. and Jullian, C. (2011) Structural Elucidation of Bioactive Principles in Floral Extracts of German Chamomille (*Matricaria Recutita L*). *Journal of the Chilean Chemical Society*, **56**, 549-553. http://dx.doi.org/10.4067/S0717-97072011000100006

[4] Abd El Raheim, D. (2014) Biological Activity of *Chrysanthemum coronarium L.* Extracts. *Annual Research & Review in Biology*, **4**, 2617-2627. https://doi.org/10.9734/ARRB/2014/10112

[5] Cavieres, M.F. (2004) Exposición a Pesticidas y Toxicidad Reproductiva y del Desarrollo en Humanos: Análisis de la Evidencia Epidemiológica y Experimental. *Revista Médica de Chile*, **134**, 873-879. http://dx.doi.org/10.4067/S0034-98872004000700014

[6] Gögera, G., Demircib, B., Ilgınc, S. and Demirciba, F. (2018) Antimicrobial and Toxicity Profiles Evaluation of the Chamomile (*Matricaria recutita L.*) Essential Oil Combination with Standard Antimicrobial Agents. *Industrial Crops and Products*, **120**, 279-285. https://doi.org/10.1016/j.indcrop.2018.04.024

[7] Jayaraj, S. and Rabindra, R.J. (1993) The Local View on the Role of Plant Protection in Sustainable Agriculture in India. In: Chadwick, D.J. and Marsh, J., Eds., *Ciba Foundation Symposium* 177—*Crop Protection and Sustainable Agriculture*, 168-180. https://doi.org/10.1002/9780470514474.ch11

[8] Ehrlich, P.R. and Ehrlich, A.H. (1997) Ehrlichs's Fables. *Technology Review*. https://www.technologyreview.com/s/400001/ehrlichs-fables/

[9] Teixeira da Silva, J.A. (2004) Mining the Essential Olis of the Anthemideae. *African Journal of Biotechnology*, **3**, 706-720.

[10] United States Environmental Protection Agency (2000) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. Vol. EPA/630/R-00/002, United States Environmental Protection Agency, Washington DC.

[11] Mahady, G.B., Pendland, S.L., Stoia, A., Hamill, F.A., Fabricant, D., Dietz, B.M. and Chadwick, L.R. (2005) *In Vitro* Susceptibility of Helicobacter Pylori to Botanical Extracts Used Traditionally for the Treatment of Gastrointestinal Disorders. *Phytotherapy Research*, **19**, 988-991. https://doi.org/10.1002/ptr.1776

[12] Gawde, A., Cantrell, C.L., Zheljazkov, V.D., Astatkie, T. and Schlegel, V. (2014) Steam Distillation Extraction Kinetics Regression Model Sto Predict Essential Oil Ield, Composition, and Bioactivity of Chamomile Oil. *Industrial Crops and Products*, **58**, 61-67. https://doi.org/10.1016/j.indcrop.2014.04.001

[13] Lucero, A., Rebolledo, C. and Buono-Core, G.E. (2012) Effect of Some Natural Uv-Absorbers on the Photostabilization of Active Ingredients in German Chamomille Floral Extracts: Part I. *Journal of the Chilean Chemical Society*, **57**, 1309-1212. http://dx.doi.org/10.4067/S0717-97072012000300024

[14] Skirvin, D. and Stavrinides, M. (2003) The Effect of Chrysanthemum Leaf Trichome Density and Prey Spatial Distribution on Predation of *Tetranychus urticae* (Acari: Tetranychidae) by *Phytoseiulus persimilis* (Acari: Phytoseiidae). *Bulletin of Entomological Research*, **93**, 343-350. https://doi.org/10.1079/BER2003243

[15] LaForge, F.B. and Markwood, L.N. (1938) Organic Insecticides. *Annual Review of Biochemistry*, **7**, 473-490. https://doi.org/10.1146/annurev.bi.07.070138.002353

