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Impact of *Chrysanthemum fontanesii* extract on sodium valproate mediated oxidative damage in mice kidney

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ABSTRACT

The objective of this study was to evaluate the potential of *n*-butanol extract obtained from flowers of *Chrysanthemum fontanesii* (*Cf*) and vitamin E against kidney oxidant injury induced by sodium valproate in mice. The evaluation was made through Histopathological examination and measuring kidney lipid peroxidation (LPO) and antioxidant parameters: reduced glutathione (GSH), glutathione peroxidase (GPx) and catalase. Severe alterations in all biomarkers were observed after injury with VPA. Treatment with *Cf* extract and vitamin E resulted in markedly decreased levels of LPO while increased levels of GSH and antioxidant enzymes activities were observed. In conclusion, *n*-butanol extract of *Cf* may be an interesting candidate for the treatment of kidney injury induced by VPA through enhancing endogenous antioxidant defenses and normalizing the kidney histopathological architecture.

INTRODUCTION

Valproic acid (VPA) is an effective and widely used anticonvulsant, associated with metabolic adverse effects such as hepatotoxicity and nephrotoxicity (Gossrau and Graf, 1989; Raza *et al*., 2000; Rahman *et al*., 2006). Although the exact biochemical mechanisms of VPA toxicity to kidney have not been well defined, several hypothesis have been proposed. The recent hypothesis suggests an involvement of peroxidative injury in VPA induced renal tubular disorder (Raza *et al*., 1997); free radical scavengers like vitamin E supplements provided adequate protection against VPA toxicity in kidney (Aktas *et al*., 2010). *Chrysanthemum* (Asteraceae) is a large genus of about 300 species (Kumar *et al*., 2005) from which *Chrysanthemum fontanesii* growing in algeria. A series of studies have demonstrated that *Chrysanthemum* species possesses antioxidants (Duh *et al*., 1999; Lin *et al*., 2010; Liu *et al*., 2012; Amrani *et al*., 2013[a]), anticancer (Bi *et al*., 2008; Jin *et al*., 2009), antimicrobial (Shunying *et al*., 2005), anti-inflammatory (Lee *et al*., 2009; Kim *et al*., 2012), immunomodulatory (Cheng *et al*., 2005), Hepatoprotective (Amrani *et al*., 2013[b]) and neuroprotective effects (Chun *et al*., 2008).Many chemical compounds have been isolated and identified from species of the *Chrysanthemum* (Benaissa *et al*., 2011, Lograda *et al*., 2013). Based on these effects this study was designed to determine the possible protective effect of the *n*-butanol extract obtained from flowers of *Chrysanthemum fontanesii* against oxidative damage of kidneys induced by VPA.

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MATERIAL AND METHODS

Plants

Chrysanthemum fontanesii which is endemic to North Africa was collected during the flowering stage in the area of bijaia, north east of Algeria. Flowers (1516 g) were powdered and macerated at room temp with EtOH–H_2O (8:2 v/v) for 48 h three times. The filtrates were combined, concentrated and successively extracted with chloroform, ethyl acetate and *n*-butanol. The organic layers were dried with Na_2SO_4. Removal of solvents under reduced pressure, $CHCl_3$ (7.20 g), EtOAc (30.50 g), *n*-butanol (59.10 g) resulted in final extracts (Benaissa *et al*., 2011).

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Animals and treatment

Female Albino Swiss mice aging from 6-8 weeks were purchased from Pasteur Institut Algiers and were maintained on 12h light/dark cycle. The animals were kept in12h light/ dark cycles, maintained in an air-conditioned room at 22–25 °C, with free access to food and water ad *libitum.* The general guidelines for the use and care of living animals in scientific investigations were followed (Council of European Communities, 1986).

Mice were divided in to five groups (Gr) consisting of 8 animals each. All substances were given for 12 days.

1-Gr.1 served as control.

2-Gr.2 received VPA intraperitoneally (ip) at single dose of 300 mg/kg body weight for 12 days.

3-Gr.3 received plant extract (100mg/kg by gavage).

4-Gr.4 received plant extract (100mg/kg) prior to VPA administration.

5-Gr.5 received vitamin E orally (100mg/kg) prior to VPA administration.

After 12 days of study, all mice (eight animals in each of five groups) were killed. Kidneys were removed, weighed, and homogenized in 5 ml of ice-cold KCl 1.15%. Homogenates (10%) were centrifuged at 3000 rpm for 15 min at 4°C, and the supernatant was kept on ice until assayed.

Determination of kidney lipid peroxidation

Lipid peroxidation, an indicator of tissue injury induced by reactive oxygen species will be measured as thiobarbituric acid reactive substance (TBARS). The amount of kidney TBARS will be measured by the thiobarbituric acid assay (TBA) as previously described by (Uchiyama and Mihara, 1978). Briefly, 0.5 ml of kidney homogenate will be reacted with 1 ml of TBA reagent containing 0.67% TBA and 3 ml of 1% phosphoric acid. Samples will be boiled for 45 minutes, cooled and centrifuged. Absorbance of the supernatants will be spectrophotometrically measured at 532 nm. TBARS concentrations will be calculated by the use of 1,3,3,3 tetra-ethoxypropane as a standard. The results will be expressed as nmol/g tissue weight.

Measurement of glutathione

Reduced glutathione (GSH) content in the kidney was measured according to the method described by (Ellman, 1959) using Ellman's reagent. This method is based on the reactive cleavage of 5, 5′-dithiobis-(2-nitrobenzoic acid) by sulfohydryl group to yield a yellow colour with maximum absorbance at 412 nm against reagent blank with no homogenate.

Evaluation of GPx activity

GPx activity in the kidney was measured according to the method described by (Flohe and Gunzler, 1984).Based on H_2O_2 reduction in the medium by GPx in the presence of GSH. Briefly 0.2ml supernatant obtained from kidneys, 0.4ml GSH (0.1 mM), 0.2ml TBS solution (Tris 50mM, NaCl 150mM PH 7.4) were added to the tubes and mixed. After 5 min incubation at 25 °C, 0.2 ml of H_2O_2 (1.3mM) was added in the mixture. The reaction was stopped after 10 min by addition of 1ml trichloroacetic acid (TCA 1 %, w/v), and then the tubes maintained at 0-5°C in an ice bath for 30min, centrifuged for 10 min at 3000 rpm. After this, 0.48ml supernatant was taken and added to each tube, and then 2.2 ml TBS solution and 0.32 ml DTNB (1mM) were added. The optical density was measured at 412 nm in the spectrophotometer after 5 min.

Evaluation of catalase activity

The enzymatic activity of catalase was measured by determining the decomposition of its substrate H_2O_2as described by(Claiborne,1985).The homogenate was centrifuged at 10000 rpm for 45 min at 4° C; the final supernatant is the source used for the evaluation of the activity of catalase. The disappearance of H_2O_2 was determined spectrophotometrically at 240 nm. Catalase activity was expressed as U/mg of protein,

In order to express the antioxidant enzyme (GPx, catalase) activities per gram of protein, total protein concentration was determined colorimetrically by using the method of(Lowry, 1951).

Histopathological examination

Kidney tissue fragments were taken and fixed in formalin 10 %. The fixed specimens were then trimmed, washed and dehydrated. These specimens were then embedded in paraffin, cut into 5µm thick sections and stained with Harris hematoxylin and eosin and examined microscopically.

Statistical analysis

Data are expressed as the mean ± SD. Statistical interferences were based on student's t-test for mean values comparing control and treated animals.

RESULTS

Effect of VPA, plant extract and vitamin E on LPO, GSH levels and GPx activityin mice kidney

Treated mice with VPA have developed kidney damage as was evident from the significant increase ($P<0.01$) of LPO, which is reversed in animals treated with both *Cf* and vitamin E (Fig.1). VPA were significantly decline in the activities of GPx($P<0.01$), catalase and GSH level ($P<0.001$). The treatment with *Cf/* or Vit E showed significant improvement in the antioxidant elements (Fig. 2, 3, 4).