[16] Casida, J.E. (1980) Pyrethrum Flowers and Pyrethroid Insecticides. *Environmental Health Perspectives*, **34**, 189-202. https://doi.org/10.1289/ehp.8034189

[17] Elliott, M. and Janes, N.F. (1978) Synthetic Pyrethroids—A New Class of Insecticide. *Chemocal Society Reviews*, **7**, 473-505. https://doi.org/10.1039/cs9780700473

[18] Casida, J.E. and Quistad, G.B. (1998) Golden Age of Insecticide Research: Past, Present, or Future? *Annual Review of Entomology*, **43**, 1-16. https://doi.org/10.1146/annurev.ento.43.1.1

[19] Amweg, E.L., Weston, D.P. and Ureda, N.M. (2005) Use and Toxicity of Pyrethroid Pesticides in the Central Valley, California USA. *Environ. Environmental Toxicology and Chemistry*, **24**, 966-972. https://doi.org/10.1897/04-146R1.1

[20] Gammon, D.W., Brown, M.A. and Casida, J.E. (1981) Two Classes of Pyrethroid Action in the Cockroach. *Pesticide Biochemistry and Physiology*, **15**, 181-191. https://doi.org/10.1016/0048-3575(81)90084-5

[21] Gray, A.J. (1985) Pyrethroid Structure-Toxicity Relationships in Mammals. *Neuro-*

toxicology, **6**, 127-137.

[22] Lawrence, L.J. and Casida, J.E. (1982) Pyrethroid Toxicology: Mouse Intracerebral Structure-Toxicity Relationships. *Pesticide Biochemistry and Physiology*, **18**, 9-14. https://doi.org/10.1016/0048-3575(82)90082-7

[23] Wolansky, M.J., Gennings, C. and Crofton, K.M. (2006) Relative Potencies for Acute Effects of Pyrethroids on Motor Function in Rats. *Toxicological Sciences*, **89**, 271-277. https://doi.org/10.1093/toxsci/kfj020

[24] Peña, C.E., Carter, D.E. and Fierro, F.A. (2001) Toxicología Ambiental: Evaluación de Riesgos y Restauración Ambiental. The University of Arizona, Tucson. https://es.slideshare.net/EdwinMamaniVilcapaza/toxicologia-ambiental-evaluacin-de-riesgos-y-restauracin-ambiental

[25] Tomei, F., Biagi, M., Baccolo, T.P., Tomao, E., Giuntoli, P. and Rosati, M.V. (1998) Liver Damage among Enviromental Disinfestation Workers. *Journal of Occupational Health*, **40**, 193-197. https://doi.org/10.1539/joh.40.193

[26] Sakr, S.A. and Hanafy, S.M. (2002) Histopathological Alterations in the Liver and Kidney of Toads (*Bufo regularis*) Intoxicated with a Pyrethroid Insecticide. *Journal of Biological Sciences*, **2**, 208-211. https://dx.doi.org/10.3923/jbs.2002.208.211

[27] Limdi, J.K. and Hyde, G.M. (2003) Evaluation of Abnormal Liver Function Tests. *Postgraduate Medical Journal*, **79**, 307-312. https://doi.org/10.1136/pmj.79.932.307

[28] Sakr, S.A., Andel, S., Hany, A. and Salina, M.E. (2004) Exploring Hepatotoxicity of Benomyl: Histological and Histochemical Study on Albino Rats. *Journal of Medical Sciences*, **4**, 77-83. https://dx.doi.org/10.3923/jms.2004.77.83

[29] Piñeiro-Carrero, V.M. and Piñeiro, E.O. (2004) Liver. *Pediatrics*, **113**, 1097-1106.