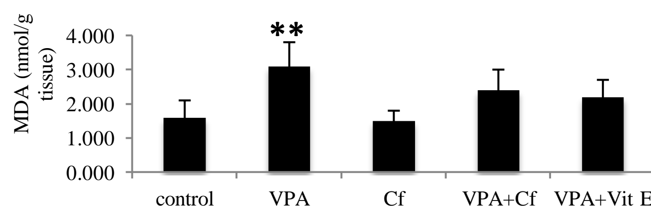

Fig. 1: Effect of VPA, plant extract and vitamin E on lipid peroxidation (TBARs content) in kidney Data are reported as means ± SD. Treatment groups were statistically compared to control group. (Students t-test, **P<0.01)

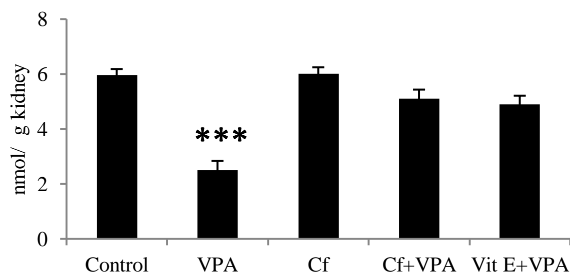

Fig. 2: Effect of VPA, plant extract and vitamin E on GSH levels in mice kidney after. 12 days of treatment. Data are reported as means ± SD.Treatment groups were statistically compared to control group(Students t-test, ***P<0.001).

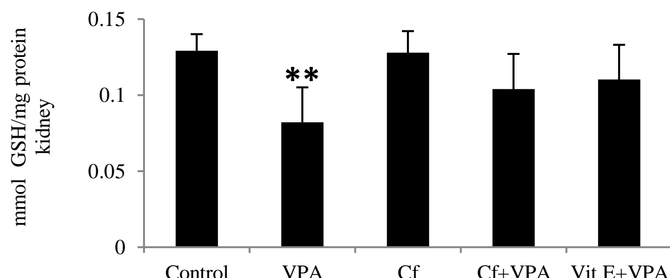

Fig. 3: Effect of VPA, plant extract and vitamin E on the antioxidant enzyme (GPx). activitiesin mice kidney after 12 days treatment. Data are reported as means ± SD., Treatment groups were statistically compared to control group. (Students t-test, **P<0.01).

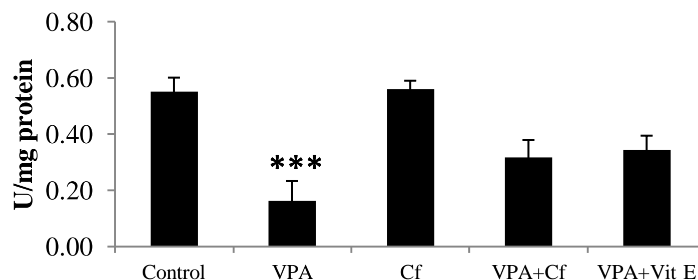

Fig. 4: Effect of VPA, plant extract and vitamin E on the antioxidant enzyme (Catalase) activities in mice kidney after 12 days treatment. Data are reported as means ± SD. Treatment groups were statistically compared to control group. (Students t-test, ***P<0.001). **Fig. 1, 2, 3 and 4:** Control; VPA, treated with 300 mg/kg valproic acid; VPA + Cf, treated with 300 mg/kg valproic acid and plant extract of *Chrysanthemum fontanesii*100 mg/kg ; VPA+Vit E, treated with 300 mg/kg valproic acid and 100mg/kg vitamin E ; VPA intraperitoneal injection. Plant extract and vitamin E were given by gavage. *: Significant p<0.05, **: Highly significant p<0.01***: Very highly significant p<0.001.

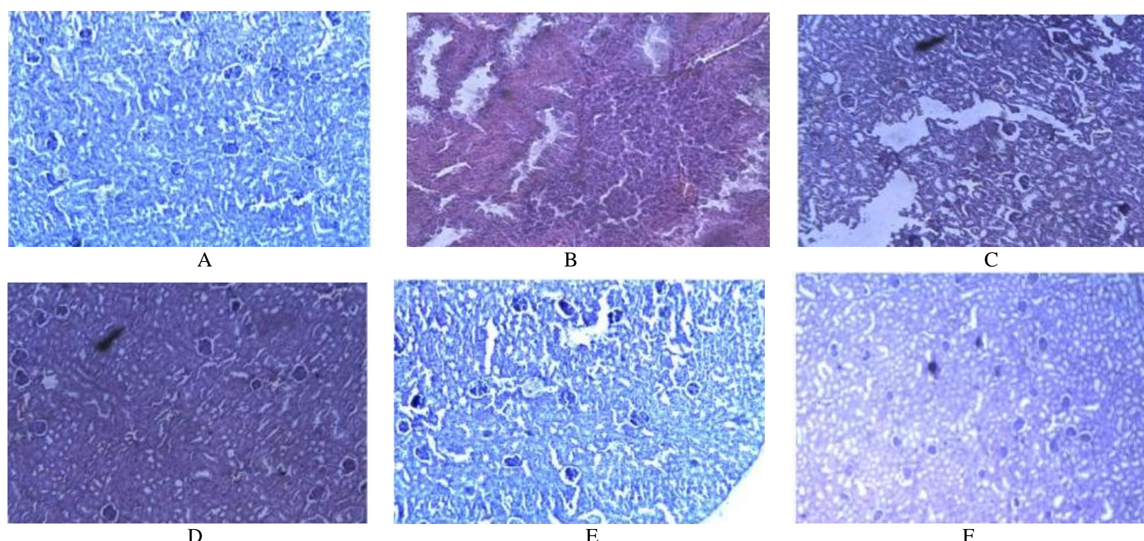

Fig. 5: Histopathologic changes affecting the kidneys of Swiss albino mice. **(A)**Normal kidney of the control group; **(B)** and**(C)**kidneys of VPA treated group showed necrosis, inflammatory cell infiltrates and oedema respectively; **(D)**kidney of *Cf* group showed a histopathological picture that closely approximate that of the control group; **(E)** and **(F)**kidneys of VPA+ Vitamin E and VPA+ Plant extract treated groups respectively showed congestion.

Histopathological examination

In the slides of kidney sections of the control group stained with Hematoxylene-Eosine, the appearance of the kidney was histologically normal (figure 5A). In kidney sections of valproic acid group; necrotic areas, inflammatory cell infiltrates, oedema and congestion were observed (fig. 5 B, C). The kidney section of plant extract treated mice, showed a histopathological picture that closely approximate that of the control group(figure 5D). In the groups of VPA+ Vitamin E and butanolic extract +VPA, sections belonging to these groups showed relatively normal ultra-structure when compared to VPA group. We noted some minor changes like Congestion (figure 5 E, F).

DISCUSSION

Results of the present study show that catalase, GPx activities and GSH level were significantly decreased in the VPA treated mice. Several studies have reported that reactive oxygen species (ROS) are considered to be important mediators of VPA induced kidney injury (Raza *et al*, 1997). The increased production of ROS causes inactivation of antioxidant enzymes. Normally GSH contributed to the intracellular antioxidant defense system as it is powerful consumer of free radicals. Glutathione (GSH) is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in tissues. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Also it is a substrate for glutathione peroxidase (GPx) and detoxifies foreign compounds and biotransformation drugs.

CAT widely distributed in all animal tissues which decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Mates and Sanddiez-jimenez, 1999). Therefore, reduction in the activity of these enzymes may result in a number of alterations do to the accumulation of superoxide radicals and H_2O_2 (Halliwell, 1994). The suppression of MDA content and the over expression of the antioxidant enzymes in mice treated with *Cf* implies that this potential antioxidant defense is reactivated by the plant extract of *Cf* with a resulting increase in the capacity of detoxification through enhanced scavenging of free radicals and acted by up regulating endogenous antioxidant defenses.

Most of these effects are considered to be due to the presence of wide range of well defined phytochemical phenolic compounds and flavonoids which have been known to be responsible of health promoting (Benaissa *et al*., 2011, Amrani *et al*., 2013[b]; 2014).

CONCLUSION

The present study revealed that the oral administration of *n*-butanol extract of *Chrysanthemum fontanesii* to valproic acid intoxicated mice exhibited significant nephroprotective effect. Hence, we suggest that *Chrysanthemum fontanesii* may be developed as successful chemotherapeutic agents.

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