[30] Hakkola, J., Tanaka, E. and Pelkonen, O. (1998) Developmental Expresión of Cytochrome P450 Enzymes in Human Liver. *Pharmacology & Toxicology*, **82**, 209-217. https://doi.org/10.1111/j.1600-0773.1998.tb01427.x

[31] Prough, R.A., Burke, M.D. and Mayer, R.T. (1978) Direct Fluorometric Methods for Measuring Mixed-Function Oxidase Activity. *Methods in Enzymology*, **52**, 372-377. https://doi.org/10.1016/S0076-6879(78)52041-7

[32] Yasui, H., Hayashi, S. and Sakurai, H. (2005) Possible Involvement of Singlet Oxygen as Multiple Oxidants in P450 Catalytic Reactions. *Drug Metabolism and Pharmacokinetics*, **20**, 1-13. https://doi.org/10.2133/dmpk.20.1

[33] Nelson, D.R., Koymans, L., Kamataki, T., Stegeman, J.J., Feyereisen, R., Waxnan, D.J., Waterman, M.R., Gotoh, O., Coon, M.J., Estabrook, R.W., Gunsalus, I.C and Nebert, D.W. (1996) P450 Superfamily Update on New Sequences, Gene Mapping, Accession Numbers and Nomenclature. *Pharmacogenetics*, **6**, 1-42. https://doi.org/10.1097/00008571-199602000-00002

[34] Nelson, D.R. (1999) Cytochrome P450 and Individuality of Species. *Archives of Biochemistry and Biophysics*, **369**, 1-10. https://doi.org/10.1006/abbi.1999.1352

[35] Raunio, H., Hakkola, J., Hukkanen, J., Lassila Paivarinka, K., Pelkonen, O., Anttila, S., Piipari, R., Boobis, A.R. and Edwards, R.J. (1999) Expression of Xenobiotic-Metabolizing CYPs in Human Pulmonary Tissue. *Experimental and Toxicologic Pathology*, **51**, 412-417. https://doi.org/10.1016/S0940-2993(99)80031-1

[36] Guengerich, F.P. (2001) Uncommon P450 Catalysed Reaction. *Current Drug Metabolism*, **2**, 93-115. https://doi.org/10.2174/1389200013338694

[37] Coon, M.J., Ding, X.X., Pernecky, S.J. and Vaz, A.D. (1992) Cytochrome P450: Progress and Predictions. *The FASEB Journal*, **6**, 669-673.

https://doi.org/10.1096/fasebj.6.2.1537454

[38] Himeo, S. and Imura, N. (2000) New Aspects of Physiological and Pharmacological Roles of Selenium. *Journal of Health Science*, **46**, 393-398. https://doi.org/10.1248/jhs.46.393

[39] Denison, M.S. and Whitlock, J.P. (1995) Xenobiotic-Inducible Transcription of Cytocchromme P450 Genes. *Journal of Biological Chemistry*, **270**, 18175-18178. https://doi.org/10.1074/jbc.270.31.18175

[40] Tamási, V., Vereckey, L., Falus, A. and Monostory, K. (2003) Some Aspects of Interindividual Variations in the Metabolism of Xenobiotics. *Inflammation Research*, **52**, 322-333. https://doi.org/10.1007/s00011-003-1186-4

[41] Monod, J., Wyman, J. and Changeux, J.P. (1965) On the Nature of Allosteric Transitions: A Plausible Model. *Journal of Molecular Biology*, **12**, 88-118. https://doi.org/10.1016/S0022-2836(65)80285-6

[42] Greenwald, R.A. (1989) Handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, 294-295. https://doi.org/10.1201/9781351072922

[43] Giray, B., Gubay, A. and Hincal, F. (2001) Cypermetrin-Induced Oxidative Stress in Rat Brain and Liver Is Prevented by Vitamin E or Allopurinol. *Toxicology Letters*, **118**, 139-146. https://doi.org/10.1016/S0378-4274(00)00277-0

[44] Zoccarato, F., Cavallini, L and Alexandre, A. (2004) Respiration-Dependent Removal of Exogenous H_2O_2 in Brain Mitochondria Inhibition by Ca^{2+}. *Journal of Biological Chemistry*, **279**, 4166-4174. https://doi.org/10.1074/jbc.M308143200

[45] Kennedy, G.L., Ferenz, R.L. and Burgess, B.A. (1986) Estimation of Acute Oral Toxicity in Rats by Determination of the Approximate Lethal Dose Rather than the LD_{50}. *Journal of Applied Toxicology*, **6**, 145-148. https://doi.org/10.1002/jat.2550060302

[46] Sumie, Y., Masanori, H., Masu, T., Kazuo, K., Jun, S. and Masao, N. (1990) A Simple Method for Screening Assessment of Acute Toxicity of Chemicals. *Archives of Toxicology*, **64**, 262-268. https://doi.org/10.1007/BF01972985

[47] Rice-Evans, C.A., Diplock, A.T. and Symons M.C.R. (1991) Techniques in Free Radical Research. In: Burdon, R.H. and van Knip-Penberg, P.H., Eds., *Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. 22, Elsevier, Amsterdam, 51-100.

[48] Videla, L.A. and Valenzuela, A. (1982) Alcohol Ingestion, Liver Glutathione and Lipoperoxidation: Metabolic Interrelations and Pathological Implications. *Life Sciences*, **31**, 2395-2407. https://doi.org/10.1016/0024-3205(82)90743-3

[49] Galleano, M. and Puntarulo, S. (1995) Role of Antioxidants on the Erythrocytes Resistance to Lipid Peroxidation after Acute Iron Overload in Rats. *Biochimica et Biophysica Acta—Molecular Basis of Disease*, **1271**, 321-326. https://doi.org/10.1016/0925-4439(95)00049-A

[50] Porter, T.D. and Coon, M.J. (1991) Cytochrome P450, Multiplicity of Isoforms, Substrates, and Catalytic and Regulatory Mechanism. *Journal of Biological Chemistry*, **266**, 13469-13472.

[51] Tanger, O., Jonsson, J. and Orrenius, S. (1973) Isolation of Rat Liver Microsomes by Gel Filtration. *Analytical Biochemistry*, **54**, 597-603. https://doi.org/10.1016/0003-2697(73)90392-8

[52] Gutiérrez, A.M., Reboredo, G.R., Arcemis, C.J. and Catalá, A. (2000) Non- Enzymatic Lipid Peroxidation of Microsomes and Mitochondria Isolated from Liver and

Heart of Pigeon and Rat. *The International Journal of Biochemistry & Cell Biology*, **32**, 73-79. https://doi.org/10.1016/S1357-2725(99)00105-3

[53] Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein Measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry*, **193**, 265-175.

[54] Okey, A.B. (1990) Enzyme Induction in the Cytochrome P450 System. *Pharmacology & Therapeutics*, **45**, 241-298. https://doi.org/10.1016/0163-7258(90)90030-6

[55] Dhawan, A., Parmar, D., Dayal, D. and Seth, P.K. (1999) Cytochrome P450 (P450) Isoenzyme Specific Dealkylation of Alkoxyresorufins in Rat Brain Microsomes. *Molecular and Cellular Biochemistry*, **200**, 169-176. https://doi.org/10.1023/A:1007026800114

[56] Guengerich, F.P., Martin, M.V., Sohl, C.D. and Cheng, Q. (2009) Measurement of Cytochrome P450 and NADPH-Cytochrome P450 Reductase. *Nature Protocols*, **4**, 1245-1251. https://doi.org/10.1038/nprot.2009.121

[57] Nims, R.W. and Lubet, R.A. (1995) Induction of Cytochrome P450 in the Norway rat, Rattus Norvegicus, following Exposure to Potential Environmental Contaminants. *Journal of Toxicology and Environmental Health*, **46**, 271-292. https://doi.org/10.1080/15287399509532035

[58] Ainslie, R., Shill, J.P. and Neet, K.E. (1972) Transients and Cooperativity. A Slow Transition Model for Relating Transients and Cooperative Kinetics of Enzymes. *Journal of Biological Chemistry*, **247**, 7088-7096.